TOWARD OPTIMAL
LABORATORY USE

HPV DNA Testing in Cervical Cancer Screening
Results From Women in a High-Risk Province of Costa Rica

Mark Schiffman, MD, MPH
Rolando Herrero, MD
Allan Hildesheim, PhD
Mark E. Sherman, MD
Maria Bratti, MD
Sholom Wacholder, PhD
Mario Alfaro, MD
Martha Hutchinson, MD
Jorge Morales, MD
Mitchell D. Greenberg, MD
Attila T. Lorincz, PhD

Context Human papillomaviruses (HPVs) are known to cause most cervical cancer worldwide, but the utility of HPV DNA testing in cervical cancer prevention has not been determined.

Objective To provide comprehensive data on the screening performance of HPV testing for the most common carcinogenic types, at different levels of analytic sensitivity.

Design Laboratory analysis conducted during 1993-1995, using 3 cytologic techniques and cervicography, followed by colposcopic examination of women with any abnormal cervical finding, to detect all high-grade intraepithelial lesions and cancer (reference standard of clinically significant disease). The HPV testing was performed subsequently with masking regarding clinical findings.

Setting Guanacaste Province, Costa Rica, a region with a high age-adjusted incidence of cervical cancer.

Participants Of 11,742 randomly selected women, 8,554 nonpregnant, sexually active women without hysterectomies underwent initial HPV DNA testing using the original Hybrid Capture Tube test; a stratified subsample of 1119 specimens was retested using the more analytically sensitive second generation assay, the Hybrid Capture II test.

Main Outcome Measures Receiver operating characteristic analysis of detection of cervical high-grade intraepithelial lesions and cancer by HPV DNA testing based on different cut points of positivity.

Results An analytic sensitivity of 1.0 pg/mL using the second generation assay would have permitted detection of 88.4% of 138 high-grade lesions and cancers (all 12 cancers were HPV-positive), with colposcopic referral of 12.3% of women. Papanicolaou testing using atypical squamous cells of undetermined significance as a cut point for referral resulted in 77.7% sensitivity and 94.2% specificity, with 6.9% referred. Specificity of the second generation assay for positivity for high-grade lesions and cancer was 89.0%, with 33.8% of remaining HPV DNA−positive subjects having low-grade or equivocal microscopically evident lesions. The higher detection threshold of 10 pg/mL used with the original assay had a sensitivity of 74.8% and a specificity of 93.4%. Lower levels of detection with the second generation assay (<1 pg/mL) proved clinically nonspecific without gains in diagnostic sensitivity.

Conclusions In this study population, a cut point of 1.0 pg/mL using the second generation assay permitted sensitive detection of cervical high-grade lesions and cancer, yielding an apparently optimal trade-off between high sensitivity and reasonable specificity for this test. The test will perform best in settings in which sensitive detection of high-grade lesions and cancer is paramount. Because HPV prevalence varies by population, HPV testing positive predictive value for detection of high-grade lesions and cancer will vary accordingly, with implications for utility relative to other cervical cancer screening methods.

See also pp 81 and 108.
progress to a high-grade preinvasive lesion (including carcinoma in situ at the most severe). High-grade lesions typically contain carcinogenic types of HPV. Once established, these lesions tend to persist. Many high-grade lesions become invasive cervical cancers, marked by higher frequency of genomic alteration. Invasive cancers are rare in the United States among women who are screened.

The Papanicolaou (Pap) test is the mainstay of cervical cancer prevention. The new cervical carcinogenesis model suggests that sensitive HPV DNA testing may be useful in cervical cancer prevention (both primary and secondary), but this is debated. Women in wealthier nations are protected (albeit imperfectly) via Pap test screening, by which microscopic cervical cellular changes caused by HPV known to precede or accompany cervical cancer are detected.

The debate on use of HPV testing to prevent cervical cancer must start with good data. Nearly all high-grade lesions and cancers contain carcinogenic HPV types, but excessive HPV testing must be avoided because infection represents common (and typically transient) processes, especially at the most sensitive level of DNA detection.

