Human Papillomavirus DNA Testing for Cervical Cancer Screening in Low-Resource Settings

Louise Kuhn, Lynette Denny, Amy Pollack, Attila Lorincz, Ralph M. Richart, Thomas C. Wright

Background: In many low-resource settings, there are barriers to cytologic screening for cervical cancer. This study evaluates human papillomavirus (HPV) DNA testing as an alternative screening method. Methods: Cervical samples from 2944 previously unscreened South African women aged 35–65 years were tested for high-risk types of HPV with the use of the Hybrid Capture I (HCI) assay. Women also had a Pap smear, direct visual inspection of the cervix, and Cervicography™. Women positive on any screening test were referred for colposcopy. Samples from women with biopsy-confirmed, low-grade squamous intraepithelial lesions (SILs) (n = 95), high-grade SILs (n = 74), or invasive cervical cancer (n = 12) and a random sample of women with no cervical disease (n = 243) were retested for HPV DNA with the use of the more sensitive Hybrid Capture II (HCII) assay. All P values are two-sided. Results: High-risk HPV DNA was detected in 73.3% and 88.4% of 86 women with high-grade SIL or invasive cancer and in 12.2% of 2680 and 18.1% of 243 women without evidence of cervical disease, with the use of the HCI and HCII assays, respectively. HPV DNA testing with the HCII assay was more sensitive than cytology for detecting high-grade SIL and invasive cancer (McNemar’s test, P = .04), and testing with the HCI assay was of equivalent sensitivity (P = .61). Cytology had a statistically significantly better specificity (96.8%) than either the HCI assay (87.8%) or the HCII assay (81.9%) (P<.01). Receiver operating characteristic curves identified test cutoff values that allow HPV DNA testing to identify 57% of women with high-grade SIL or cancer, while classifying less than 5% of women with no cervical disease as HPV DNA positive. Conclusions: HPV DNA testing has a sensitivity equivalent to, or better than, that of cytology. Since HPV DNA testing programs may be easier to implement than cytologic screening, HPV testing should be considered for primary cervical cancer screening in low-resource settings.

in younger women, and detection of HPV DNA does not necessarily imply the presence of SIL or invasive cervical cancer.

Another reason why HPV DNA testing has not been adopted for routine cervical cancer screening is that the earlier commercially available HPV DNA molecular diagnostic tests lacked the required sensitivity. Recently, robust, highly sensitive HPV DNA tests have become commercially available. These tests have sensitivities equivalent or superior to the sensitivity of cytology for detecting high-grade SIL and cervical cancer (11–13). The availability of these highly sensitive tests offers the potential for replacement of conventional cytologic screening in settings where cytologic screening programs have not been successful.

There are many barriers to conventional cytologic cervical cancer screening programs in low-resource settings (14). One of these barriers is the nature of the screening test. Cytologic screening programs require trained cytotechnicians, high-quality laboratories that maintain adequate quality-control programs, an infrastructure for transporting smears to the laboratory and results back to the clinical site, and the capacity to recall, diagnose, and treat women with abnormal results. It is possible that HPV DNA testing, particularly if a rapid test that could be performed on site were developed, might be easier to implement in low-resource settings.

In this article, we report results of a study conducted in Khayelitsha, South Africa, that evaluated HPV DNA testing as a primary screening test for cervical cancer in a low-resource setting.

**Subjects and Methods**

**Participants**

A total of 2944 women, 35–65 years of age, who had not previously had a Pap smear, were enrolled at a primary care clinical site in Khayelitsha, which is a periurban, informal settlement located outside Cape Town, South Africa. All of the women were volunteers and were informed of the study through posters placed in community health centers and a variety of community-based outreach programs that included presentations at churches, community meetings, and radio programs. A total of 2957 women presented for the study, of whom 2944 were eligible and were enrolled. Written informed consent was obtained from the women after meeting with a health educator, and the women were then administered a questionnaire. The study was approved by the Institutional Review Boards of Columbia University (New York, NY) and the University of Cape Town (South Africa).

