In recent years, the array of options used in screening for cervical cancer has been expanded substantially by the development of new technologies such as liquid-based cytology (LBC) and by testing for human papillomavirus (HPV). Also, empirical data about the natural history of HPV and the effect of various strategies for screening and triage of abnormal cytology results have allowed for robust scrutiny of evidence-based screening algorithms. These changes have resulted in several organizations substantially revising their prior screening guidelines for cervical cancer.

**Epidemiology Of Cervical Cancer**

Cervical cancer mortality has decreased substantially by the detection of precursor lesions and earlier-stage cancers by means of Papanicolaou testing. However, invasive cervical cancer remains the cause of death for almost 4000 women each year in the United States, with most cases occurring in unscreened (and suboptimally screened) women. Virtually all cases of squamous cell cervical cancer arise in the context of prior infection with a high-risk type of HPV (ie, one known to increase the chance of cervical neoplasia). Cervical HPV infection is acquired sexually. The peak incidence and prevalence of HPV infection occur in women younger than 25 years, but most infections (70%-80%) in younger women are transient and do not progress to cervical neoplasia.
When infection and cervical abnormalities progress, the vast majority do so in an orderly fashion from less severe to more severe lesions before transitioning to an invasive cancer. Glandular lesions may be an important exception to this rule. Reliable early detection of cervical adenocarcinoma or the precursor lesion, adenocarcinoma in situ (AIS), remains a challenge. Because glandular lesions follow a less predictable clinical course and because the sensitivity for detecting glandular lesions is believed to be decreased compared with squamous lesions, all interpretations of Papanicolaou test results suggesting a glandular cell abnormality require meticulous and cautious follow-up.

Behavioral risk factors for squamous cell cervical cancer include earlier age at onset of sexual intercourse and larger number of lifetime partners. Cigarette smoking is the most important nonsexual risk behavior, independently increasing the risk 2- to 4-fold in several studies.

Initiation And Frequency Of Screening

Screening is defined as testing of a healthy individual. It is important to remember that the following discussion should not be generalized to the evaluation of a patient with signs or symptoms of cervical disease or to the follow-up of a woman with prior abnormal results from Papanicolaou testing. Screening recommendations attempt to balance the potential for good (prevention of cancer) against the potential for harm, in this case needless worry, expense, or intervention.

Both the American Cancer Society (ACS) and the US Preventive Services Task Force (USPSTF) recommend that all women begin annual Papanicolaou testing approximately 3 years after onset of sexual activity or at age 21 years (whichever occurs first). Although screening women who have never been sexually active has little value, this recommendation is based on the generally high prevalence of sexual activity by that age and on concerns that clinicians may not always obtain accurate sexual histories.

Women with atypical squamous cells of undetermined significance (ASCUS) or a low-grade squamous intraepithelial lesion (LSIL) whose initial evaluation reveals no dysplasia should be screened again at 6 and 12 months for LSIL or at 12 months for ASCUS, followed by screening every year (or every 2 years if using LBC and following ACS guidelines) until 5 years from the last abnormal result. Women with a history of higher-grade lesions are considered under “surveillance” rather than being “screened” and should be monitored at shorter intervals under the guidance of a gynecologist.

The most recent ACS guidelines recommend different screening intervals (previously noted) for the conventional and the LBC Papanicolaou tests. The increased sensitivity of LBC methods results in improved detection of all categories of dysplasia, with potential for increased detection of lesions of questionable clinical significance (such as a transient low-grade dysplasia).
The ACS guidelines recommend expanding the testing interval to every 2 years when using LBC screening for those women who would otherwise receive annual testing by the traditional method. With either method, screening should be performed at least every 3 years. Thus, when using LBC, a woman initially would be screened every 2 years and, after 3 consecutive normal test results (over 6 years), she would continue to be screened at 2- to 3-year intervals. (However, the recommendation for different testing intervals based on conventional vs LBC preparation of the slides has not yet been accepted universally.)

Women who are at high risk of cervical cancer should be screened more frequently. Specifically, immunocompromised women (including those with human immunodeficiency virus [HIV] or lymphoproliferative disorders or those taking long-term corticosteroids or organ transplant immunosuppression) generally should continue annual screening, as should women with intrauterine exposure to diethylstilbestrol (DES). Women with a history of recent cervical intraepithelial neoplasia (CIN) 2 or 3 or any prior diagnosis of invasive cervical cancer generally continue surveillance (no longer screening) Papanicolaou testing annually (or more frequently in some settings). Those with a history of CIN 2 or 3 may stop screening when criteria listed in the “When to Stop Screening” section are met.

