Clinical Policy Bulletin:  
Cervical Cancer Screening and Diagnosis

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Policy

I. Consistent with guidelines from the U.S. Preventive Services Task Force and the American College of Obstetricians and Gynecologists, Aetna considers annual cervical cancer screening with conventional or liquid-based Papanicolaou (Pap) smears a medically necessary preventive service for nonhysterectomized women age 21 years and older.

II. Aetna considers Pap screening medically necessary beginning in adolescence in HIV-infected women. The ACOG guidelines on cervical cancer in adolescents (2010) recommend that adolescents with HIV have cervical cytology screening twice in the first year after diagnosis and annually thereafter.

III. Aetna considers Pap screening medically necessary in sexually active immunocompromised adolescent women, including those who have received an organ transplant or those with long-term steroid use. According to ACOG guidelines (2010), sexually active immunocompromised adolescents, including those who have received an organ transplant or those with long-term steroid use, should undergo screening after the onset of sexual activity and not wait until 21 years of age. The testing should be done at 6-month intervals during the first year of testing and then annually thereafter.

IV. Aetna considers Pap screening medically necessary beginning in adolescence in women diagnosed with cervical dysplasia or cervical cancer, with testing twice in the first year after diagnosis and annually thereafter.

V. Aetna considers Pap smears medically necessary beginning in adolescence in sexually active women who have been exposed in utero to diethylstilbestrol (DES). Testing should begin after the onset of sexual activity, and should be done at 6-month intervals during the first year of testing and then annually thereafter.

VI. Aetna considers Pap smear screening experimental and investigational for all other women under 21 years of age because they have no proven value for these younger women.

VII. Aetna considers Pap smear screening not medically necessary for women who have undergone complete (total) hysterectomy for benign disease (e.g., no
evidence of cervical neoplasia or cancer) or have absent cervix.

Note: Medically necessary cervical cancer screening is covered under plans that cover routine physical exams, routine gynecological exams and/or routine Pap smears. Please check benefit plan descriptions for details.

VIII. Aetna considers diagnostic Pap smears medically necessary when any of the following conditions is met:

A. Pap smear is accompanied by a diagnosis of a malignancy of the female genital tract (i.e., cervix, ovary, uterus, or vagina); or

B. There is a description of symptoms or a disease requiring diagnosis by a Pap smear, for example:
   1. Abnormal vaginal bleeding or discharge
   2. Chronic cervicitis
   3. Vaginal tumor; or

C. Following gynecological surgery for cancer; or
D. Member has been exposed to diethylstilbestrol (DES); or

E. Member has any of the following risk factors for cervical cancer:
   1. History of cervical, vaginal or vulvar cancer
   2. HIV infection
   3. History of genital HPV infection
   4. Immunosuppression
   5. Multiple sexual partners
   6. Previously abnormal Pap smear
   7. Previous sexually transmitted disease.

Aetna considers diagnostic Pap smears experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

IX. Aetna considers automated liquid-based thin-layer slide preparation methods (e.g., ThinPrep® PapTest™, SurePrep™ Liquid Based Pap Test, AutoCyte PREP System™) medically necessary as an alternative to conventional Pap smears when the criteria for conventional Pap smears are met.

X. Aetna considers automated cervical cancer slide interpretation systems (e.g., FocalPoint Slide Profiler (formerly AutoPap), PAPNET) a medically necessary adjunct to cervical cancer screening.

XI. Aetna considers testing for high-risk strains of human papilloma virus (HPV) DNA using Food and Drug Administration (FDA)-approved techniques (e.g., Hybrid Capture II, cobas HPV PCR) medically necessary for adult women 21 years of age or older with any of the following indications:

A. Assessment of women with atypical squamous cells of undetermined significance (ASCUS). This is consistent with the National Cancer Institute’s interim guidelines for managing abnormal cervical cytology as well as the position of the American Society for Colposcopy and Cervical Pathology (ASCCP) for the management of ASCUS.

B. Follow-up of women with ASCUS who have a previously positive HPV DNA
test and negative colposcopy results within the past 2 years.
C. Follow-up of women with low-grade squamous intra-epithelial lesions (LSIL) who have had negative colposcopy results within the past 2 years.
D. Follow-up of women with atypical squamous cells: Cannot exclude high-grade SIL (ASC-H) who have negative colposcopy results within the past 2 years.
E. Use in combination with Pap smears for screening women aged 30 years and older. If this combination is used for screening, it is not considered medically necessary to re-screen women who receive negative results on both tests more frequently than every 3 years. This policy is consistent with guidelines from the American College of Obstetricians and Gynecologists (2009).
F. Assessment of women with atypical glandular cells not otherwise specified (AGC NOS).
G. Follow-up of women with AGC NOS who have had negative colposcopy results within the past 2 years.

Note: The medically necessary indications for HPV DNA testing are not affected by pregnancy status.