Each of the 10 to 15 carcinogenic genital HPV types implies a different risk to the patient, with greatest burden of risk attributed to type 16. However, gradations between the risks overlap and if costs permit, testing for the whole group for maximal sensitivity seems desirable, although restriction to fewer types would increase specificity incrementally.

However, the analytic sensitivity level that optimizes clinical effectiveness of HPV testing is not known. Early HPV assays were insensitive and identified only a few types.

This study was conducted to provide comprehensive data on HPV testing performance for carcinogenic types at various test positivity cut points. We evaluated HPV testing in screening for high-grade lesions and cancer and estimated HPV test sensitivity and specificity and resultant rates of referral to colposcopy over a possibly useful range of thresholds used previously for screening.

**METHODS**

**Study Population**

Women were randomly selected for recruitment into a National Cancer Institute (NCI)-sponsored cervical cancer screening study conducted in Guanacaste, Costa Rica, as reported. A follow-up phase is under way, but enrollment data only are presented here. The cohort was assembled in 1993-1994 via a door-to-door survey of all adult women residing in randomly chosen censal segments of Guanacaste. Local and NCI institutional review boards approved the study. Subjects provided written informed consent. A total of 11,742 women were identified, of whom 10,738 were eligible for the study (ie, age ≥18 years, full-time residents, mentally competent, and not pregnant) and 10,049 (93.6%) were interviewed. Pelvic examinations for 583 virgins were not done and 291 women refused or were physically unable to undergo examination. Thus, pelvic examination was completed on 9,175 participants, representing more than 90% of the eligible, nonvirgin population.

Analysis is further restricted to 8,554 women with no history of hysterectomy. Despite equivalent age-adjusted HPV DNA prevalences, only 2 women with hysterectomy had cytologic evidence of a high-grade lesion (not histologically confirmed) and 2 more of a low-grade lesion.

Apart from younger age, the final group of 8,554 women resembled the full cohort. Age range was 18 to older than 90 years, with a median age of 37 years. Most women in this predominantly rural province had fewer than 6 years of formal education, although literacy was nearly universal. More than three quarters were married. Median age at first intercourse was 18 years, with slightly more than 50% reporting 1 lifetime sexual partner and few women with more than 3. In prior studies, men in the region reported larger numbers of sexual partners. Of the women, 70% had 3 or more pregnancies, with about 40% reporting at least 5 pregnancies. Few women (11%) had ever smoked. Most women (87%) had a previous Pap test, although screening had been relatively ineffective in reducing cervical cancer incidence, apparently because of inadequate specimen preparation and interpretation, subject participation, and management of abnormal findings. Based on unpublished Costa Rican government serologic surveys (Gisela Herrera, MD, written communication, November 1999), prevalence of human immunodeficiency virus infection in Guanacaste was so low that its role was not considered in this study.

**Clinical Specimens**

For the conventional Pap test, exfoliated cervical cells collected with a Cervex brush (Unimar, Wilton, Conn) were prepared as conventional smears fixed with Pap Perfect (Medscand, Hollywood, Fla) and stained by an optimized Pap method in Costa Rica. Residual cells on the brush were rinsed in vials containing 20 mL of PreservCyt (Cytotec Corporation, Marlborough, Mass) and prepared as Thin-Prep cytologic specimens in the United States. A second cell specimen obtained with a Dacron swab was placed in Specimen Transport Medium (Digene Corporation, Silver Spring, Md) and shipped frozen to the United States for HPV DNA testing using the Hybrid Capture Tube (HCT) test and its successor, Hybrid Capture II (HC II, Digene Corporation). Finally, the cervix was rinsed with 5% acetic acid and 2 Cervigrams (photographs of the cervix) were obtained (National Testing Laboratories, Fenton, Mo) for visual screening.

**Clinical Evaluation**

Conventional smears were screened in Costa Rica by an expert Costa Rican cytopathologist (M.A.) and then reevaluated in the United States (M.E.S.) using the PAPNET (NSI, Suffern, NY) system, a semiautomated, computer-assisted screening device. The Thin-Prep slide was stained using a modified
Pap method and screened, then interpreted by a cytopathologist expert in ThinPrep cytology (M.H.). The 3 cytologic diagnoses (conventional, PAPNET-assisted, and ThinPrep) were made as per the Bethesda system as within normal limits or reactive cellular changes (negative), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, or carcinoma. Cervicography results were classified by an expert (M.D.G.) as normal (including atypical) or positive with graded severity.