**When To Stop Screening**

Women older than 65 years (USPSTF recommendation) or 70 years (ACS recommendation) who are not at high risk may safely stop screening if they have had 3 or more documented, technically satisfactory normal results from Papanicolaou testing and have had no abnormal results within the past 10 years.

There are exceptions to these guidelines for women at higher risk. Women with a history of CIN 2 or 3 should continue screening regardless of age or history of hysterectomy until they have had 3 or more technically satisfactory normal results from Papanicolaou testing and have had no abnormal results within the past 10 years. Women with a history of cervical cancer or in utero DES exposure should continue screening indefinitely for as long as they are in reasonably good health, regardless of age or history of hysterectomy. Immunosuppressed women (including those taking corticosteroids or those infected with HIV) should continue screening indefinitely for as long as they are in reasonably good health, regardless of age, but may stop after hysterectomy if this would otherwise be appropriate (see next paragraph).

Should women be screened after hysterectomy? Women who have undergone total hysterectomy for benign disease and have documented surgical pathology showing normal cervical epithelium or at most low-grade dysplasia (CIN 1) and who were screened appropriately before hysterectomy need not be screened. This recommendation is based on the extremely low yield of significant disease and the potential harms of false-positive results in this population. Women with a history of CIN 2 or 3 (or for whom prior pathology reports are unavailable) should continue screening until the criteria (discussed previously) associated with 3 consecutive normal results from Papanicolaou testing within 10 years have been met. Women with a history of cervical cancer or a history of in utero DES exposure should continue screening indefinitely. For various reasons, cervix-sparing hysterectomy (supracervical hysterectomy or subtotal hysterectomy) is once again in vogue in certain regions in the United States. Women who have undergone subtotal hysterectomy should continue (or discontinue) screening as would women in their risk group who have not undergone a hysterectomy.
Liquid-Based Cytology

Options for Papanicolaou testing now include conventional (slide smear) or LBC analysis. Recommendations increasingly favor the LBC methods for reasons discussed subsequently.

With LBC, the sample is suspended in a liquid that is centrifuged, and then cells are recovered from the centrifuged sediment. This technique provides a cleaner, more accurate preparation for microscopic analysis than conventional Papanicolaou testing for various reasons (including improved transfer of cellular material, more uniform distribution on the slide, a decrease in obscuring background factors, and less air-drying artifact). In most studies, LBC preparation has a slightly higher sensitivity than does preparation based on the conventional method, with approximately equivalent specificity. The LBC preparation also has been found to result in a higher rate of “satisfactory” test results. The ACS considered the higher sensitivity (more frequent detection of actual disease), increased cost, and chance of increased harm (patient anxiety and possible treatment of clinically insignificant lesions) with LBC Papanicolaou tests when formulating their recommendation to increase the screening interval for LBC methods to every 2 years.

Another advantage of LBC is the ability to perform HPV testing on the same sample when indicated (eg, for ASCUS).

It is important to note that optimal specimen collection for LBC Papanicolaou testing requires use of a plastic rather than a wooden spatula. The endocervix is sampled with the usual brush instrument or with a plastic spatula that incorporates an endocervical “broom.” The collection device(s) must be swirled in the collection medium for the recommended 30 seconds and then discarded or the ends of the collection devices are cut or broken off and submitted in the collection medium, depending on manufacturer’s directions. Physicians should review instructions for the particular test used in their own clinical settings.
**HPV DNA Testing**

Testing for high-risk types of HPV can help in the treatment of women with ASCUS by identifying those at higher risk of harboring or developing neoplasia. Most cytology laboratories are set up to allow this test to be performed on the same specimen already collected for LBC testing. This “reflex” testing is not available with traditional Papanicolaou testing. (Note that the types of HPV that cause genital warts are associated with only a minimal increase in cervical cancer risk and that testing for these low-risk types has no role in cervical cancer screening.) Currently, only 1 Food and Drug Administration–approved HPV test is available. The Hybrid Capture II assay (Digene Diagnostics, Gaithersburg, Md) tests for the 13 high-risk HPV types most commonly associated with high-grade dysplasia and cancer.

Recently, the Food and Drug Administration approved the use of HPV DNA testing as an adjunct to cytology for primary cervical cancer screening. This decision was based on several large studies that indicated increased sensitivity for detection of high-grade lesions compared with screening with cytology alone. Guidelines from both the ACS and the American College of Obstetricians and Gynecologists have noted this as a reasonable alternative screening strategy when used only in women aged 30 years or older and no more frequently than every 3 years. These restrictions take advantage of the extremely high negative predictive value (99%-100%) of combined cytology and HPV DNA testing for high-grade lesions while decreasing the costs and anxiety associated with overdiagnosis and overtreatment of women with transient HPV infections of no clinical consequence.

The introduction of HPV testing in primary screening requires careful education of the patient and clinician. Much confusion in this early phase of implementation has centered on 2 misperceptions. First, HPV infection is common in all sexually active women, even in the absence of classic epidemiological risk factors for cervical disease. Women with positive HPV test results must be helped to understand that this is not an indicator of infidelity. Also, positive test results for high-risk HPV types do not mean that a cytologic abnormality is present. It is possible, and indeed common, to have high-risk HPV infection with no detectable cytologic abnormality. Such women should be regarded as being at higher risk of dysplasia and cancer, but dysplasia is not an inevitable consequence of HPV infection.

**Treatment Of Patients With Abnormal Results From Papanicolaou Testing**

To more effectively communicate results to clinicians, the National Cancer Institute issued a revision in 2001 of the “Bethesda System” terminology used to report cervical cytology. The major types of intraepithelial lesions in that classification are listed in Table 1. A consensus conference also was convened that year to develop evidence-based guidelines for treating women with abnormal results from Papanicolaou testing. These guidelines form the basis of the following discussion, and the consensus conference recommendations are summarized in Table 2.

**Specimen Adequacy**

Cervical cytology reports often comment on limitations of the specimen. Fortunately, guidelines for these situations have been published. Patients whose Papanicolaou test results are interpreted as “Negative for intraepithelial lesion but lacking endocervical or transformation zone component” generally have been found to be at no higher risk than those with the components present. Recheck in 1 year is advised. Also, women whose Papanicolaou test results are interpreted as “Negative but partially obscured by... (blood, inflammation, air-drying artifact)” generally have been found to be at no increased risk. Again, recheck in 1 year is advised. (Note that LBC substantially decreases the frequency of this problem.) Women whose test results are interpreted as “Unsatisfactory for evaluation because of... [any reason]” are more likely than average to have a high-grade lesion present, and recheck in less than 6 months (preferably 2-4 months) is advised.
### Table 1. Terminology Used in Reporting Cervical Cytology*

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Expansion</th>
<th>Risk and follow-up†</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGC</td>
<td>Atypical glandular cells</td>
<td>Very high risk that cervical or endometrial cancer or precursor lesion is present</td>
</tr>
<tr>
<td>AIS</td>
<td>Adenocarcinoma in situ</td>
<td>Very high risk that cervical cancer or precursor lesion is present</td>
</tr>
<tr>
<td>ASC-H</td>
<td>Atypical squamous cells, cannot exclude HSIL</td>
<td>Higher risk; requires colposcopy</td>
</tr>
<tr>
<td>ASCUS</td>
<td>Atypical squamous cells of undetermined significance</td>
<td>Very low risk that cervical cancer is present, but requires follow-up</td>
</tr>
<tr>
<td>CIN</td>
<td>Cervical intraepithelial neoplasia</td>
<td>Not a Pap test result, but rather a histologic finding on biopsy; precancerous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lesion of the cervix; grades range from 1 (low dysplasia) to 3 (severe dysplasia)</td>
</tr>
<tr>
<td>HSIL</td>
<td>High-grade squamous intraepithelial lesion</td>
<td>Rare, but high risk; requires vigilant follow-up</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low-grade squamous intraepithelial lesion</td>
<td>Low to moderate risk; requires follow-up in most patients</td>
</tr>
</tbody>
</table>

*Pap = Papanicolaou test; the Pap test is a method of detecting cervical cytopathologic abnormalities that may suggest cancer or cancer precursor lesions.
†Based on abnormal Pap test results.

### Table 2. Guidelines for Treating Women With Abnormal Results From Papanicolaou Testing*

<table>
<thead>
<tr>
<th>Pathology report</th>
<th>Intermediate considerations</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&quot;Negative for intraepithelial lesion but lacking endocervical or transformation zone component&quot; (or &quot;No endocervical cells identified&quot;)</td>
<td>Continue regular screening</td>
</tr>
<tr>
<td></td>
<td>&quot;Negative but partially obscured by...&quot;</td>
<td>Recheck in 1 year</td>
</tr>
<tr>
<td></td>
<td>&quot;Unsatisfactory for evaluation&quot;</td>
<td></td>
</tr>
<tr>
<td>Atypical squamous cells of undetermined significance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most patients</td>
<td>Reflex test for high-risk HPV types—test is positive</td>
<td>Continue regular screening</td>
</tr>
<tr>
<td></td>
<td>Reflex test for high-risk HPV types—test is negative</td>
<td>Recheck in 1 year</td>
</tr>
<tr>
<td></td>
<td>Other (less preferred) options</td>
<td>Recheck within 6 months</td>
</tr>
<tr>
<td>Special patient populations</td>
<td>Immunosuppression (HIV, lymphoproliferative disorder, or pharmacological)</td>
<td>Either colposcopy now or repeat Pap test in 4 months</td>
</tr>
<tr>
<td></td>
<td>Postmenopausal with vaginal atrophy</td>
<td>Colposcopy</td>
</tr>
<tr>
<td>Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion</td>
<td></td>
<td>Consider intravaginal estrogen followed by repeated Pap test in 4-6 months (see text for details)</td>
</tr>
<tr>
<td>Low-grade squamous intraepithelial lesion</td>
<td></td>
<td>Colposcopy</td>
</tr>
<tr>
<td>High-grade squamous intraepithelial lesion</td>
<td></td>
<td>Colposcopy</td>
</tr>
<tr>
<td>Atypical glandular cells and adenocarcinoma in situ</td>
<td></td>
<td>Colposcopy</td>
</tr>
</tbody>
</table>

*HIV = human immunodeficiency virus; HPV = human papillomavirus; Pap = Papanicolaou testing.
Atypical Squamous Cells Of Undetermined Significance

The most common abnormality on Papanicolaou testing is ASCUS. Approximately 5% of patients with ASCUS results will harbor CIN 2 or 3 (moderate to severe dysplasia) on biopsy, whereas 0.1% to 0.2% will have invasive cervical cancer. Thus, some type of follow-up or further testing is appropriate but need not be aggressive. Traditionally, options were limited to immediate colposcopy or repetition of Papanicolaou testing at 4- to 6-month intervals until 2 consecutive normal results were obtained, with immediate colposcopy if ASCUS reappeared (or more serious results appeared) on any subsequent tests. Immediate colposcopy has the advantage of prompt confirmation of the presence or absence of disease. However, it has the disadvantages of cost, discomfort, and the anxiety associated with being referred for specialty care (with the attendant implications of potential serious disease).

The ability to test for high-risk HPV types offers a third and arguably the best option for treating patients with ASCUS results. With this protocol, women who test positive for high-risk HPV types are referred for colposcopy, whereas those testing negative are advised to undergo screening again in 1 year. This method of triage for results that indicate ASCUS is highly sensitive, with a well-documented negative predictive value of 98% or more. This method is preferable when it can be performed as a reflex test (initially LBC is used so that HPV testing is performed without an additional patient visit when ASCUS results are present). A recent analysis showed that reflex HPV testing is more cost-effective than alternative strategies for follow-up of ASCUS results.

In the setting of reflex HPV testing for ASCUS cytology, should a woman infected with a high-risk HPV type ever be tested for HPV again? In young women (in whom HPV infection usually remits spontaneously), repeated HPV testing with subsequent Papanicolaou testing may continue to be useful for interpreting ASCUS results. Repeated HPV testing in older women (in whom the HPV infection is more likely to be chronic) is probably of less value, but a discriminating age cutoff has not been identified. Postmenopausal women with clinical or cytologic evidence of atrophy who have ASCUS are at lower risk of clinically important neoplasia than are premenopausal women. Although use of reflex testing for HPV generally is preferred in this population as well, a reasonable alternative suggested by some guidelines is to treat with vaginal estrogen for 4 to 6 months and then repeat Papanicolaou testing 1 week after therapy is completed. The Papanicolaou test should be repeated again in 4 to 6 months. If results from both follow-up tests are normal, then routine screening can be resumed. If results are abnormal from either follow-up test, the patient should be referred for colposcopy.

Another exception includes women who are immunosuppressed due to HIV, lymphoproliferative disorder, corticosteroid use, or posttransplantation immunosuppression. All immunosuppressed women with ASCUS results should be referred for colposcopy. In HIV-positive patients, colposcopy should be performed regardless of CD4 count, HIV viral load, or use or nonuse of antiretroviral therapy.