XII. Aetna considers HPV testing experimental and investigational for the following indications:

A. Use as a primary screening test for cervical cancer in women younger than 30 years of age. According to evidence-based guidelines from the U.S. Preventive Services Task Force (2003), the medical literature does not support HPV testing as a screening test for cervical cancer for younger individuals whose cervical cytology is normal or is unknown.
B. For selecting candidates for cervical cancer vaccine. The Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices does not recommend HPV testing to select persons for cervical cancer vaccine.
C. For testing members with definitively positive cervical cytology, other than follow-up of women with ASC-H, LSIL or AGC NOS and negative colposcopy.
E. Testing of men.
F. Use for indications other than detection of cervical cancer, such as testing for infection following exposure to HPV.
G. For use in girls and women less than 21 years of age.
H. Use for all indications other than those listed in section XI above.

XIII. Aetna considers cervicography or speculoscopy (Pap-Sure) experimental and investigational for the screening or diagnosis of cervical cancer because of a lack of adequate clinical studies related to their use for these indications.

XIV. Aetna considers video colpography experimental and investigational for cervical cancer screening or diagnosis because of a lack of adequate evidence of its effectiveness for these indications.

XV. Aetna considers spectroscopy/optical detection systems (e.g., the Luma cervical imaging system) experimental and investigational for cervical cancer screening or diagnosis because of insufficient evidence of their effectiveness for these indications.
XVI. Aetna considers the Resolve™ laboratory testing kit (Gynecor™, Glen Allen, VA) experimental and investigational for cervical cancer screening or diagnosis because of insufficient evidence of its effectiveness for these indications.

XVII. Aetna considers the use of methylation markers for cervical cancer screening experimental and investigational because of insufficient evidence of their effectiveness.

XVIII. Aetna considers fluorescence in-situ hybridization (FISH) testing (e.g., the Ikonisys oncoFISH cervical test) for cervical cancer screening or diagnosis experimental and investigational because of insufficient evidence of its effectiveness.

See also CPB 0650 - Polymerase Chain Reaction Testing: Selected Indications.

Background

Pap smears consist of cells removed from the cervix, which are specially prepared for microscopic examination. The cells are removed by brushing or scraping the cervix during a pelvic examination and then placing the cells on one or more glass slides. Each slide typically contains hundreds of thousands of cells. All Pap smears should be sent to an accredited laboratory to be stained, examined under a microscope, and interpreted. The test is used as the principal screening test to detect cervical cancer in asymptomatic women. It can detect precancerous changes or cancer of the cervix or vagina. A Pap test will only rarely detect cancer of the ovaries or endometrial cancer. It can also find some infections of the cervix and vagina.

The American Academy of Family Physicians recommends that all women who are or have been sexually active, or who have reached age 18, should have annual Pap smears. The American Cancer Society, National Cancer Institute, and American Medical Association recommend that cervical cytology screening should begin within 3 years of onset of sexual activity or age 21. The recommendation allows less frequent Pap testing after 3 or more annual smears have been normal, at the discretion of the physician. For women who have had repeated negative tests, the marginal gain from screening more often than every 3 years decreases sharply. However, because of the difficulty in identifying patients at increased risk for cervical cancer, most physicians will recommend a Pap test be performed at least once-yearly.

The American College of Obstetricians and Gynecologists (ACOG, 2009) recommend that cervical cytology screening begin at age 21 regardless of age at onset of sexual activity and from age 21 to 29, testing is recommended every 2 years but should be more frequent in women who are HIV-positive, immunosuppressed, were exposed in-utero to diethylstilbestrol (DES), or have been treated for cervical intraepithelial neoplasia (CIN) grade 2, 3 or cervical cancer. The ACOG guidelines on cervical cancer in adolescents (2010) recommend that adolescents with HIV have cervical cytology screening twice in the first year after diagnosis and annually thereafter. Sexually active immunocompromised adolescents, including those who have received an organ transplant or those with long-term steroid use, should undergo screening after the onset of sexual activity and not wait until 21 years of age. The testing should be done at 6-month intervals during the first year of testing and then annually thereafter. Beginning at age 30, women who have 3 consecutive negative screens and who do not fit the criteria above for more frequent screening, may be tested every 3 years. Co-testing with cervical cytology and high-risk HPV typing is also appropriate and if both tests are negative, re-screening in 3 years is recommended.
After age 65, there is no clear consensus on the need for Pap smears in women who have had previous adequate screening. The American Academy of Family Physicians recommends that at age 65, screening may be discontinued if there is documented evidence of previously negative smears; however, these recommendations are currently under review. The ACOG (2009) recommend discontinuation of screening after age 65 or 70 in women with 3 or more negative consecutive tests and no cervical abnormalities during the previous 10 years. Women with histories of CIN grade 2, 3 or cancer should undergo annual screening for 20 years after treatment. The American College of Physicians (ACP) recommends Pap smears every 3 years for women aged 20 to 65, and every 2 years for women at high-risk. The ACP also recommends screening women aged 66 to 75 every 3 years if not screened in the 10 years before age 66.

Pap testing need not be performed for women who had a hysterectomy for benign disease; however, women who had a hysterectomy performed in which the cervix was left intact probably still require screening. However, a recent study by Sirovich and Welch (2004) indicated that many U.S. women who have undergone hysterectomy are undergoing Pap smear screening despite the U.S. Preventive Services Task Force's recommendation that Pap smear screening is unnecessary for women who have undergone a complete hysterectomy for benign disease.

A federally funded survey of 1,100 clinicians (internists, family practitioners, or obstetrician-gynecologists) found that only about 1/5 consistently follow guidelines for Pap testing (Yabroff et al, 2009). While over 80% said that at least one set of screening guidelines (e.g., U.S. Preventive Services Task Force) was "very influential" in their practices, only 22% recommended guideline-consistent care for every clinical scenario included in the survey. Obstetrician-gynecologists were less guideline-concordant than the other specialties. Of note, 1/3 of participants recommended annual Pap testing for an 18-year old who hadn't had sexual intercourse, while almost 1/2 continued to recommend Pap testing for a women whose cervix had been removed for benign reasons.

Repeat Pap smears may be indicated 3 to 4 months following local treatment of vaginal infection/inflammation, and 2 to 3 months following a Pap test suggestive of mild dyskaryosis or if the initial Pap smear results were unsatisfactory due to inadequate sampling.

A standardized method of reporting cytology findings was developed by the National Cancer Institute called the "Bethesda System". In the Bethesda System, atypical squamous cells fall into 2 categories: (i) **atypical squamous cells of undetermined significance (ASCUS)** and (ii) **atypical squamous cells: cannot exclude HSIL (ASC-H)**. Cervical cancer precursors fall into 2 categories: (i) **low-grade squamous intraepithelial lesions (LSIL)** and (ii) **high-grade squamous intraepithelial lesions (HSIL)**. Low-grade squamous intraepithelial lesions include CIN 1 (mild dysplasia) and the changes of HPV, termed **koilocytic atypia**. High-grade squamous intraepithelial lesions include CIN 2 and CIN 3 (moderate dysplasia, severe dysplasia, and carcinoma in situ). Other classification systems in use include the Dysplasia/CIN System and the Papanicolaou System.

Currently there are no formal guidelines for anal Pap smear screening. The most recent recommendations from the Centers for Disease Control and Prevention (Workowski and Berman, 2006) stated: "Routine testing for anal cytologic abnormalities or anal HPV infection is not recommended until more data are available on the reliability of screening methods, the safety of and response to treatment, and programmatic considerations."

**Automated Liquid-Based Thin-Layer Slide Preparation** (e.g., ThinPrep, SurePath, AutoCyte PREP)
To decrease the number of false-negative Pap smears, new technologies for preparing the Pap smear have been approved by the U.S. Food and Drug Administration (FDA).

The ThinPrep® PapTest™ (Cytyc Corp., Marlborough, MA), and SurePath (TriPath Imaging Inc., Burlington, NC) are automated liquid-based thin layer slide preparation techniques. With the ThinPrep System, a conventional Pap smear is not performed. Using a spatula and a brush or a cervical broom, the cervical area is sampled and the devices are rinsed in a fixative solution. The slide is then automatically made in the laboratory, which decreases the possibility of air-drying artifacts. It is then stained and read by a technician or a cytopathologist. SurePath (formerly known as AutoCyte PREP) is another liquid-based thin-layer sample preparation system that uses centrifugation to separate cells from obscuring material, and automatically prepares and stains cytology slides.

An assessment of liquid-based cervical cytology systems by the Institute for Clinical Systems Improvement (ICSI, 2003) concluded that liquid-based cytology is an acceptable alternative to conventional Pap testing for cervical cancer screening. The ICSI technology assessment made the following findings:

- For the detection of pre-invasive cervical lesions, liquid-based cytology is comparable to conventional Pap; there is no evidence of a change in the rate of cancer detection when liquid-based samples are analyzed.
- For minor grade lesions, there is evidence of a higher detection rate with liquid-based cytology. As a result, liquid-based cytology acts to normalize the rate of detection of atypical squamous cells of undetermined significance (ASCUS) so that pathologists can reach the 3% to 5% ASCUS rate expected (Bethesda criteria). More accurate detection of ASCUS helps to better identify patients who need further testing. Inter-observer validity is higher with LBC.
- Of 11 studies cited in the ICSI technology assessment that presented test results as either satisfactory, satisfactory but limited by, or unsatisfactory, 8 found a higher rate of satisfactory samples with liquid-based cytology. Between 75.6% and 97.7% of liquid-based cytology preparations were satisfactory compared with 60.5% to 97.5% with conventional Pap preparations.
- The ICSI report cited the results of a meta-analysis of 15 studies that reported a sensitivity of 80% for liquid-based cytology and 72% for conventional Pap testing predominantly for the detection of low-grade squamous intraepithelial lesions or more severe (LSIL+) by histology and/or independent pathology review of slides with a Pap test result of LSIL+. Specificity did not differ between conventional and liquid-based cytology preparations.

A technology assessment by the Canadian Coordinating Office for Health Technology Assessment found that "evidence (based primarily on results from split-sample trials) suggests that compared with Pap smears, the use of [liquid-based cytology] reduces the proportion of unsatisfactory specimens and generates fewer false negatives for ordinary populations, but not for high-risk populations" (Noorani et al, 2003).

In its updated guidelines on cervical cancer screening, the American Cancer Society has stated that liquid-based thin-layer Pap smears are an acceptable alternative to conventional Pap smears (Saslow et al, 2002). "As an alternative to conventional cervical cytology smear, cervical screening may be performed every two years using liquid-based cytology; at or after age 30, women who have had 3 consecutive, technically satisfactory normal/negative cytology results may be screened every 2 to 3 years (unless they have a history of in utero DES exposure, are HIV+, or are immunocompromised)."

An assessment by the Danish Centre for Evaluation and Health Technology Assessment...
(DACEHTA, 2005) concluded that "no scientific basis has been found to suggest any difference in clinical or health economic effect between liquid based cytology (LBC) and conventional Pap smear (CPS)." The report noted that "[i] the objective is to improve the clinical or health economic effectiveness, the report demonstrates that an increased coverage rate and an expansion of the age interval included in screening programmes for cancer of the uterine cervix from 59 to 69 years of age would be the more efficient strategy."

A recent large-scale clinical trial found that liquid-based cytology does not perform better than conventional Pap tests in terms of relative sensitivity and PPV for detection of cervical cancer precursors. In a randomized double-blind controlled trial, Siebers and colleagues (2009) compared liquid-based cytology with conventional cytology for detection of cervical cancer precursors in women (n = 89,784) aged 30 to 60 years participating in the Dutch cervical screening program. A total of 122 practices were assigned to use liquid-based cytology and screened 49,222 patients and 124 practices were assigned to use the conventional Pap test and screened 40,562 patients. Patients were followed for 18 months. The adjusted detection rate ratios for CIN grade 1+ was 1.01 (95 % confidence interval [CI]: 0.85 to 1.19); for CIN grade 2+, 1.00 (95 % CI: 0.84 to 1.20); for CIN grade 3+, 1.05 (95 % CI: 0.86 to 1.29); and for carcinoma, 1.69 (95 % CI: 0.96 to 2.99). The adjusted positive predictive value (PPV) ratios, considered at several cytological cut-offs and for various outcomes of CIN did not differ significantly from unity.

Automated System (FocalPoint, PAPNET)

Automated slide analysis devices (e.g., PapNet (Neuromedical Systems Inc.), FocalPoint (formerly AutoPap) (TriPath Technologies, Inc., AutoCyte SCREEn (AutoCyte, Inc.)) are designed to partially automate screening of Pap smears. The primary focus of current research is on use of image analysis as a primary screening device, where Pap smear slides are translated into digitalized images for automated image analysis. Slides that are identified by automated image analysis as possibly abnormal are passed on for manual interpretation. Slides that are identified by automated image analysis as very unlikely to contain abnormal cells may not be examined manually, or a random sample may be spot checked manually. Automated slide analysis devices may also be used to rescreen slides that are reported as negative or inadequate.

Although it is not known whether programs employing automated slide analysis are more effective than manual screening in detecting more cervical cancers, automated slide analysis devices have become standard of care. An Agency for Healthcare Research and Quality technology assessment of cervical cancer screening techniques (McCroy et al, 1999) concluded that there is substantial uncertainty about the estimates of sensitivity and specificity of cervical cancer screening using automated slide analysis devices compared with conventional manual screening, which in turn results in substantial uncertainty about the estimates of the effectiveness and cost-effectiveness of these techniques. "Although it is clear that both thin-layer cytology and computerized rescreening technologies provide an improvement in effectiveness at higher cost, the imprecision in estimates of effectiveness makes drawing conclusions about the relative cost-effectiveness of thin-layer cytology and computerized rescreening technologies problematic."

A technology assessment for the Minnesota Health Technology Advisory Committee (1999) concluded: "Studies of these methods demonstrate that computer-assisted cervical cancer screening and rescreening modestly improves detection of false-negative smears as compared with conventional manual screening. The majority of false-negative smears detected are low-grade squamous intraepithelial lesions (LGSIL), reactive or reparative changes, or atypical squamous cells of undetermined significance (ASCUS) rather than the
more serious premalignant or malignant lesions. Some studies have shown that computer-assisted Pap smear screening may marginally improve health outcome for some patients. The net health benefits of computer-assisted screening have not been proven. Studies examining the cost-effectiveness of the new technologies indicate that the cost-benefit of computer-assisted rescreening technologies is less favorable than any manual rescreening alternatives.

An assessment of the use of automated slide analysis devices in cervical cancer screening conducted by the Research Triangle Institute Evidence-Based Practice Center for the Agency for Healthcare Research and Quality (Hartmann et al, 2002) concluded that "overall, the quality of this literature is poor for the purposes of making decisions about choice of screening systems in US populations. No randomized trials or prospective cohort studies relate use of a screening modality over time to outcomes for individual women. The cost-effectiveness of use of new technologies has only been estimated, not measured directly."

More recently, the U.S. Preventive Services Task Force (USPSTF, 2003) reached the following conclusions regarding cervical cancer screening using automated slide analysis devices: "The USPSTF concludes that the evidence is insufficient to recommend for or against the routine use of new technologies to screen for cervical cancer. The USPSTF found poor evidence to determine whether new technologies, such as liquid-based cytology, computerized rescreening, and algorithm based screening, are more effective than conventional Pap smear screening in reducing incidence of or mortality from invasive cervical cancer. Evidence to determine both sensitivity and specificity of new screening technologies is limited. As a result, the USPSTF concludes that it cannot determine whether the potential benefits of new screening devices relative to conventional Pap tests are sufficient to justify a possible increase in potential harms or costs.

An assessment for the European Cervical Cancer Screening Network's Guidelines for Quality Assurance in Cervical Cancer Screening (Nieminen, 2003) summarized the current evidence for automated cervical cancer slide analysis devices: "There are several articles published concerning the performance of automation assisted screening. They show generally a better sensitivity with at least same specificity than conventional screening. Most of these articles have been retrospective (quality control) and/or relatively small numbers of smears have been studied. However, randomized, prospective public health trials in primary screening setting have been published very few. The show equal or slightly better performance compared to manual conventional screening …. When implementing the new methods, it is needed to carefully ascertain and evaluate the performance of the method in primary (public health) screening up to the final invasive end points with randomized prospective studies."

An assessment for the National Coordinating Centre for Health Technology Assessment (Willis et al, 2005) concluded: "As in previous health technology assessments on this subject, the conclusion is that the available evidence on test performance, impact on process and cost-effectiveness is still insufficient to recommend implementation of automated image analysis systems. The priority for action remains further research."

Wain (1997) has commented that "[t]he performance of automated techniques in quality assurance should be assessed against other methods of quality assurance, such as random re-screening of a mandated proportion of smears, directed re-screening of 'high-risk' groups and 'rapid re-screening'."

In its updated guidelines on cervical cancer screening, the American Cancer Society expert review panel (Saslow et al, 2002) only considered screening technologies with sufficient published clinical data, and excluded cervical cancer screening with automated slide
analysis devices from its consideration.

**HPV Testing**

Human papillomavirus (HPV) has been associated with the development of CIN and invasive cancer of the cervix. Recent prospective studies have shown that abnormal Pap smears that are positive for oncogenic HPV strains are much more likely to be associated with abnormal colposcopic findings than abnormal Pap smears that are HPV negative. There is no proven value for testing for additional "low-risk" strains of HPV that have not been associated with substantially elevated cancer risk.

HPV testing has been used as an adjunctive reflex test in women with ASCUS to identify those at highest risk for cervical cancer, who should go on to receive definitive colposcopy. HPV testing of patients with ASCUS can be used to identify patients at highest risk of underlying cervical dysplasia, and minimize the number of unnecessary colposcopic examinations in women who have no disease. Women with ASCUS who have a positive HPV and no lesions on colposcopy should be followed-up with repeat Pap testing at 6 and 12 months or with HPV testing at 12 months. Current guidelines do not recommend reflex testing of women with squamous intraepithelial lesions (HSIL, ASC-H, or LSIL). However, guidelines from the American Society of Colposcopy and Cervical Cytology (Wright et al, 2002; Wright et al, 2007) recommend that women with LSIL or ASC-H with no lesions on colposcopy should be followed-up with repeat Pap testing at 6 and 12 months or with HPV testing at 12 months.

HPV testing has also been proposed as a primary screening test to be performed simultaneously with Pap smear screening. Digene Corp. received FDA approval for a test that combines the Pap smear with a genetic exam for 13 oncogenic strains of HPV. Aetna, however, does not cover HPV testing as a screening test for cervical cancer for women less than 30 years of age because the evidence is insufficient to determine whether HPV screening reduces the incidence of or mortality from invasive cervical cancer. Aetna's policy is consistent with updated recommendations of the U.S. Preventive Services Task Force (USPSTF) (2003). The USPSTF concluded that the evidence is insufficient to recommend for or against the routine use of human papillomavirus (HPV) testing as a primary screening test for cervical cancer. The USPSTF found "poor evidence to determine the benefits and potential harms of HPV screening as an adjunct or alternative to regular Pap smear screening."

The ACOG (2009) concluded, based on "good and consistent scientific evidence" that the use of a combination of cervical cytology and HPV DNA screening is appropriate for women aged 30 years and older. According to ACOG (2009), if this combination is used, women who receive negative results on both tests should be re-screened no more frequently than every 3 years. ACOG's recommendation was based on the results of studies that demonstrated that women aged 30 years and older who had both negative cervical cytology test results and negative high-risk type HPV-DNA test results were at extremely low-risk of developing CIN 2 or CIN 3 during the next 4 to 6 years. ACOG guidelines explain that any woman aged 30 years or older who receives negative test results on both cervical cytology screening and HPV DNA testing should be re-screened no more frequently than every 3 years. The ACOG guidelines state that the combined use of these modalities has been shown to increase sensitivity but also decrease specificity and increase cost. However, ACOG estimated that the increase in screening interval will offset the cost of this new screening regimen.

The ACOG guidelines (2009) stated that the combination of cytology and HPV DNA screening should be restricted to women aged 30 years and older because transient HPV infections are common in women younger than 30 years, and a positive test result may
lead to unnecessary additional evaluation and treatment. The ACOG guidelines on cervical cancer in adolescents (2010) stated that HPV testing is not recommended at any time in adolescents because of the high prevalence of HPV infection in adolescents and there is little utility in HPV testing in this population; there are no clinical situations, screening, triage, or follow-up that require HPV testing in this population and if conducted, a positive test result should not influence management. The guidelines stated that there is no role for HPV testing in the patient before HPV vaccination. Furthermore, the ACOG guidelines (2010) stated that adolescents who have low- to high-grade precancerous lesions (dysplasia) -- with the exception of cervical intraepithelial neoplasia 3 (CIN 3) -- generally should be managed by periodic observation. The guidelines stated that re-screening can be delayed until age 21 when the Pap test results show regression of the dysplasia, but annual screening also is an acceptable alternative.

Published studies of cervical cancer screening using a combination of cytology and HPV DNA tests have predominantly employed conventional Pap smears for assessment of cervical cytology. Although there are no studies directly comparing the screening performance of HPV-cytology combination testing using a conventional Pap versus liquid-based cervical cytology, available indirect evidence suggests that there is no clinically significant difference in the screening performance of HPV-cytology combination testing regardless of whether conventional or liquid-based cervical cytology is used (Lorincz and Richart, 2003).

The National Cancer Institute is currently sponsoring a multi-center 5-year clinical trial directed at determining the role of HPV testing in the management of cervical disease. Interim guidelines for the management of abnormal cytologic findings in the cervix were developed at a work-shop sponsored by the NCI, which concluded that HPV testing can be used as an adjunctive test to help identify patients at low- or high-risk of developing CIN and cancer. The American Society of Colposcopy and Cervical Pathology has also issued guidelines for the management of ASCUS which incorporated HPV testing and typing to determine which women with ASCUS should undergo colposcopy.

There are no current guidelines recommending HPV testing of men (CDC, 2006; Workowski and Berman, 2006). There is no FDA-approved HPV test for men, and there are no studies demonstrating benefit of testing men for HPV infection. Unlike with cervical ASCUS, HPV typing has not been shown to aid in predicting which patients with anal ASCUS are at risk for high-grade anal intraepithelial neoplasia (AIN) (Panther et al, 2003).

Saqi and colleagues (2006) evaluated the potential role of HPV DNA testing on atypical glandular cells (AGC) cases. Hybrid Capture 2 (Digene Corp.) testing was performed on 144 cervical/endo-cervical AGC specimens. A total of 103 of 144 cases had follow-up; 60/103 (58.3 %) were high-risk HPV negative and 43/103 (42.3 %) were high-risk HPV positive. Of 43 HPV-positive patients, 37 had adenocarcinoma in situ (AIS), ASCUS, or cervical squamous intra-epithelial neoplasia, while only 1 patient without high-risk HPV had a squamous intraepithelial neoplasia. Furthermore, most high-risk HPV positive AGC cases harbored high-grade squamous intra-epithelial lesion rather than AIS. The authors concluded that their findings support HPV DNA testing of all AGC specimens to detect cervical, especially squamous, neoplasia.

The American Society for Colposcopy and Cervical Pathology (ASCCP)'s guidelines for the management of women with abnormal cervical cancer screening tests (Wright et al, 2007) noted that HPV testing is incorporated into the management of AGC after their initial evaluation with colposcopy and endometrial sampling. The recommended post-colposcopy management of women with AGC is to repeat cytologic testing combined with HPV DNA testing at 6 months if they are HPV DNA positive and at 12 months if they
are HPV DNA negative. Referral to colposcopy is recommended for women who subsequently test positive for HPV DNA or who are found to have ASCUS or greater on their repeat cytologic tests. If both tests are negative, women can return to routine cytologic testing.

In a randomized study, Mayrand et al (2007) examined if testing for DNA of oncogenic HPV is superior to the Pap test for cervical cancer screening. These investigators compared HPV testing, using an assay approved by the FDA, with conventional Pap testing as a screening method to identify high-grade CIN in women aged 30 to 69 years. Women with abnormal Pap test results or a positive HPV test (at least 1 pg of high-risk HPV DNA per milliliter) underwent colposcopy and biopsy, as did a random sample of women with negative tests. Sensitivity and specificity estimates were corrected for verification bias. A total of 10,154 women were randomly assigned to testing. Both tests were performed on all women in a randomly assigned sequence at the same session. The sensitivity of HPV testing for CIN of grade 2 or 3 was 94.6 % (95 % CI: 84.2 to 100), whereas the sensitivity of Pap testing was 55.4 % (95 % CI: 33.6 to 77.2; p = 0.01). The specificity was 94.1 % (95 % CI: 93.4 to 94.8) for HPV testing and 96.8 % (95 % CI: 96.3 to 97.3; p < 0.001) for Pap testing. Performance was unaffected by the sequence of the tests. The sensitivity of both tests used together was 100 %, and the specificity was 92.5 %. Triage procedures for Pap or HPV testing resulted in fewer referrals for colposcopy than did either test alone but were less sensitive. No adverse events were reported. The authors concluded that as compared with Pap testing, HPV testing has greater sensitivity for the detection of CIN.

An assessment by the California Technology Assessment Forum (CTAF, 2008) found HPV testing for primary cervical cancer screening did not meet CTAF criteria. The CTAF assessment found that, although incorporation of HPV screening can lead to earlier detection of carcinoma in situ lesions, whether or not this will result in reduced cervical cancer incidence and mortality is not known. The CTAF assessment noted, in addition, that the 2 trials that have published long term follow-up used a PCR test that is not currently available in the United States. The CTAF assessment concluded that the evidence is insufficient to recommend for or against the incorporation of HPV testing into cervical cancer screening programs.

Thus, whether HPV testing can replace conventional Pap cytologic testing for cervical cancer screening awaits results from randomized controlled trials and/or recommendations from leading national medical organizations.

Sankaranarayanan and colleagues (2009) began to measure the effect of a single round of screening by testing for HPV, cytologic testing, or visual inspection of the cervix with acetic acid (VIA) on the incidence of cervical cancer and the associated rates of death in the Osmanabad district in India. In this cluster-randomized trial, 52 clusters of villages, with a total of 131,746 healthy women between the ages of 30 and 59 years, were randomly assigned to 4 groups of 13 clusters each. The groups were randomly assigned to undergo screening by HPV testing (n = 34,126), cytologic testing (n = 32,058), or VIA (n = 34,074) or to receive standard care (n = 31,488, control group). Women who had positive results on screening underwent colposcopy and directed biopsies, and those with cervical pre-cancerous lesions or cancer received appropriate treatment. In the HPV-testing group, cervical cancer was diagnosed in 127 subjects (of whom 39 had stage II or higher), as compared with 118 subjects (of whom 82 had advanced disease) in the control group (hazard ratio for the detection of advanced cancer in the HPV-testing group, 0.47; 95 % CI: 0.32 to 0.69). There were 34 deaths from cancer in the HPV-testing group, as compared with 64 in the control group (hazard ratio, 0.52; 95 % CI: 0.33 to 0.83). No significant reductions in the numbers of advanced cancers or deaths were observed in the cytologic-testing group or in the VIA group, as compared with the control group. Mild adverse events
were reported in 0.1 % of screened women. The authors concluded that in a low-resource setting, a single round of HPV testing was associated with a significant reduction in the numbers of advanced cervical cancers and deaths from cervical cancer.

In an editorial that accompanied the afore-mentioned article, Schiffman and Wacholder (2009) stated that "[i]n developed nations, HPV testing at extended screening intervals could eventually replace repeated cytologic testing as the primary screening method. Cytologic testing might be used to stratify risk further by identifying HPV-positive women at highest risk for cancer. In these countries, a widespread transition from a good method (frequent cytologic testing) to a better one (less frequent HPV screening) will require high-quality testing that is widely available and properly priced, the establishment of correct screening intervals and related health messages, and the promulgation of clinical guidelines and reimbursement policies to avoid overtreatment of benign infections". Schiffman and Wacholder also noted that, "[i]n the United States, switching to primary HPV screening will be contentious, partly because lengthening the interval between cervical screenings seriously disrupts established gynecologic clinical practice".

Davis (2009) stated that "[i]n showing that a single round of HPV screening (compared with cytologic or VIA screening) had the most marked effect on preventing cervical cancer deaths, these results have tremendous international health implications: Single rounds of HPV screening are much easier to implement than repetitive cytologic screening, particularly in resource-poor countries where cervical cancer is relatively common. Nonetheless, we should not rush to apply such findings to populations with optimal resources. Clinicians in the U.S. should continue to screen as recommended by the American Society for Colposcopy and Cervical Pathology".

Cervicography and Speculoscopy

Cervicography is a procedure in which the cervix is swabbed with an acetic acid solution to identify acetowhite changes in the cervix. With cervicography, a photograph of the cervix is taken with a special camera (Cerviscope), and is sent to trained technicians for evaluation (National Testing Laboratories, St. Louis, MO). The technicians determine whether the visual image is most compatible with normal, atypia/metaplasia, intraepithelial neoplasia, or cancer. In contrast, speculoscopy (PapSure) uses a chemiluminescent light to aid naked-eye or minimally magnified visualization of acetowhite changes on the cervix. Both cervicography and speculoscopy have been used as an adjunct to Pap smear for cervical cancer screening and as a triage method to identify which patients with low grade atypical Pap smears need further evaluation by colposcopy and biopsy. According to practice guidelines from the ASCCP, "there have been insufficient large scale controlled studies related to their use in the triage of LGISL [low grade squamous intraepithelial lesion] to recommend either for or against their use" (Cox et al, 2000). An International Academy of Cytology (IAC) Task Force (van Niekerk et al, 1998) concluded that "[t]he role of cervicography, or high resolution photography, as a screening device remains to be defined." The IAC Task Force also noted that "[t]here are, at present, insufficient data for the evaluation of speculoscopy...." The U.S. Preventive Services Task Force (1996) concluded that "[t]here is insufficient evidence to recommend for or against routine screening with cervicography ... although recommendations against such screening can be made on other grounds."

Video Colpography

Video colpography (video colposcopy) has been used for imaging the vagina and cervix, and has been proposed for use as a method of cervical cancer screening. In this procedure, a video camera is used to create computerized digital images of the cervix, vaginal fornices and endocervical canal; the system may be interfaced with a computer for
image manipulation. The images are evaluated by a video screener for signs of cervical cancer. Etherington et al (1997) stated that video colpography has potential advantages as a portable and rapid method of cervical imaging. The investigators stated that video colpography has potential of use in fields of teaching, audit and screening of women with low-grade smear abnormalities. Etherington et al (1997) compared video colpography with colposcopy in a pilot study involving 50 women referred for colposcopy. The investigators reported that the video colpography images were satisfactory or good in 47 (94 %) cases, and there was agreement between colposcopist and video screener in 86 % of cases. The investigators stated that, if the technique had been used in a primary health care setting as a secondary screening method for women with low-grade cervical smear abnormalities, 61 % would have avoided referral for colposcopy. The investigators concluded that "before the technique can be implemented as part of the screening process, it needs to be evaluated in a larger series ..."

Spectroscopy/Optical Detection Systems

The Luma cervical imaging system (MediSpectra, Inc., Lexington, MA) is an optical detection system approved by the FDA in March, 2006 as an adjunct to colposcopy to identify areas of the cervix with the highest likelihood of high-grade CIN on biopsy. The Luma system shines a light on the cervix and analyzes how different areas of the cervix respond to the light. The system produces a color map that distinguishes between healthy and potentially diseased tissue to indicate where biopsy samples should be taken.

In a pilot study, Huh and colleagues (2004) investigated the in-vivo optical detection of high-grade CIN. Cervical scanning devices collected intrinsic fluorescence and broadband white light spectra and video images from 604 women during routine colposcopy examinations. A statistically significant dataset was developed of intrinsic fluorescence and white light-induced cervical tissue spectra that was correlated to histopathologic determination. On the basis of a retrospective analysis of the acquired data, a classification algorithm was developed, validated, and optimized. Intrinsic fluorescence, back-scattered white light, and video imaging each contributed complementary information to diagnostic algorithms for high-grade cervical neoplasia. Over 10,000 measurements were made on colposcopically identified tissue from more than 500 subjects and were the basis for algorithm training and testing. Algorithm performance demonstrated a sensitivity of approximately 90 %. This performance was confirmed by various training methods. With the use of a multi-variate classification algorithm, optical detection is predicted to detect 33 % more high-grade CIN (2/3+) than colposcopy alone. The authors concluded that full cervix optical interrogation for the detection of high-grade CIN is feasible and appears capable of detecting more high-grade CIN than colposcopy alone.

A multi-center controlled trial (Alvarez et al, 2007) evaluated the performance of the Luma system as an adjunct to colposcopy among women (n = 193) referred for the evaluation of an abnormal cervical cytology result. Initial colposcopy identified 41 cases of CIN 2+ for a true positive (TP) rate of 21.2 %. Adjunctive use of the Luma system identified an additional 9 cases of CIN 2+ which corresponds to an incremental optical detection TP rate of 4.7 % (95 % CI: 2.2 % to 8.7 %). Adjunctive use of the Luma system resulted in a 22.0 % (95 % CI: 6.1 % to 37.8 %) relative gain in the number of women with CIN 2+ compared
to colposcopy alone. The false-positive (FP) rate for initial colposcopy was 51.8 % (100 of 193 women). An additional 35 subjects had a Luma system-directed biopsy that was not diagnosed as CIN 2+, yielding an incremental FP rate of 18.1 % (95 % CI: 13.0 % to 24.3 %). The authors concluded that the adjunctive use of the Luma system with colposcopy provided a significant increase in the detection of CIN 2+ in women referred for the evaluation of abnormal cytology results.

There is insufficient evidence of the effectiveness of an optical detection system as an adjunct to colposcopy for in vivo identification and localization of cervical intraepithelial for cervical cancer screening or diagnosis. Furthermore, there are no recommendations or guidelines for its use from any professional medical society. Post-approval studies are currently underway to further assess the long-term efficacy of the Luma system.

A spectroscopy system may be referred to as an optical detection system. