High Risk Human Papillomavirus Testing:
Guidelines for Use in Screening, Triage, and
Follow-up for the Prevention and Early
Detection of Cervical Cancer

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Abstract
The changes in cervical cytology characterization agreed on by the Bethesda committee meeting in 2001 created a category of atypical findings that has caused some management confusion. By description, the characterization of cervical cytology as only atypical implies a less worrisome prognosis. However, more than 40% of high-grade (CIN II or III or cancer) will be discovered within this category. The development and Food and Drug Administration approval of the Hybrid Capture 2 (HC-2; Digene Corporation, Gaithersburg, MD) for detecting high-risk human papillomavirus (HR-HPV) subtypes and the subsequent level I evidence supporting use of this test in the triage of women with atypical cytology has revolutionized the management of this cytology. With this success has come numerous additional uses for HR-HPV testing in the treatment and follow-up of women with a variety of cytologic abnormalities. This article reviews the literature on uses of HR-HPV testing in this population, with reference to currently accepted guidelines. (JNCCN 2004;2:589–596)

The introduction of the Papanicolaou (Pap) smear in late 1941 is credited with the drastic reduction in the incidence and mortality rates of squamous cell carcinoma of the cervix in the United States. Approximately 22% of cases now are adenocarcinoma. Incidence rates of adenocarcinoma continue to rise due to poor detection of preinvasive glandular lesions using the Pap test. Recent estimates from the American Cancer Society report an incidence of 8.3 cases of cervical cancer per 100,000 women, for a total of 10,520 cases and approximately 3,900 deaths per year. In recent years, the classification of cervical cytology has undergone multiple revisions in nomenclature, in large part to clarify results in the equivocal categories. The most recent Bethesda revision in 2001 identifies four categories of cytologic abnormality that can be applied to cervical screening. These categories include: atypical cells of undetermined significance (ASC), low-grade squamous intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), and atypical glandular cells (AGC). Also, a sub-category of ASC designated as atypical cells cannot rule out high-grade (ASC-H).

The management of HSIL is the least controversial; patients are referred for colposcopy with directed biopsy and generally undergo treatment. LSIL cytology is almost synonymous with human papillomavirus (HPV) infection, with 80% to 85% of cases being high-risk (HR)-HPV positive. Women with these findings should be referred for colposcopy. The treatment of patients with atypical cytologic categories has been clarified with the addition of sensitive DNA testing for HPV types that are most associated with cervical cancer. The cause of cervical neoplasia is HPV, and its presence and persistence can now be used to predict outcome and assist the determination of management guidelines. The development and Food and Drug Administration (FDA)
approval of the Hybrid Capture2 (HC2) (Digene Corporation, Gaithersburg, MD) system for HR-HPV detection, along with the recent publication of many well-designed clinical trials that investigate the use of HR-HPV testing in women with HPV, have revolutionized the initial management of atypical cytology as well as provided new testing modalities for evaluation after colposcopy and treatment. The contribution of highly sensitive HR-HPV testing to the overall reduction of cancer incidence or mortality is not yet known. Potential uses of this test are continuing to be proposed, and modifications are to be expected.

This goal of this article is to review the role of HR-HPV testing in initial triage of women with equivocal cytologic abnormalities to colposcopy, in post-colposcopy surveillance, in post ablative or excisional therapy surveillance, and as a primary screening modality. The literature concerning the use of HPV in patients with ASC–H and AGC is also discussed separately.