**Testing**

A gynecologic examination was performed on the participants by a nursing sister. The examination included four screening tests performed sequentially: 1) a Pap smear for which an Accellon sampler (MedScand, Hollywood, FL) was used; 2) a sample for HPV DNA testing obtained by breaking the tip from the Accellon sampler into an HPV specimen collection tube (Digene Corporation, Silver Spring, MD) after the Pap smear had been prepared; 3) direct visual inspection of the cervix following application of 5% acetic acid with and without 2.5× magnification (Edmund Scientific, Barrington, NJ); and 4) a 35-mm photograph of the cervix (Cervigram™; National Testing Laboratories, St. Louis, MO). All of the women were asked to return to the clinic 2–6 days after the initial examinations.

HPV DNA status was determined at the University of Cape Town with the use of the first-generation Hybrid Capture I (HCI) HPV DNA assay (Digene Corporation). The HPV DNA test was run according to the manufacturer’s instructions by use of the tube-based format and probes for “high oncogenic risk” HPV types (i.e., types 16, 18, 31, 33, 35, 45, 51, 52, and 56) (15). HPV determinations were read out as the ratio of relative light units (RLU) of HPV DNA in the sample to that of a positive control set at 10 pg/mL HPV DNA (corresponding to approximately 100,000 HPV genome copies per test). The HPV DNA test results were missing for one woman; she was excluded.

For purposes of referral to colposcopy, a “positive” result on the HPV DNA screening test was defined as high levels of high-risk HPV DNA (high cutoff level >10 times the positive control or approximately >100 pg/mL HPV DNA with the use of the HCI assay). When they returned for their results, women with a positive HPV DNA screening test result or with positive results on their initial direct visual inspection examination underwent on-site colposcopy. Biopsy samples were taken from minor-grade colposcopic lesions (Reid score <3) (16). High-grade lesions (Reid score ≥3) (16) were electrosurgically excised. An endocervical curettage was performed if no lesions were visible.

All laboratory tests were performed without knowledge of the results of the other tests. Pap smears were evaluated at the University of Cape Town, and Cervigrams were evaluated at the National Testing Laboratories. Women whose cytology results were diagnosed as low-grade SIL, high-grade SIL, or cancer (smears diagnosed as having atypical squamous cells of undetermined significance [ASCUS] were considered to be negative) or with positive Cervigrams (atypical Cervigrams were considered to be negative) were traced and recalled for colposcopy. Of the biopsy specimens, loop excision specimens, and endocervical curettage specimens were evaluated blindly at Columbia University and were diagnosed using a modification of the Bethesda System terminology (17).

A total of 2861 (97.2%) of 2943 women with HPV DNA results could be classified by cervical disease status, among whom 12 (0.4%) women with invasive cervical cancer, 74 (2.6%) with high-grade SIL (cervical intraepithelial neoplasia [CIN] 2 or 3), and 95 (3.3%) with low-grade SIL (CIN 1) were identified by histology (17). Of 2943 women, 2101 (71.4%) had four negative testing results or with no SIL or cancer detected at colposcopy. Of 842 women with at least one positive screening test referred for colposcopy, 760 (90.3%) underwent colposcopy; 579 women who underwent colposcopy had no histologic evidence of cervical disease. Therefore, a total of 2680 women (91.1%) of the 2943 women with HPV DNA results were classified as having “no cervical disease” (e.g., either negative on the four screening tests and not referred for colposcopy or referred for colposcopy and found to have no histologic evidence of SIL of any grade or invasive cervical cancer).

A subset of Accellon cervical samplers that had been stored at 4 °C was later tested at Columbia University with the use of the second-generation Hybrid Capture II (HCII) HPV DNA assay (Digene Corporation). The subset test consisted of all women with a histologic diagnosis of low-grade SIL (CIN 1), high-grade SIL (CIN 2 or 3), or invasive cervical cancer and an approximately 10% random sample (n = 243) of all the women with no positive screening test results or with a positive screening test(s) but no SIL or cancer detected at colposcopy and biopsy. The HCII assay uses probes for 13 high-risk HPV types (types 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, and 68) and a microtiter plate-based assay format. The chosen analytic sensitivity limit of the HCII assay for high-risk HPV types was 1 pg/mL.