Other Abnormalities On Papanicolaou Testing

Patients with abnormalities other than ASCUS on Papanicolaou testing should be referred for specialty care. The following brief summary is a supplement to specialty consultation for primary care physicians. (Most of this information is abridged from a report of a 2001 consensus conference sponsored by the American Society for Colposcopy and Cervical Pathology.)

Low-Grade Squamous Intraepithelial Lesion.

On subsequent cervical biopsy, 15% to 30% of women with LSIL will have CIN 2 or 3. Referral for colposcopy is indicated for all premenopausal patients. Because 83% of women with LSIL test positive for high-risk HPV types anyway, HPV testing is not indicated in this setting. The only exception to this recommendation is that postmenopausal women with LSIL who have evidence of atrophy on examination may be treated in the same way as those with ASCUS.
Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion.
The probability of finding CIN 2 or greater on biopsy ranges from 24% to 94% in such patients. Patients should be referred for prompt colposcopy.

High-Grade Squamous Intraepithelial Lesion. This cytologic interpretation is uncommon, accounting for only 0.45% of Papanicolaou test results. However, there is a 75% chance of having CIN 2 or 3 on biopsy and a 1% to 2% chance of invasive cancer. Thus, aggressive follow-up with colposcopy and endocervical evaluation is indicated. Because of the high risk of clinically important neoplasia, close follow-up is necessary, even if results from the initial colposcopic evaluation were negative.

Atypical Glandular Cells and AIS. This category is associated with a substantially greater risk of cervical neoplasia than ASC or LSIL, with up to 50% of results indicating CIN 2 or 3 on biopsy and 5% to 10% of results revealing AIS or invasive adenocarcinoma. Aggressive treatment is indicated, with immediate referral for colposcopy, endocervical curettage, and endometrial biopsy. This treatment often is followed by further procedures such as cone biopsy and dilation and curettage if the diagnosis remains uncertain.

Summary

Cervical cancer screening with Papanicolaou testing should begin at age 21 years (or within 3 years after onset of vaginal intercourse, if earlier) and cease after hysterectomy for benign conditions or after age 65 years (USPSTF recommendation) or 70 years (ACS recommendation) in women with adequate recent screening who are not otherwise at high risk of cervical cancer.

The maximum screening interval should be 3 years, with more frequent screening at onset and in high-risk situations (HIV, prior dysplasia, chronic immunosuppression).

Although more expensive, LBC has the advantages of improved sensitivity for LSIL and higher-grade lesions as well as fewer “unsatisfactory” and “obscured by blood/inflammation” readings and provides the ability to perform HPV testing on the same specimen (when indicated).

The use of HPV testing as an adjunct to cervical cytology for screening is an acceptable strategy, as long as it is restricted to women older than 30 years, and combined screening is done no more frequently than every 3 years. When available, the preferred strategy for management of ASCUS in most situations is reflex HPV testing (high-risk types only) on the original LBC specimen collected for the Papanicolaou test. When this is unavailable, other options include repeated Papanicolaou testing at 4- to 6-month intervals until 2 consecutive normal results are obtained, immediate colposcopy, and HPV testing at the next Papanicolaou testing.

Women with ASC-H (atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion) and LSIL test interpretations should undergo colposcopy.

Minimal follow-up requirements for women with high-grade squamous intraepithelial lesion, atypical glandular cells, and AIS interpretations include colposcopy and endocervical curettage.
References


The complete article is available on-line at URL http://www.mayoclinicproceedings.com.
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**Question:** Is it really necessary to remove the plasma from cells within 1 hour for homocysteine analysis?

**Answer:** Yes it is critical to do so in order to provide accurate results. Homocysteine levels in whole blood can increase at ambient temperatures if the plasma is not removed immediately. Homocysteine levels in plasma can increase by 10% in the first hour, 35% within 4 hours and 75% within 12 hours if not removed from cells.

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**Question:** Why is plasma preferred over serum for homocysteine analysis?

**Answer:** The whole blood collected to obtain a serum sample must be allowed to clot. This process may take too long at ambient temperatures where the analyte is not stable. As a result, serum homocysteine levels may be elevated as much as 10%. Therefore, EDTA plasma specimens are preferred.

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**Question:** Is it necessary to put the sample on wet ice after collection?

**Answer:** It is important to keep the whole blood sample at cool (4 degrees C) temperatures prior to centrifugation. Within 1 hour of blood draw at ambient temperature, homocysteine levels may increase 10% or more in whole blood. By keeping the sample cold, homocysteine is stabilized for 4-6 hours insuring adequate time for processing to occur.
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**Practical Spirometry**
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