**Colposcopic Referral**

As screening results became known, patients with any of the following conditions were referred for colposcopy: (1) physical examination findings suspicious for cancer, (2) cytologic diagnosis of ASCUS or more severe abnormality rendered on conventional smear in Costa Rica, the PAPNET-assisted review in the United States, or the ThinPrep slide in the United States, or (3) a positive cervicography result. An experienced gynecologist (J.M.) performed colposcopically directed biopsies of visible lesions with guidance from the cervicography. Biopsies were prepared as hematoxylin-eosin stained sections in Costa Rica and diagnosed locally for clinical purposes.

Patients with a high-grade histologic diagnosis (cervical intraepithelial neoplasia 2/3) or carcinoma, and those with a cytologic diagnosis of high-grade lesion rendered by 2 observers, were referred for large loop excision of the transformation zone, cold knife cone, or hysterectomy. Patients with a single initial cytologic diagnosis of high-grade lesion confirmed on review, but not associated with a high-grade or cancer biopsy result, were also referred for large loop excision if a lesion was identified colposcopically, there was no treatment contraindication, and the patient consented. The physicians in Costa Rica made all follow-up and final treatment decisions.

Of the 8554 women with no hysterectomy history, 2147 were referred to colposcopy and 96.6% participated. As a quality control measure, a 2% random sample of cohort subjects was referred to colposcopy to test the screening protocol sensitivity. No low- or high-grade lesions were found in women in the random sample having normal screening results (n = 128), suggesting that the combined screening protocol was sensitive in identifying abnormalities.

**Final Diagnoses**

Final case diagnoses were based on combining screening diagnoses and review (M.E.S.) of pathologic biopsy material from colposcopic and treatment visits. Final case diagnoses were made without knowledge of HPV test results and roughly followed the Bethesda system groupings. Glandular diagnoses were rare and subsumed under the appropriate squamous diagnosis. "Disease" was defined as cancer and high-grade lesions. All 12 cases of cancer were histologically confirmed. Of 128 high-grade lesions, 119 (93%) were biopsy confirmed. For the remaining 9, there was agreement on at least 2 of 3 cytologic diagnoses.

The 189 "low-grade lesion" diagnoses were based mainly on firm cytologic agreement vs biopsy confirmation. Because we view low-grade lesions to be typically transient and benign, we combined low-grade diagnoses with equivocal and normal categories for receiver operating characteristic curve (ROC) analysis, vs cancers and high-grade precursor lesions. A diagnosis of "equivocal" was assigned to 661 cases with various test result combinations not meriting diagnosis of a definite lesion such as a single cytologic diagnosis of low-grade lesion not corroborated by the other techniques, a positive cervicography result with normal cytology and histopathology findings, or equivocal results following review of all available tests (M.E.S.). The "negative" diagnostic category included 7564 patients with either completely negative screening results or cytologic diagnoses of atypical cells followed by normal colposcopic diagnoses.

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**HPV Testing**

All testing was rigorously masked by the NCI principal investigator. Initially, we used the currently available HCT because of its standardization (reflected in Food and Drug Administration [FDA] clearance) and ability to quantify HPV DNA.

The HCT test was performed on all available specimens (8539 of 8554) with a modified reported procedure. It provides a positive or negative test result at a threshold of about 10 pg/mL HPV DNA. An aliquot of Specimen Transport Medium was denatured to produce single-stranded DNA and reacted with a cocktail of 11 full-length RNA probes recognizing oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58. Hybrids consisting of target HPV bound to RNA probes were bound or “captured” on sides of tubes coated with antibodies recognizing DNA-RNA hybrids. Adding a second antibody tagged with alkaline phosphatase permitted detection of bound hybrid by a chemiluminescent readout. Test specimens in which light emission (expressed as relative light units [RLUs]) equalled or exceeded the mean of positive controls (PCs) consisting of 10 pg/mL HPV 16 DNA run in triplicate were categorized as positive (RLU/PC ≥ 1.0). Higher viral levels could be estimated as the ratio of test signal over positive control, but estimation of much lower levels was not possible because of nonlinear downward extrapolation.

The test also used a separate set of probes for 5 low-risk HPV types (6/11, 42, 43, and 44) that were rarely positive (<1% of the population) and did not associate strongly with high-grade lesions. These results are not presented.

To evaluate levels of oncogenic HPV types below the reliable detection threshold for HCT (10.0 pg/mL), we retested a subset of 1119 specimens with the HC II test,24 which subsequently received FDA approval for clinical use at the 1.0-pg/mL detection threshold. The HC II test is similar to the HCT procedure with only a few differences. The HC II test uses...
Data Analysis

Specimens were selected for HC II testing based on 6 sampling strata. (1) To permit direct calculation of assay sensitivity, we were able to test specimens from 126/128 high-grade lesions and all 12 cancers. We also tested all specimens from the 189 women with low-grade lesions. (2) We randomly sampled a fifth of women (20.6%) with equivocal (n = 661) final diagnoses. (3) We reassayed random samples of varying percentages among strata of women with negative (n = 7564) final diagnoses following different screening result combinations and focused most on the 96 women with an initial, unconfirmed atypical cytologic diagnosis who were HCT positive for carcinogenic types on previous testing and/or reported 5 or more sexual partners (68.8% retest). (4) We retested 42.9% of the 599 women with an unconfirmed atypical cytologic diagnosis alone, and (5) 8.6% of the 591 women with negative screening diagnoses who were HCT positive for carcinogenic types and/or reported 5 or more sexual partners. (6) The retesting included 4.5% of the 6278 women with completely negative findings on screening tests, negative HCT results for carcinogenic types, and 4 or fewer reported lifetime partners.

To estimate population-wide percentages, sampling strata were reconstituted. To apply HC II data to the population, sensitivity percentages were calculated directly (as all high-grade lesions and cancers were tested). To compute prevalence in the population, numbers of HPV-positive specimens for each of 6 sampling strata were divided by sampling fractions to derive the number of estimated HPV-positive test results derived from that stratum. Numbers of positive test results expected from each expanded stratum were added to obtain a total estimate of positive test results, which was divided by the number of women in the population to obtain the percentage of positive tests in the population.

Based on HCT testing and HC II estimates, an ROC analysis was done. For plausible thresholds of positivity for HPV testing, we cross-tabulated percent sensitivity of detecting high-grade lesions and cancer (y-axis) with 1 – specificity (x-axis). One minus specificity represents the percentage of women without high-grade lesions or cancer who would have been referred to colposcopy given that choice of an HPV positivity cut point (x-axis). A curve of (x, y) points indicating how HPV testing would perform along a curve of possible diagnostic thresholds was generated. The theoretical optimal cut point (x = 100% sensitivity, y = 0% nonspecificity) would detect only high-grade lesions and cancers without additional referrals (which would represent false-positives). In practice, all cut points suffer from imperfect specificity or unnecessary referrals. To complement the analysis of specificity, we calculated for each cut point the closely related statistic percentage referred to colposcopy. We also presented percentage referral because it provided a direct estimate of number of women requiring colposcopic evaluation given HC II test performance at each cut point. Because the percentage of women with high-grade cervical lesions and cancers is typically small in population-based screening programs (<2% here), the 2 statistics are similar.

Statistical significance of paired and independent proportions was tested using standard contingency table methods and association between viral load and lesion grade was assessed by analysis of variance of the log-transformed RLU/PC values.

RESULTS

The HC II test positivity at the 1.0-pg/mL cut point was strongly associated with screening results and final diagnoses. Percentages of HPV positivity were 5.0% in women with negative screening diagnoses and no risk factors, 10.9% with initial unconfirmed atypical cytologic diagnoses alone, 28.7% with equivocal final diagnoses, 31.4% with negative cytologic diagnoses plus HCT positivity and/or reporting 5 or more lifetime partners. (6) The retesting included 4.5% of the 6278 women with completely negative findings on screening tests, negative HCT results for carcinogenic types, and 4 or fewer reported lifetime partners. (6) The retesting included 4.5% of the 6278 women with completely negative findings on screening tests, negative HCT results for carcinogenic types, and 4 or fewer reported lifetime partners. (6) The retesting included 4.5% of the 6278 women with completely negative findings on screening tests, negative HCT results for carcinogenic types, and 4 or fewer reported lifetime partners.
more sexual partners, 54.5% with initial unconfirmed atypical cytologic diagnoses plus HCT positivity and/or 5 or more sexual partners, and 65.1% with low-grade lesions.