Because both HPV assays are quantitative, we used two different HPV DNA levels to define a “positive” result for the purposes of the analysis. The first cutoff level classifies only samples with relatively high levels of high-risk HPV DNA (RLU >10x the positive control) as being positive. The second cutoff classifies samples with lower levels of HPV DNA (RLU >1x the positive control) as being positive.

**Statistical Analysis**

Chi-squared tests were used to compare proportions (e.g., sensitivity and specificity). Quantitative HPV DNA determinations were analyzed on a log10 scale and were compared with the use of Kruskal–Wallis tests. Ratios of the prevalence of HPV DNA among women with disease to women without disease and 95% confidence intervals (CIs) were calculated (18). Agreement between the two HPV tests was estimated by use of kappa coefficients. McNemar’s test was used to compare the sensitivity and specificity of HPV DNA testing with those of cytology (19, 20). Although we cannot estimate true sensitivity and specificity (because a few women with disease may have been missed by all four screening tests), we can directly compare the sensitivity and specificity of HPV DNA testing with those of the other screening tests. Receiver operating characteristic (ROC) curves (21) were calculated to investigate the consequences of shifting cutoff values used to define each HPV test as positive. The ROC curves were used to estimate the estimates from the ROC curve; values to identify all of the histologically confirmed cases of severe disease (high-grade SIL or invasive cancer) detected in the course of the study against decreases in
the specificity. Women with no positive screening tests or who had at least one positive screening test but no histologic evidence of disease following colposcopy were considered to be free from disease for the calculation of specificity (i.e., women with low-grade SIL were excluded from this analysis). Since the HCII assay was performed on only a stratified sample of the study population (including a random sample of all women with no positive screening test results), the estimated HPV DNA prevalence with the use of this test was calculated as a weighted average of the stratum-specific prevalences. All P values were two-sided and were considered to be statistically significant at .05.

RESULTS

HPV DNA Prevalence

High-risk HPV DNA was detectable with the use of the HCI assay in 16.2% (95% CI = 14.9%–17.6%) and high levels (>10× the positive control) were measured in 6.1% (95% CI = 5.3%–7.1%) of 2943 women screened with the use of the HCI assay. The weighted average of the prevalence of HPV DNA (any level) with the use of the HCI assay was 22.0% (95% CI = 17.4%–26.6%). Within the age distribution in this study (35–65 years), the prevalence of HPV DNA positivity (any level) with the use of the HCI assay was lowest in women aged 40–49 years (Fig. 1). High levels of HPV DNA with the use of the HCI assay (>10× the positive control) also had a clear U-shaped age distribution.

Positive Predictive Value of HPV DNA Testing

Of 180 women with high levels of HPV DNA (>10× the positive control) detected with the use of the HCI assay, 171 (95%) underwent colposcopy. Cancer was detected in five (2.9%) of these women, high-grade SIL in 38 (22.2%), and low-grade SIL in 44 (25.7%). Although detection of lower levels of HPV DNA by the HCI assay was not a criterion for referral for colposcopy in this study, 97 (32.8%) of 296 women with HPV DNA detectable at levels higher than 1× but lower than 10× the positive control received colposcopy because of other positive tests (viz., Pap smears, direct visual inspection of the cervix, or Cervicography™). When we combined all 268 women with any level of detectable HPV DNA (>1× the positive control) seen for colposcopy, cancer was detected in 10 (3.7%), high-grade SIL (CIN 2 or 3) in 53 (19.8%), and low-grade SIL (CIN 1) in 62 (23.1%). Thus, the positive predictive value of detection of HPV DNA testing >1× the positive control was 46.6% for low-grade SIL or higher and 23.5% for high-grade SIL or higher. In comparison, the positive predictive value of cytology was 58.8% for low-grade SIL or higher and 31.9% for high-grade SIL or higher.

Estimated Sensitivity and Specificity of HPV DNA Testing

With the use of the HCI assay, high-risk HPV DNA was detected at the low cutoff level (RLU >1× the positive control) in 10 (83.3%) of 12 women with invasive cervical cancer, in 71.6% of 74 women with histologically confirmed high-grade SIL, in 65.3% of 95 women with low-grade SIL, and in 12.2% of 2680 women with no cervical disease (Table 1). Thus, the estimated sensitivity of the HCI assay for detection of high-grade SIL or higher was 73.3% (95% CI = 62.6%–82.2%), and the estimated specificity was 87.8% (95% CI = 86.6%–89.0%).

