Interim Guidance for the Use of Human Papillomavirus DNA Testing as an Adjunct to Cervical Cytology for Screening

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Human papillomavirus (HPV) DNA testing was recently approved by the Food and Drug Administration for use as an adjunct to cytology for cervical cancer screening. To help provide guidance to clinicians and patients when using HPV DNA testing as an adjunct to cervical cytology for screening, a workshop was cosponsored by the National Institutes of Health–National Cancer Institute, American Society of Colposcopy and Cervical Pathology (ASCCP), and American Cancer Society. Consensus was reached based on a literature review, expert opinion, and unpublished results from large ongoing screening studies. The conclusions of the workshop were that HPV DNA testing may be added to cervical cytology for screening in women aged 30 years or more. Women whose results are negative by both HPV DNA testing and cytology should not be rescreened before 3 years. Women whose results are negative by cytology, but are high-risk HPV DNA positive, are at a relatively low risk of having high-grade cervical neoplasia, and colposcopy should not be performed routinely in this setting. Instead, HPV DNA testing along with cervical cytology should be repeated in these women at 6 to 12 months. If test results of either are abnormal, colposcopy should then be performed. This guidance should assist clinicians in utilizing HPV DNA testing in an effective manner, while minimizing unnecessary evaluations and treatments. (Obstet Gynecol 2004;103:304–9. © 2004 by The American College of Obstetricians and Gynecologists.)

Infection of the uterine cervix with one of approximately 15 “high-risk” types of human papillomavirus (HPV) is required for the subsequent development of virtually all cervical cancers.1 However, HPV infections are extremely common in sexually active women and most are transient and benign. Unfortunately, some HPV-infected women do not spontaneously clear their infections and instead develop persistence. Persistent infections with a high-risk type of HPV can result in the development of high-grade cervical cancer precursors or even cervical cancer if the precursors are not identified through screening and subsequently treated.

Molecular testing for high-risk types of HPV has been available for clinical use for some time. Previously, the Food and Drug Administration (FDA) approved the Hybrid Capture 2 HPV DNA Test (HC2; Digene Diagnostics, Gaithersburg, MD) for use in the management of women with equivocal cytology results (atypical squamous cells of undetermined significance [ASC-US]). Based on the results of large clinical studies, HPV testing is now considered the preferred approach to managing women of all ages with ASC-US results on liquid-based cytology.2 Recently, FDA also approved the use of the “high-risk” panel of HC2 (that detects HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) as an adjunct to cervical cytology screening in women aged 30 years or more. Although the availability of HPV testing as an adjunctive method to cervical cytology for screening is an important advance, there are currently insufficient data to prove that a combination of HPV testing and cervical cytology will improve outcomes, reduce costs, or be more acceptable to women than screening using cytology alone. Because of limited data and resultant uncertainty, there are conflicting recommendations as to...
testing as an adjunct to cervical cytology screening.\cite{4,5}

The American College of Obstetrics and Gynecology acknowledges the FDA approval of HPV DNA testing for use as an adjunct to cervical cytology screening for primary screening, and guidelines have not yet been developed for its use. Therefore a consensus workshop was convened to develop interim guidance based on the available evidence and expert opinion for the use of HPV DNA testing in conjunction with cervical cytologic screening and the management of women with specific combinations of test results. The workshop was cosponsored by the National Institutes of Health–National Cancer Institute, American Society of Colposcopy and Cervical Pathology (ASCCP), and the American Cancer Society and was held February 22–23, 2003, in Tucson, AZ. This guidance should be used in conjunction with the new guidelines for cervical cancer screening that have recently been developed by various organizations and societies.\cite{3–5}

### POTENTIAL BENEFITS AND LIMITATIONS OF HPV DNA TESTING AS AN ADJUNCT TO CYTOLOGY

Many of the studies that were used to support the FDA approval of Hybrid Capture 2 as an adjunct to cervical cytology screening are presented in Table 1 (personal communications, C. Ferreccio and M. Schiffman for Costa Rica results, 2003; S. Womack and K. Shah for Baltimore results, 2003; J. Cuzick for United Kingdom results, 2003).\cite{6–9} In these large clinical screening trials, 85–100% of cases of concurrently diagnosed, histologically confirmed cervical intraepithelial neoplasia grades 2 and 3 or cancer (CIN2\(^+\)) were found to be positive for high-risk types of HPV. Sensitivity was even higher for the identification of CIN 3 or cancer (CIN3\(^+\)). Testing for high-risk types of HPV identifies more women with CIN2\(^+\) than does a single cervical cytology. When HPV testing is added to cervical cytologic screening, the sensitivity of the combination is somewhat improved compared with HPV testing used alone. In all of the studies, except for one, the sensitivity obtained by using the combination of methods exceeded 92%, and in the majority sensitivity exceeded 95%. The negative predictive values for not having CIN2\(^+\) that were obtained with the combination of methods ranged from 0.988 (South Africa) to 0.999–1.000 (Germany, United Kingdom, Mexico, United States, and China). It is important to recognize that only one of these studies shown in Table 1 was conducted in the United States. However, other studies from the United States have confirmed a very high sensitivity of HPV DNA testing for the identification of women with CIN2\(^+\) in a screening setting.\cite{10}