As shown in Figure 1, HPV DNA testing by HC II was strongly associated with the detection of high-grade lesions and cancers in the study population. The steep leftward rise of the observed curve far exceeded chance, which, on this type of plot, would have been seen as a linear increase with the approximate (x, y) relationship on the diagonal of % sensitivity = % referred.

The HCT test performance at the fixed cut point of 10 pg/mL (for carcinogenic types) coincided closely with the estimated performance from HC II test data (Table and Figure 1). Sensitivity of HCT testing for detection of high-grade lesions and cancer was 74.8%, and specificity was 93.4%. The sensitivity of HC II testing at a 10 pg/mL cut point was 72.3% and specificity, 94.0%. Thus, sampling scheme and extrapolated population estimates from the HC II testing were corroborated by the nearly complete HCT results.

The HC II test cut point for detection of high-grade lesions and cancer most closely balancing high sensitivity (88.4%) with specificity (89.0%) was about 1.0 pg/mL, at which all cancers and all high-grade lesions defined only by cytology were detected. As the threshold decreased from 10 pg/mL to 1 pg/mL, there was a pronounced increase in sensitivity, a steeply vertical rise indicating that sensitivity gains were achieved with little specificity loss in this diagnostic range. However, an infection point in the ROC curve was evident at positivity thresholds approaching 1.0 pg/mL. At positivity thresholds lower than 1.0 pg/mL, specificity losses became pronounced while further sensitivity advances were marginal.

The HPV testing at the 1.0-pg/mL threshold was more sensitive (88.4% sensitive, 89.0% specific, 12.3% referred) but less specific than conventional Pap testing using the ASCUS cut point for colposcopy referral (77.7% sensitivity, 94.2% specific, and 6.9% referred). Differences in sensitivity, specificity, and referral were significant by McNemar test for paired data (P < .001).

When analysis was restricted to women positive (>1.0 pg/mL) with the HC II test, severity of disease diagnosis was still associated with the rough estimate of viral load provided by the RLU/PC data. In HPV-positive women, those with cancer had a median DNA positivity of 100.7 pg/mL. Corresponding medians for other diagnostic categories were 84.6 (high-grade), 76.8 (low-grade), and 46.9 (equivocal), and 13.0 pg/mL (normal). Group means showed a similar trend, the main distinction being between women with and without lesions. Overall association was significant by analysis of variance (P < .001).

In the 8,414 women without high-grade lesions or cancer, 927 were estimated to be HPV-positive for carcinogenic types at the 1.0-pg/mL threshold while 7,487 were estimated to be HPV-negative. The 927 women with apparently false-positive HPV results included 313 (33.8%) with a final diagnosis less severe than a high-grade lesion but still an equivocal or low-grade lesion vs 537 (7.2%) of the 7,487 women estimated to have true-negative or missing HPV results with these diagnoses (P < .001). Median age of 37 years and prior lack of treatment may account for the relatively low numbers of low-grade compared with high-grade lesions.

We repeated the ROC curves by age tertiles because HPV infection is sexually transmitted and thus the acute and transient infections (unrelated to prevalent high-grade lesions and cancer) peak at young ages. As shown in the Table and Figure 2, HPV testing performance is optimal at older ages where sensitivity is high in all age groups, but specificity is improved with older age because prevalence of acute, benign human papillomavirus infection decreases with age. Asterisks indicate HPV 16 positive controls used at 0.1, 0.2, and 1.0 pg/mL cut points; values between control values were interpolated (see “HPV Testing” section).

Table. Sensitivity and Specificity of Human Papillomavirus DNA Hybrid Capture Tube (HCT) or Hybrid Capture II (HC II) Testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Population</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Referral, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT, 10 pg/mL*</td>
<td>All women</td>
<td>74.8</td>
<td>93.4</td>
<td>7.7</td>
</tr>
<tr>
<td>HC II, 1.0 pg/mL</td>
<td>Stratified sample of all women†</td>
<td>88.4</td>
<td>89.0</td>
<td>12.3</td>
</tr>
<tr>
<td>HC II, 1.0 pg/mL Ages 18-30†</td>
<td></td>
<td>92.9</td>
<td>80.2†</td>
<td>21.0</td>
</tr>
<tr>
<td>HC II, 1.0 pg/mL Ages 31-40†</td>
<td></td>
<td>80.8</td>
<td>90.3</td>
<td>11.2</td>
</tr>
<tr>
<td>HC II, 1.0 pg/mL Ages ≥41†</td>
<td></td>
<td>93.2</td>
<td>94.0</td>
<td>7.1</td>
</tr>
</tbody>
</table>

*The 8,554 women tested with HCT included 11 with cervical cancer and 128 with high-grade cervical lesions, 1 more case than for HC II. Sensitivity and specificity of HCT were calculated directly.
†The women tested with HC II (n = 1119) included virtually all women with cervical cancer (n = 12/12), high-grade cervical lesions (n = 126/128), or low-grade cervical lesions (n = 189/189) and a stratified random sample of equivocal and negative diagnostic categories. Sensitivity estimates for HC II were computed directly as percent HPV positive in those with high-grade lesions and cancer, in all women, or within age stratum. Specificity estimates of HC II were based on percent HPV DNA positivity and sampling fractions for each sampling stratum.

Figure 2. Receiver Operating Characteristic Curve for Hybrid Capture II (HC II) Test, for Detection of High-Grade Cervical Lesions and Cancer According to Age

Percent sensitivity for detection of cervical high-grade lesions and cancer is plotted on the y-axis, against the value (1 – specificity), closely linked to percent colposcopic referral, at that same positivity threshold. Sensitivity is high in all age groups, but specificity is improved with older age because prevalence of acute, benign human papillomavirus infection decreases with age. Asterisks indicate HPV 16 positive controls used at 0.1, 0.2, and 1.0 pg/mL cut points; values between control values were interpolated (see “HPV Testing” section).
number of women with high-grade lesions (31.0%) in our population. The youngest woman with cancer was younger than 25 years old.

Given that HPV testing has been proposed for triage of equivocal cytologic diagnoses, a secondary analysis of association of HC II test results with detection of high-grade lesions and cancer in women with ASCUS diagnoses was done. Small numbers did not permit firm conclusions and results varied by source of ASCUS diagnosis. Only 5 cases of high-grade lesions or cancer were associated with an ASCUS diagnosis on conventional Pap test, and all were HPV positive at the 1.0 pg/mL cut point (binomial, 97.5% confidence interval [CI], 47.8%-100%). ASCUS was more commonly diagnosed by the pathologist using ThinPrep technology. Of the 13 ASCUS diagnoses with associated high-grade lesions or cancer, 9 (69.2%; 95% CI, 38.6%-90.9%) were HPV positive at the 1.0 pg/mL cut point.

**COMMENT**

The success of HPV testing for triage or general screening will depend on proper determination of the analytic cut point. Prior debates on the value of HPV DNA testing may have suffered from inadequate understanding of test cut points and HPV type range being assayed. Many earlier studies had insensitive, type-restricted testing protocols. Other, extremely sensitive tests may have generated a sense that HPV is ubiquitous and tests lack clinical utility. In this high-risk population, we showed that HPV testing with HC II at 1.0 pg/mL detected almost 90% of 126 testable (out of 128) high-grade lesions and 100% of 12 cancers, with referral rate to colposcopy of about 12%. About a third of the apparently false-positive HPV results at the 1.0 pg/mL cut point were associated with definite or equivocal low-grade cytologic lesions defined as “nondisease” for this analysis. Sensitivity of HC II testing for detection of high-grade lesions and cancer is likely to be high in all populations because the assay probes for the main carcinogenic types found worldwide. However, as shown here, even potentially oncogenic HPV-type infections are found commonly in women with low-grade, equivocal, or normal diagnoses. Thus, percent referral to colposcopy based on HC II testing, and specificity and positive predictive value of this assay for detection of high-grade lesions and cancer, will depend on HPV infection population prevalence, which depends largely, in turn, on age-specific societal sexual practices. Thus, any application of general testing will require careful planning, using ROC or equivalent methods, to avoid excessive referrals to colposcopy based on detection of HPV infection in its usually benign state.