Associations between HPV and disease were assessed by use of the prevalence ratio—i.e., ratio of the proportion of women who were HPV DNA positive in each grade of disease to the proportion of women who were HPV DNA positive and had no evidence of cervical disease. Compared with women with no evidence of disease, the prevalence of high-risk HPV DNA detected with the HCI assay at a low cutoff level was 6.8-fold higher (95% CI = 5.20–8.97) in women with cancer, 5.9-fold higher (95% CI = 4.93–7.00) in women with high-grade SIL, and 5.4-fold higher (95% CI = 4.48–6.40) in women with low-grade SIL. Although the prevalence of HPV DNA positivity was lower in all disease categories when high cutoff levels were used (>10× the positive control) to define a positive result, the magnitude of associations between HPV DNA and disease was stronger. Prevalence ratios for detection of HPV DNA at high cutoff levels (RLU >10× the positive control) were 13.5 (95% CI = 6.67–27.16) for women with cancer, 16.6 (95% CI = 12.21–22.54) for women with high-grade SIL, and 15.0 (95% CI = 11.05–20.25) for women with low-grade SIL.

High-risk HPV DNA prevalence in all disease categories was higher when measured by the HCII assay than when measured by the HCI assay (Table 1). With the use of the HCII assay at the standard cutoff level (RLU >1× the positive control) to define a positive result, HPV DNA was detected in all 12 (100%) women

**Fig. 1.** Prevalence of human papillomavirus (HPV) DNA detected with the use of the Hybrid Capture I assay in 2943 cervical samples by age category. **Hatched bars** indicate the proportion of samples classified as positive with the use of a low cutoff value (relative light units [RLU] >1× the positive control). **Solid bars** indicate the proportion of samples classified as positive with the use of a high cutoff value (RLU >10× the positive control).
with cancer, in 86.5% of women with histologically confirmed high-grade SIL, and in 18.1% of women with no cervical disease. Thus, the estimated sensitivity of the HCl assay for detection of high-grade SIL or higher was 88.4% (95% CI = 76.9%–92.6%), and the estimated specificity was 81.9% (95% CI = 73.3%–86.6%). The sensitivity of HPV DNA testing was roughly equivalent between the HCl assay in which a high cutoff level was used to define a positive result (RLU >10× the positive control) (77.9%) and the HCl assay in which a standard cutoff level was used (RLU >1× the positive control) (73.3%). Associations between HPV DNA and disease were stronger with the HCl assay in which the higher cutoff level was used than with the HCl assay in which the standard cutoff level was used. Prevalence ratios were 10.1 (95% CI = 6.93–14.80) for women with cancer, 7.5 (95% CI = 5.03–11.26) for women with high-grade SIL, and 6.0 (95% CI = 3.94–9.04) for women with low-grade SIL relative to women without cervical disease with the use of the HCl assay at a high cutoff level (RLU >10× the positive control) to define a positive result.

In comparison, the estimated sensitivity of cytology for detection of high-grade SIL or higher was 78.3% (95% CI = 67.9%–86.6%), and the estimated specificity was 96.8% (95% CI = 96.1%–97.4%). With the use of McNemar’s test, the sensitivity of cytology was not significantly different from that of HPV DNA testing with the HCl assay at a standard cutoff value (two-tailed $P = .61$) and was significantly worse than that of HPV DNA testing with the HCl assay at a standard cutoff value ($P = .04$). The specificity of cytology was significantly better than either the HCl assay ($P<.01$) or the HCl assay ($P<.01$) at standard cutoff values.

There were no statistically significant differences in the sensitivity of HPV DNA testing across different age categories between 35 and 65 years (data not shown).

**Table 1. Prevalence of high-risk types of human papillomavirus (HPV) DNA detected with the use of the Hybrid Capture I (HCl) assay and the Hybrid Capture II (HCII) assay among 12 women with invasive cervical cancer, 74 women with histologically confirmed high-grade SIL, 95 women with low-grade SIL, 2680 women with no disease (by HCl assay), and 243 women with no disease (by HCII assay)**

<table>
<thead>
<tr>
<th>Disease category</th>
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<th>HCII assay</th>
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<tbody>
<tr>
<td></td>
<td>No. of patients</td>
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<td>Cancer</td>
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</tr>
<tr>
<td>High-grade SIL</td>
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<td>71.6</td>
</tr>
<tr>
<td>Low-grade SIL</td>
<td>95</td>
<td>65.3</td>
</tr>
<tr>
<td>No disease††</td>
<td>2680</td>
<td>12.2</td>
</tr>
</tbody>
</table>

*SIL = squamous intraepithelial lesion; RLU = relative light units.
†RLU of sample >1× the positive control (i.e., 10 pg/mL HPV DNA for the HCl assay and 1 pg/mL HPV DNA for the HCII assay).
‡RLU of sample >10× the positive control.
§RLU of sample >100× the positive control.
††Women were classified as having “no disease” if they had no positive screening tests or if they had one or more positive screening tests and no histologic evidence of cervical neoplasia following colposcopy.

**Agreement Between the HCl Assay and the HCII Assay**

In the 424 samples that were tested by both the HCl assay and the HCII assay, the quantities of HPV DNA measured by the two tests were statistically significantly correlated ($r = .81; P<.0001$), although the mean levels of HPV DNA were approximately 10-fold higher with the HCII assay (Fig. 2). Since the HCII assay used a 10 pg/mL and the HCII assay used a 1 pg/mL positive control, agreement between the two tests was assessed with the use of a standard cutoff (RLU >1× the positive control) for the HCl assay and a higher cutoff (RLU >10× the positive control) for the HCII assay. Agreement was excellent (kappa = 0.77). Among women with histologically confirmed high-grade SIL, 10.8% were missed by both tests, 2.7% were detected by the HCl assay but not by the HCII assay, and 17.6% were detected by the HCII assay but not by the HCl assay. Among women with no evidence of disease, 9.5% were classified as positive by both tests, 4.5% were classified as positive by the HCl assay but not by the HCII assay, and 8.6% were classified as positive by the HCII assay but not by the HCl assay.

**HPV DNA Quantity by Disease Severity**

A gradient in mean RLU levels (corresponding to the number of copies of HPV DNA present) by disease severity was observed when the HCII assay was used. The geometric mean levels (± standard deviation) were 24.9 ± 28.6 in women with low-grade SIL, 62.5 ± 18.91 in women with high-grade SIL, and 113.8 ± 6.92 in women with cancer (Fig. 3, A). With the HCII assay, trends were similar but weaker (no statistically significant differences in mean RLU levels) (Fig. 3, B). However, when only women with RLU greater than 1× the positive control were considered, the mean RLU levels did not differ by disease severity. With the HCII assay, the geometric mean RLU levels of samples with RLU greater than 1× the positive control were 150.0 ± 8.78 in women with low-grade SIL, 149.3 ± 7.90 in women with high-grade SIL, and 113.8 ± 6.92 in women with cancer.

**ROC Curves**

ROC curves comparing the relative performance of the HCl assay with that of the HCII assay are shown in Fig. 4. The area
under the ROC curve (higher values indicate better overall performance) was 0.88 for the HCII assay and 0.83 for the HCI assay. However, when the RLU cutoff values of the two tests were set such that the prevalence of HPV DNA positivity in the population was below 8% (equivalent to a specificity of 92%), the corresponding capacities of the two tests to identify cases of confirmed high-grade SIL or cancer were almost identical. The HCII assay offered improved sensitivity to detect cases of high-grade SIL and cancer over that of the HCI assay when lower specificities (i.e., lower cutoff values on the HPV DNA test) were allowed (Fig. 4).

The HCII assay had an estimated sensitivity of 88% for detection of high-grade SIL and cancer if the estimated specificity was allowed to decrease to 82%, a circumstance that occurred at the lowest test threshold (RLU >1× the positive control). The HCI assay reached an estimated sensitivity plateau around 73% detection of high-grade SIL or cancer, at which point the estimated specificity was 88% (Table 2). At an estimated specificity of 95% (i.e., when the HPV DNA prevalence was 5% in women at low risk for having cervical disease), either test could achieve estimated sensitivities of 57%.