HPV Test
The only available test currently approved by the FDA for testing of HR-HPV is the HC2. HC2 uses a ribonucleic acid probe to bind to high and intermediate risk HPV DNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Antibodies to DNA–RNA hybrids are then added with a chemiluminescent marker, resulting in the identification as well as semiquantitation of HR-HPV subtypes and allowing for detection of HR-HPV subtypes as low as 1 pg/mL. There is currently no rationale for identification of low risk HPV types. HC2 has been the subject of a large number of studies comparing the sensitivities for prediction of CIN III between HC2, liquid phase, and conventional Pap smears. HC2 has consistently showed a sensitivity of 90% to 100% in detection of CIN III as compared with 50% to 75% for conventional Pap smears and approximately 85% to 88% for liquid-based cytology (LBC). The expectation is that HPV type-specific testing will eventually be available commercially. Physicians would then be able to more accurately assess individual risk based on type-specific HPV persistence, because each of the 13 types do not carry the same risk, and HC2 positivity does not show whether serial positive results are caused by the same infection.

HPV Triage in Patients with Atypical Squamous Cells
The Bethesda system ASC interpretation is reserved for samples that are not quite normal but not quite diagnostic of squamous intraepithelial lesion (SIL). Not surprisingly, this category comprises a wide variety of histologic diagnoses, from normal to invasive cancer and follow-up strategies needed to acknowledge this ambiguity. The challenge here is that the “atypical” criteria comprise approximately 2 million of the 3.5 million abnormal Pap results reported in the United States each year. The cumulative risk of CIN II or III is somewhere between 6.4% and 11.9%, and the risk of invasive cancer is between 0.1% to 0.2% (1/1,000–2/1,000). Although the individual risk of CIN II or III or cancer is low, a sizeable proportion of the total number of women ultimately found to have these histologic diagnoses will come from this category of cytologic result. HR-HPV triage offered a method by which the truly high-risk atypical cytologic screens could be identified, and the rest spared the inconvenience and cost of further diagnostic evaluation.

The National Cancer Institute’s ASCUS and LSIL Triage Study (ALTS) evaluated three different management strategies for patients with these Pap smears. These patients were randomly assigned to immediate colposcopy, triage to colposcopy via the detection of HR-HPV (HC2) or HSIL cytology result, or conservative management with repeat cytology every 6 months and referral to colposcopy on cytologic diagnosis of HSIL. With a sample size of almost 3,500 ASCUS patients, the ALTS trial identified a cumulative diagnosis of CIN III in 8% to 9% of each study arm. They found that a single enrollment HPV test identified 92.4% of all women diagnosed with CIN III. A somewhat lower sensitivity was seen in for two repeat cytology evaluations with a threshold referral to colposcopy of ASCUS. In addition to the increased sensitivity, HPV dramatically reduced the number of women referred to colposcopy to 55.6% as compared with 67.1% referred with repeat cytology. Therefore, reflex HR-HPV triage for women with ASC reduces the number of patients referred for colposcopy because the HR-HPV negative patients are returned to routine screening. This screening reduces the overall cost of evaluation by not only eliminating a large number of colposcopic examinations, but also by eliminating a second cervical cytology and office visit. Triage also eliminates the delay in diagnosis of CIN III that necessarily occurs in a repeat cytology schema. Based on ALTS and other data, reflex HR-HPV testing should be considered in evaluating women with ASC. The alternative is two-repeat cytology every 6 months.
with referral for colposcopy for results of ASC or worse (Figure 1).

HPV Triage in ASC-H
Although HR-HPV identification has allowed for the effective triage of patients with ASC-US (undetermined significance) to colposcopy or routine follow-up, it is not currently recommended for similar use in ASC-H (cannot exclude HSIL). The ability to triage patients with ASC-US is predicated on a cumulative risk of high-grade histology (CIN II or III) of 6.4% to 11.9%9,13 and a concomitant high sensitivity and negative predictive value of HR-HPV testing to identify patients with significant cervical pathology. Rowe et al.19 attempted to look at rates of HR-HPV presence in women with ASC-H and found that 37% of these samples were positive, suggesting a role for HR-HPV testing in the triage of ASC-H. However, this study contained only a small number of ASC-H samples (16 of 371) and did not correlate with histologic diagnosis.19 A finding of ASC-H has a much higher association with underlying high-grade histology (24% to 66.3% of cases, depending on the series).20,21 This variability is based in large part on the inability to standardize ASC-H cytologic diagnosis between communities.