In HPV-positive women, those with lesions appeared to have higher viral loads as measured via RLU/PC values. There may be utility in semiquantitative measurement of high HPV viral load in lesion management. However, viral load distinctions are prone to variability, given that test results are dependent on numbers of viral particles per infected cell, lesion size and position, the tendency of the lesion to exfoliate relative to surrounding epithelium, and specimen adequacy. Nonetheless, better viral load measurements might be useful clinically and attempts to refine measurements, particularly by validation with a standard denominator of numbers of epithelial cells collected, are worthwhile.

The HC II assay targets 13 carcinogenic HPV types, but detects some lower-risk types such as 53 and 66 (albeit with lower efficiency). We could not assess whether another assay detecting a more restricted range of carcinogenic HPVs could maintain sensitivity and have increased specificity. Geographic variation in the etiologic fraction of cancers caused by less prevalent HPV types does exist; thus, a test for all regions could be difficult to develop.

The ROC analysis could be expanded to explore age restriction effects and to compare screening tests, used singly or in combination. Overall, HPV testing was more sensitive than conventional Pap testing (88.4% vs 77.7%) for high-grade lesions and cancer but less specific (89.0% vs 94.2%). The combined sensitivity/specificity of our Costa Rican cytopathologist collaborator (M.A.) was at the high end of these values in reported literature for conventional Pap tests. Also, thin-layer cytology was especially accurate, matching HC II test performance for sensitivity and specificity when performed by an expert cytopathologist. A variety of 2-technique screening combinations approached 100% sensitivity for high-grade lesions and cancer (data not shown). Such combinations, in addition to higher expense of multiple tests, would generate high referral rates. However, such nonspecificity might be acceptable in wealthy nations, particularly if the screening interval could be lengthened because of more sensitive, and thus more reassuring, screening results.

Cytology will likely continue to be the major screening method for cervical cancer prevention in the United States, but it has proven difficult to standardize at the highest levels of expertise. We are actively working on novel cervical cancer diagnostic assays in Costa Rica and in US centers participating in an HPV testing study of management of low-grade and equivocal cytologic abnormalities. In the US trial, we have seen that HPV assays such as HC II can be optimized and performed routinely at regional laboratories. Using masked sets of retested specimens, correlations of RLU/PC values between laboratories are consistently high (Pearson $r=0.90$), yielding agreement rates regarding HPV positivity approaching 95% (Cosette Wheeler, PhD, written communication, October 1999). Also, HC II test performance is similar to the other major approaches to HPV testing based on consensus primer polymerase chain reaction assays. Optimized HPV testing protocols have converged independently on a common set of HPV types, detected at a comparable threshold, an important landmark for scientists interested in population studies of HPV.
and cervical cancer prevention. Human papillomavirus testing should be considered as a viable cervical cancer screening method that has come of age technically. Thus, cervical cancer is more than ever a virtually preventable disease.

Author Affiliations: Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Md (Dr Schiffman, Hildesheim, and Wacholder); International Agency for Research on Cancer, Lyon, France (Dr Herrero); Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, Md (Dr Sherman); Costa Rican Social Security Administration, San Jose, Costa Rica (Drs Bratti, Alfaro, and Morales); Women's and Infants' Hospital, Providence, Ri (Dr Hutchinson); Omnia, Blue Bell, Pa (Dr Greenberg); and Digene Corp, Silver Spring, Md (Dr Lorincz).

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Corresponding Author: Mark Schiffman, MD, MPH, Interdisciplinary Studies Section, Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Room 7066, 6120 Executive Blvd, Rockville, MD 20852 (e-mail: schiffmm@exchange.nih.gov).

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