**DISCUSSION**

This study was designed to evaluate HPV DNA testing as a primary cervical cancer screening method for low-resource settings. HPV DNA testing offers a number of theoretical advantages over cytologic screening in select settings. In contrast to cervical cytology, which is highly subjective, HPV DNA tests are standardized and provide a quantitative determination of the amount of HPV DNA present in a sample. A midlevel technician can test a large number of samples for HPV DNA each day, whereas cytologic screening requires highly trained cytotechnicians who can evaluate only 55–80 specimens daily (22). In addition, the results of an HPV DNA test should not be adversely influenced by cervical inflammation and cervicovaginitis, which are common conditions in women in low-resource settings. However, the ultimate clinical acceptability of a screening test depends on the sensitivity and specificity of the test in the target population.

The prevalence of high-risk HPV DNA was very high in this unselected population of older, urban African women. High-risk HPV DNA was detected in 16% of women with the use of the HCI assay and in 22% of women with the use of the HCII assay with the standard cutoff (RLU >1× the positive control) to define a positive result, and relatively high levels of high-risk HPV DNA were present in many of these women. In subsequent surveys of women from this community, we have found that approximately 8% of the women in this age group are seropositive for human immunodeficiency virus, 6% have chlamydia or gonorrhea infections, and 18% have trichomonas vaginalis infections. Therefore, this population is clearly at high risk for sexually transmitted diseases.

Of the two HPV DNA assays evaluated in this study, the second-generation HCII assay was superior—in some respects—to the first-generation HCI assay. High-risk HPV DNA was detected in 88% of women with high-grade SIL or cancer with the use of the HCII assay compared with 73% with the use of the HCI assay. When the cutoff value (RLU ratio of sample:positive control) of the HCII assay was shifted to a point at which its sensitivity was equivalent to that of the HCI assay, the specificity of the HCII assay was marginally better than that of the HCI assay. The better performance of the HCII assay compared with the HCI assay is also reflected in the somewhat stronger associations between HPV prevalence and cervical disease observed with the use of the HCII assay compared with the HCI assay. From a logistic point of view, however, the HCI assay is a simpler and easier test to perform. Moreover, although the HCI assay was not able to achieve the same degree of sensitivity as that of the HCII assay, at specificities likely to be practical for a primary screening test in a low-resource setting, the HCI assay performed equivalently to the HCII assay.

In the context of screening, good sensitivity (i.e., the capacity
of the test to detect all women with the condition of interest) has to be balanced against the test’s specificity. Specificity is particularly important in cervical cancer screening, since screening involves large numbers of otherwise healthy women, and positive screening test results require a follow-up colposcopic evaluation that is both uncomfortable and costly. Specificity takes on added importance in low-resource settings where colposcopy is not available and where all women who are classified as positive by a screening test may undergo treatment. One of the advantages of HPV DNA testing in these settings is that the specificity of the test can be altered by the adjustment of the cutoff level used to define a positive result. For example, if overtreatment of 10% of women with no evidence of cervical disease was acceptable, then the HCII assay could detect 79% of the cases of high-grade SIL and cancer. If overtreatment of only 5% of women with no evidence of disease was acceptable, then either the HCI assay or the HCII assay could identify 57% of the cases of high-grade SIL or cancer.

Our study has several strengths and limitations. Its strengths are that it evaluated the performance of HPV DNA testing in the context of a screened population in a low-resource setting. The population of women included in this study has access only to poor or nonexistent routine gynecologic services and experiences very high rates of other sexually transmitted infections. Evaluation of screening test performance in a population other than the target population can produce considerable and unmea-

![Fig. 3. A) Amounts of human papillomavirus (HPV) DNA measured among 12 women with invasive cervical cancer, among 74 women with histologically confirmed high-grade squamous intraepithelial lesion (SIL) (cervical intraepithelial neoplasia [CIN] 2 or 3), among 95 women with histologically confirmed low-grade SIL (CIN 1), and among a random sample of 243 women with no evidence of cervical disease measured with the use of the Hybrid Capture II assay. B) Amounts of HPV DNA measured among 12 women with invasive cervical cancer, among 74 women with high-grade SIL, among 95 women with low-grade SIL, and among 2681 women at low risk of cervical disease measured with the use of the Hybrid Capture I assay.](http://jnci.oxfordjournals.org/)

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surable bias, such as when screening tests are evaluated in women referred to colposcopy clinics. The main limitation of the study was that the “criterion standard” for detection of cervical disease—i.e., colposcopy followed by pathology diagnosis—was not applied to all study participants. Colposcopy was not performed in women with four negative screening tests, which results in a form of verification bias (19,20). To examine the magnitude of this verification bias, we considered the sensitivity and specificity of HPV DNA testing, using cytology as the “criterion standard,” and found that its performance was similar to that observed when judged against our combination of screening test or colposcopy negativity. Thus, we believe that the extent of verification bias in our study is small. The positive predictive value of the HCI assay at a high cutoff value is unbiased in our design.

The sensitivity of HPV DNA testing was equivalent to or better than that of cytology in this study. Furthermore, within our study, cytology performed exceptionally well—in fact, better than has been previously reported in the literature. Two meta-analyses of published studies (23,24) have estimated the sensitivity of cytology in screening settings to be between 49% and 67%, with specificities ranging from 62% to 77%. In our study, the estimated sensitivity of cytology was 78% and the specificity was 97%. However, the estimated specificity of HPV DNA testing with the use of standard cutoff values was lower than that of cytology in our study. Comparable specificities could be achieved only if the sensitivity of HPV DNA testing was allowed to decrease to around 57% by use of more stringent, higher cutoff values. The high specificity of cytology observed in our study is also better than that reported in the literature; other studies have estimated from ROC analyses that the sensitivity of cytology tends to drop to within the 20%–35% range if specificity achieved 90%–95% (24). The smears in our study were taken by carefully trained and monitored nurses and were processed in a laboratory in which personnel knew that they were part of a research study.

The finding that the performance of HPV DNA testing in a high-risk, previously unscreened population is equivalent to that of expert cytology has important implications for countries that have not yet developed national cervical cancer screening programs. In many settings, it may prove easier to establish clinical laboratories for large-scale HPV DNA testing than to establish

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**Table 2.** Results of the receiver operating characteristic curve analysis of estimated sensitivity and specificity of human papillomavirus (HPV) DNA testing* at different cutoff values with the use of the Hybrid Capture I (HCI) assay or the Hybrid Capture II (HCII) assay†

<table>
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</tbody>
</table>

*Sensitivity is estimated as the proportion of women with histologically confirmed high-grade squamous epithelial lesion or cancer (n = 86) who would be defined as HPV DNA positive at various cutoff values of the HPV DNA test. 1 − specificity (i.e., the false-positive rate) is estimated as the proportion of women whom we defined as having no disease (n = 2680) (which includes those with no positive screening tests or with no histologic evidence of disease following colposcopy) who would be defined as HPV DNA positive at various cutoff values of the HPV DNA test. Women with biopsy-confirmed low-grade squamous epithelial lesions are excluded from this analysis.

†Shown in bold are the estimated proportions classified as HPV positive when the cutoff to define a positive test is >1× the positive control.

‡Relative to standard kit cutoff level of 10 pg/mL for the HCI assay and 1 pg/mL for the HCII assay.
high-quality cytology laboratories. HPV DNA testing requires less skilled technicians and is easier to perform than cervical cytology. Therefore, it may be more feasible to set up HPV DNA testing on site (e.g., in outpatient clinics or day hospitals) than to provide on-site cytology services. A final advantage of HPV DNA testing over cytologic screening for resource-poor settings is that HPV DNA testing identifies not only women who currently have high-grade cervical disease but also women who are at greatest risk of developing the disease in the future. HPV screening would thus allow follow-up efforts to be targeted to women at greatest risk for the disease. Cost-effectiveness analysis of HPV DNA testing is urgently needed to evaluate its application in various health care settings.

REFERENCES


NOTES

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