The patient’s best interest is probably for the colposcopist to communicate directly with the cytopathologist on these rare and uncertain diagnoses. Therefore, these patients are currently best served by referral to immediate colposcopy as outlined by the guidelines of the American Society for Colposcopy and Cervical Pathology (ASCCP).18 If these patients have no lesion identified or biopsies consistent with CIN I or less, they may undergo surveillance as outlined for ASC with cytology at 6 and 12 months or HR-HPV testing alone at 12 months.18 In some institutions, the use of HR-HPV testing may help to standardize cytologic interpretations and guide colposcopy referral in this population.

HR-HPV Triage in LSIL
HR-HPV testing is not a cost-effective initial triage tool for women with Pap results showing LSIL. The ALTS trial found that more than 80% of patients with LSIL showed positive results for HR-HPV using HC2 testing. Given this fact, any cost savings obtained by a reduction in colposcopy referral would be far outweighed by the excess expense of reflex HPV. Therefore, HR-HPV testing in women with LSIL is not currently recommended, and these patients are referred directly to colposcopy.17,18,22 The use of HR-HPV testing in adolescent patients and in the post-colposcopy management of a finding of less than or equal to CIN I will be discussed subsequently.

HPV Triage in AGC
AGC comprises only 0.10% to 0.30%23–27 of all cytology results. The rarity of this lesion has precluded its evaluation in relation to HPV on any large scale. One interesting study performed within the Kaiser system in Oregon evaluated 137 cases of AGC-US referred for colposcopy. The investigators performed HC2 HPV tests on all subjects and found that all cases of adenocarcinoma in situ (5 of 137) and 92% of all CIN II or III (12 of 137) were positive for HR-HPV. This resulted in a positive predictive value (PPV) and sensitivity for combined AIS and CIN II or III of 41% and 94%, respectively.25

A second study correlated the presence of HR-HPV in patients with AGC pap smears with histologic findings. HR-HPV has a sensitivity of 83%, a specificity range of 78% to 82%, a PPV of 57% to 61%, and a negative predictive value (NPV) of 91% of 95% to finding significant pathological abnormalities.26 Although these studies may direct us toward a new role for HPV in the triage of AGC, larger, prospective

Figure 1 American Society for Colposcopy and Cervical Pathology (ASCCP) algorithm for management of ASC, including use of HR-HPV as a triage tool for colposcopy. ASC/ASC-US = atypical squamous cells of undetermined significance; HR-HPV = high-risk human papillomavirus.

Source: The Consensus Guidelines algorithms originally appeared in and are reprinted from The Journal of Lower Genital Tract Disease volume 6, issue 2, 2002, and are reprinted with the permission of ASCCP (American Society for Colposcopy and Cervical Pathology). No copy of the algorithm may be made without the prior consent of ASCCP.
trials are required and currently ongoing with the National Cancer Institute (NCI)-funded Gynecologic Oncology Group. Current recommendations from the ASCCP are referral for colposcopically directed biopsy, endocervical sampling, and, in addition, endometrial biopsy for women over 35 years of age or with abnormal bleeding. Many women will be recommended to undergo cervical excisional procedures if no etiologic lesion is identified, and review of cytology is concerning for an endocervical neoplastic process. 

With our present knowledge, women with AGC who are HR-HPV negative and in whom no lesion can be found on colposcopic examination, endocervical curettage, and endometrial biopsy appear to be at low risk. The use of HR-HPV testing may allow young nulliparous women to avoid a cervical excisional procedure if they want to prioritize preserving childbearing capacity (Figure 2).

**HPV testing in Post-Colposcopy Evaluation**

Two-year follow-up data from the ALTS trial showed the poor sensitivity of a single initial colposcopic examination to identify all women with CIN II-III. This study showed the need for a surveillance mechanism for women with cytologic abnormalities who have undergone colposcopy and whose findings either by colposcopic visualization or directed biopsy were either negative or CIN I (CIN I or less). Except in special circumstances, histologic diagnosis of CIN II or III is generally treated with either ablative or excisional therapy.

Analysis of the ALTS data showed that patients with LSIL and HR-HPV-positive ASCUS were equally likely to have a cumulative histologic diagnosis of CIN II or III (26%) over 2 years. At first colposcopy, 17.9% of women with LSIL or ASC/HR-HPV+ were identified to have CIN II or III and were referred for appropriate therapy. Of the remaining patients, who had no visible lesion, a negative biopsy, or CIN I identified at initial colposcopy (CIN I or less) were found to have a risk of between 11.3% and 13% for development of CIN II or III over a 2-year period. A follow-up scheme was developed to determine which patients need a repeat colposcopic examination. An isolated HPV at 12 months was compared with cytology at 6 and 12 months. Isolated HR-HPV testing at 12 months was found to have a high sensitivity (92.2%), with a 55% referral back to colposcopy. The addition of cytology did not improve sensitivity; however, it did increase the referral to colposcopy and reduce the specificity. Practically speaking, however, a practitioner would need the cytology in the event of a positive HR-HPV result because this will help dictate treatment after colposcopy. Ideally, if a patient is followed up with HR-HPV testing alone, reflex cytology could be performed only for patients with positive results so as to aid post-colposcopy decision-making without adding to the number of colposcopy referrals.

Reflex cytology mechanisms are not current practice. Therefore, current recommendations are for HR-HPV evaluation at 12 months or repeat cytology at 6 and 12 months with referral back to colposcopy for ASC or worse (Figure 3). 

**ASC in Menopausal Women and Adolescent Girls**

Women at the extremes of age warrant special consideration regarding colposcopic referral and appropriate surveillance. Menopausal women with ASC have rates of HR-HPV infection of 31% to 50%. This is far less than reported in younger age ranges (<71% for ages 18–22 and 65% in ages 23–28 years).
The finding of ASC in a menopausal woman may be a false positive related to atrophy as physiologic estrogen begins to decline. Therefore, given an increasing rate of misclassification after menopause and a decreasing prevalence of HPV infection, one strategy is for HR-HPV testing with referral for colposcopy if results are positive. An alternative strategy is to treat menopausal women who are found to have an ASC cytology with vaginal estrogen preparation and then repeat the cervical cytology after the completion of estrogen therapy. If the cytologic appearance normalizes with estrogen therapy, the patient can return to routine clinical follow-up treatment.

Adolescent patients also present treatment and surveillance challenges. Rates of HR-HPV in sexually active adolescents are extremely high. In one study of sexually active teenagers between 13 and 21 years old, 81% of those who presented for care at a family planning clinic were positive for HR-HPV. Of 105 patients who did not have HR-HPV at enrollment in the study, approximately 50% went on to develop the infection during a median follow-up of 60 months.

Given the very high rates of HPV infection among adolescents and the almost nonexistent progression to carcinoma, surveillance may be tailored to the individual patient. For individuals for whom adherence may be reasonably assured, HPV testing at 12 months from the incident cytology is a reasonable option. This allows the adolescent to avoid the anxiety of a colposcopic examination by considering the often transient nature of adolescent HPV infections. Recognizing both the extremely high rate of adolescent HR-HPV infection and the transient nature of these infections, the current recommendations from the American Cancer Society, mirrored in the NCCN Guidelines, changed the screening recommendations for this age group to begin Pap testing at age 21 or after 3 years of sexual activity, which may allow initial HPV infections to resolve.

HPV Testing as Surveillance after Excision or Ablation

The presence or persistence of HPV after excisional or ablative therapy has been shown to be predictive for recurrence of cervical intraepithelial neoplasia, suggesting a role for HPV testing as surveillance after treatment. Multiple studies have shown that the absence of HPV on post-treatment testing has a 99% to 100% negative predictive value (NPV) for remaining free of intraepithelial neoplasia. Sensitivities from these studies range from 79.7% to 100%, and positive predictive values approach 100% for predicting recurrent or persistent disease based on positive HR-HPV testing after treatment.

Current recommendations from the 2001 ASCCP consensus conference include testing for HR-HPV at least 6 months after treatment to allow enough time for clearance. In the absence of compelling risk factors (large lesion, positive conization margins, or endocervical involvement) HPV testing should occur 12 months after treatment.

HPV as Primary Screen in Women 30 Years of Age or Older

Recognizing the excellent sensitivity of HPV testing and the decrease in HPV prevalence with age, experts now recommend considering the use of both cytology and HR-HPV as primary cervical cancer screening in women 30 years of age and older. This indication for
HR-HPV testing has recently gained approval from the Food and Drug Administration. If women undergo combined testing and both cytology and HR-HPV are negative, no further routine cervical screening is indicated for 3 years. In the instance of normal cytology and a positive test for HR-HPV, the incidence of CIN II or III is much lower than the ASC/HC-HPV combination, and these patients may be offered repeat cytology at 6 and 12 months or repeat HR-HPV testing at 12 months only. The expectation is that the use of HR-HPV screening in women 30 years of age and older may improve the detection of preinvasive glandular lesions, allowing for a decrease in the incidence of adenocarcinoma of the cervix. This combined test is ideal for use in unscreened or under-screened populations. It should also be considered for women less likely to undergo annual screening and allows for increased intervals between testing. Women not needing annual visits for contraception or obstetrical care include women who have undergone tubal ligation or whose partner has undergone vasectomy; as well as infertile and menopausal women. These women may be best served by testing less frequently using the combination of HR-HPV and cytology, giving a higher sensitivity and improved reassurance.

Summary
The reduction in incidence and mortality from cervical cancer in women living in countries with established cervical cytology screening combined with treatment of CIN II or III has been well established. The integration of HPV DNA detection, the causative virus, into the prevention strategies has more recently been widely adopted in the United States. The contribution of the use of the highly sensitive HR-HPV DNA testing to overall reduction of cancer incidence or mortality is not yet known. Potential uses of this test are continuing to be proposed, and modifications are to be expected. The currently available test (HC2) allows for a positive or negative result based on the presence or absence of any or the 13 high-risk HPV types. The future may bring a type-specific test, which may allow determination of new infection versus persistent infections. Persistence of type-specific HPV DNA in the cervix for more than 2 years, rather than a single HC2, may eventually allow prediction of risk of CIN III or precursor.

Although this development may be clinically useful in predicting which women at increased risk for developing CIN II or III, it could also cause psychologic distress for patients. Disclosing to patients that they have been infected with a sexually transmitted virus may evoke a myriad of emotions and requires a great deal of patience and compassionate counseling on the part of the physician to alleviate some of this anxiety. The current HC2 allows us to tell patients whether or not they have one of the 13 HR-HPV subtypes. It does not tell us when the infection took place or how long they have been infected. Many practitioners discuss the infection as one to which the majority of sexually active young people will be exposed, thereby reducing some of the stigmas associated with a sexually transmitted disease (STD). However, with the development of type-specific tests, we will have the ability to discern new infections from previous infections. Again, although this has clinical utility, it also comes with psychologic costs. We are not suggesting that women be shielded from this information, but we are suggesting that physicians be prepared to deal with the emotional consequences of technologic advances.

Current uses for HR-HPV testing are: (1) as primary screening in women 30 years of age or older, when used in conjunction with cervical cytology; (2) as triage of ASC cytology to further colposcopic evaluation; (3) for evaluating adolescent patients with ASC at 12 months after incident Pap tests; (4) in post-colposcopy surveillance at 12 months, for women with CIN I or less identified on initial colposcopy; and (5) in post-conization surveillance at 6 to 12 months. At the present time, HR-HPV is not recommended for triage of patients with AGC or ASC-H, although this may be a future use.

References
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