Another potential benefit of using HPV testing as an adjunct to cervical cytology screening is that it identifies not only women with concurrent cervical disease, but also those at risk of developing disease in the future. In contrast, cervical cytology mainly identifies concur-

#### Table 1. Performance of Hybrid Capture 2 and Cervical Cytology for Screening in Women Aged 30 Years or More in Cross-Sectional Studies

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>CIN2(^+) (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>npv, Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>7592</td>
<td>1.01</td>
<td>33.8</td>
<td>85.7</td>
<td>93.5</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>10358</td>
<td>0.9</td>
<td>72.2</td>
<td>96.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Mexico</td>
<td>6115</td>
<td>1.41</td>
<td>57.0</td>
<td>94.2</td>
<td>97.7</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>6176</td>
<td>1.75</td>
<td>80.4</td>
<td>86.3</td>
<td>92.2</td>
</tr>
<tr>
<td>South Africa</td>
<td>2925</td>
<td>3.56</td>
<td>74.0</td>
<td>84.9</td>
<td>87.0</td>
</tr>
<tr>
<td>China</td>
<td>1936</td>
<td>4.34</td>
<td>94.0</td>
<td>97.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Baltimore, MD</td>
<td>1040</td>
<td>0.48</td>
<td>60.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

CIN2\(^+\) = cervical intraepithelial neoplasia grades 2 and 3 or cancer; HPV = human papillomavirus; NPV = negative predictive value.

Data are modified from Salmeron et al,\cite{6} Belinson et al,\cite{7} Wright et al,\cite{8} and Petry et al\cite{9} and personal communications from C. Ferreccio and M. Schiffman (Costa Rica data, 2003), S. Womack and K. Shah (Baltimore, MD, data, 2003), and J Cuzick (United Kingdom data, 2003).

* For CIN2\(^+\).*
urrent disease. In a cohort study conducted in Portland, OR, 23,000 women were followed-up for up to 10 years. Detection of high-risk HPV at enrollment was highly associated with a subsequent diagnosis of CIN 3, a finding that is consistent with other large cohort-studies. The cumulative incidence of biopsy-confirmed CIN3+ among women who were high-risk HPV positive at enrollment and who were followed-up for up to 45 months was 4.4% (95% confidence interval 3.4, 5.4). In contrast, the cumulative incidence among women who were high-risk HPV DNA negative was only 0.24% (95% confidence interval 0.2, 0.3) at 45 months and 0.87% after 10 years.

It is also useful for laboratories to use HPV testing for quality assurance of cytology. As a general rule, the prevalence of high-risk HPV DNA positivity is typically higher than 85% for high-grade squamous intraepithelial lesions, 75–85% for low-grade squamous intraepithelial lesions, and approximately 50% for ASC-US.

Although there are clear potential benefits to the use of HPV testing as an adjunct to cervical cytology for screening, very real concerns have been voiced about its potential negative impact, if misused. Because infection with HPV is so common, a large number of women will be identified who are high-risk HPV DNA positive, but few of these women will have cervical cancer or a high-grade precursor lesion. In 2002, it was estimated that there were only 13,000 cases and 4,100 deaths from cervical cancer among the approximately 105 million women aged more than 20 years in the United States. More-over, approximately half of cervical cancers occur among women not participating in regular screening. In marked contrast, at any time 10–20% of the adult population has transient, clinically insignificant HPV infections that would be identified through widespread screening. Even by restricting HPV testing to women aged 30 years or more, 5–15% of women will be high-risk HPV DNA positive. These women will require counseling regarding their risk of cervical disease, the source of their infection, and their infectivity. Counseling is made more complicated by the lack of an effective treatment for persistent HPV and incomplete understanding of viral latency. It is also possible that some HPV DNA-positive women may undergo unnecessary intensive follow-up or treatment such as loop electrosurgical excisional procedure. To balance sensitivity against risk of over-use, the frequency of screening will need to be reduced when using HPV testing in combination with cervical cytology, and reasonably conservative follow-up strategies must be developed for HPV DNA–positive, cytology-negative, women.

### INTERIM GUIDANCE FOR THE USE OF HPV DNA TESTING AS AN ADJUNCT TO CERVICAL CYTOLOGY FOR SCREENING

The following guidance on the use of HPV DNA testing as an adjunct to cervical cytology (either conventional or liquid-based Papanicolaou tests) is based on published and unpublished data presented at the workshop, as well as the collective experience of the participants (see Appendix). It should be stressed that low-risk types of HPV are not associated with an increased risk of cervical cancer, and testing for low-risk types should not be a part of routine screening.

#### Target Populations

**Age to initiate use of HPV DNA testing as an adjunct to cervical cytology for screening:** HPV DNA testing may be added to cervical cytology (either conventional or liquid-based) for screening in women aged 30 years or more.

Most newly acquired HPV infections clear spontaneously, and there is a diminishing number of new sexual partners with increasing age. Therefore the prevalence of HPV DNA positivity in the population drops with increasing age from a peak in the late teens or early 20s. In contrast, the incidence of CIN 2,3 rises and peaks among women in their late 20s and early 30s. Cervical cancer is rare in women aged less than 25 years. Relatively few large, cervical cancer screening studies have evaluated the performance of HPV DNA testing in women aged less than 30 years, but in general, it is expected that the specificity of HPV DNA testing would be considerably lower in younger, as opposed to older, women. In a study of 8,554 women in Costa Rica, the sensitivity and specificity of Hybrid Capture 2 with the high-risk probe mixture for the identification of cases of CIN 2,3 and cancer among women aged 41 years or more were 93% and 94%, respectively. In contrast, sensitivity remained at 93% but specificity decreased to 80% among women aged 18 to 30 years. There is still considerable uncertainty as to the precise rate of decline in age-specific HPV prevalence in the U.S. population aged 30 to 40 years. Until such data are available, initiating HPV DNA testing with cervical cytology for screening at 30 years of age should provide a reasonable balance between sensitivity and unnecessary testing, procedures, and treatment. This recommendation is the same as that recently adopted by the American Cancer Society. It is important to note that the use of HPV DNA testing to determine which women with ASC-US cytology results require colposcopy is indicated in women of all ages.

**Age to discontinue use of HPV DNA testing as an adjunct to cervical cytology for screening:** Use of the combination of
HPV DNA testing and cervical cytology should be discontinued at the same age, and under the same circumstances, as cervical cytology screening.

The performance of HPV testing for identifying women with CIN2+/H11001 does not decrease with increasing age. Therefore it appears reasonable to discontinue the use of HPV testing in combination with cervical cytology for screening at the same time cervical cytology is stopped. The U.S. Preventive Services Task Force recommends against routine screening using cervical cytology in women aged more than 65 years if they have had a recent negative cytology and are not otherwise at high risk for cervical cancer.3 The American Cancer Society recommends that screening using cervical cytology be discontinued in women aged 70 years or more who have had 3 or more recent negative cervical cytology examinations and no abnormal cytology results within the preceding 10 years.4 A negative HPV test result at a woman’s last screening would provide additional reassurance before discontinuing screening.

Women who should not receive HPV DNA testing in combination with cervical cytology: HPV DNA testing should not be added to cervical cytology for screening in women aged less than 30 years, women who are immunosuppressed for any reason including infection with human immunodeficiency virus (HIV), and following total hysterectomy (with removal of the cervix) for benign gynecologic disease.

Immunosuppressed women are at increased risk for CIN2+ and infections with high-risk types of HPV.19 Since screening interval should not be extended in this population, there would be little benefit from the use of HPV DNA testing as an adjunct to cervical cytology.4 Both the recent American Cancer Society and the U.S. Preventive Services Task Force guidelines recommend not screening women who have had a total hysterectomy for pathologically documented benign gynecologic disease (CIN 2,3 is not considered “benign”).3,4 This is based on multiple studies showing that abnormal cytology results are uncommon, often falsely positive, and rarely of clinical importance in this clinical setting. The same rationale applies to the lack of utility of HPV testing.

Management of Specific Combinations of Test Results

Women whose results are negative by both HPV DNA testing and cytology (either conventional or liquid-based): Women who are high-risk HPV DNA negative and have a cervical cytology result of “negative for intraepithelial lesion or malignancy” should not be rescreened before 3 years. This recommendation (Figure 1) reflects the very high negative predictive value for underlying CIN2+ in women in whom both tests are negative. Rescreening includes cytology alone (either conventional or liquid-based) or HPV DNA testing along with cytology. When cytology is used alone for rescreening, the screening interval should be based on recently published guidelines for cytologic screening in women aged 30 years or more with a history of multiple negative cervical cytologic examinations. The 7 large screening studies pre-
Women whose results are cytology negative but who are high-risk HPV DNA positive: The risk of CIN 2,3 or cancer in women who are high-risk HPV DNA positive and have a satisfactory, negative cervical cytology result is very low. Therefore, colposcopy should not be performed in this setting routinely. HPV DNA testing along with cervical cytology should be repeated at 6 to 12 months. If either test is abnormal, colposcopy should be performed. Women who do not have CIN 2,3 or worse identified at colposcopy should be followed-up by using HPV DNA testing together with cervical cytology at 12 months. If the woman is persistently high-risk HPV positive, colposcopy with careful evaluation of the vagina and vulva should be performed.

The prevalence of CIN2+ identified at follow-up colposcopy (median of 6 months after enrollment) was only 4.2% among cytology-negative, HPV DNA–positive women in a French screening study. After a median follow-up of 6 months, 60% of the HPV DNA–positive women had become HPV DNA negative. These data and confirmatory unpublished results from at least 2 other cohort studies with longer follow-up suggest that most cytologically negative, HPV DNA–positive women have transient HPV infections and are at lower risk for having CIN2+ than women with a cytological result of ASC (5–17% risk of CIN2+ when not stratified by HPV status). Therefore these women should be followed conservatively by repeating both tests in 6 to 12 months. Women who are found to have persistent high-risk HPV infections on repeat testing are at increased risk for having high-grade cervical intraepithelial neoplasia and should undergo a colposcopic examination. Women who are negative on both repeat tests are at low risk for having, or developing, CIN2+ and can be rescreened at 3 years.

Women whose results are positive for high-risk types of HPV but who have atypical squamous cells of undetermined significance: Women who are high-risk HPV DNA positive and have a cytology result of atypical squamous cells of undetermined significance should have colposcopy performed. These recommendations are derived from recent national consensus guidelines (available at http://jama.ama-assn.org).2

Women whose results are negative for high-risk types of HPV but who have abnormal cervical cytology: Women who are high-risk HPV DNA negative and have a cytology result of ASC-US can be followed-up with repeat cytology testing in 12 months. Women who are high-risk HPV DNA negative and have a cytologic epithelial cell abnormality of atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion, low-grade squamous intraepithelial lesions, or high-grade squamous intraepithelial lesions should undergo a colposcopic examination.

These recommendations are derived from recent national consensus guidelines (available at http://jama.ama-assn.org).2

Limitations
The current interim guidance is restricted to the use of HPV DNA testing as an adjunct to cervical cytology screening. Comprehensive guidelines on the use of cytology for cervical cancer screening and the management of cytologic abnormalities and CIN have recently been developed by a variety of different groups including the American Cancer Society, the U.S. Preventive Services Task Force, the American College of Obstetricians and Gynecologists, and the American Society for Colposcopy and Cervical Pathology (ASCCP).2–5

No single screening test or combination of tests is 100% perfect. The sensitivity of the combination of cervical cytology and HPV DNA testing for identifying CIN 2,3 and cervical cancer in the large clinical studies has been 83–100%. Therefore, some women will develop cervical cancer even if routinely screened with a highly sensitive test or combination of tests. In addition, a balance must be maintained between enhancing screening sensitivity and the undesirable consequences of unnecessary testing, procedures, and treatment. The specificity of the combination of cervical cytology and HPV DNA testing has been 70–96% in the different studies.

Clinicians need to remember that almost all women will have an HPV infection at some point in their lifetimes and that having multiple positive tests does not necessarily indicate persistent infection with the same type of high-risk HPV because serial infections with different types are common.

Future Studies
Additional research is needed in several areas to develop effective strategies for the following:

• Communicating to patients and providers about HPV infection, related cervical disease, and management
• Defining better the optimal age to start and stop the use of HPV testing as an adjunct to cervical cytology screening
• Determining the best clinical management of positive test results
• Monitoring the use of HPV testing and outcomes in clinical practice over the next several years

Finally, comprehensive technology assessments utilizing analytic modeling methods are needed to evaluate the long-term population-based impact and the potential health economic consequences of this new technology.

REFERENCES


APPENDIX

In addition to the listed authors, the following were invited workshop participants: Walter Kinney, MD, University of California at Davis, Permanente Medical Group, Sacramento, California; Herschel Lawson, MD, Centers for Disease Control and Prevention, Atlanta, Georgia; Louise Magruder, Food and Drug Administration, Rockville, Maryland.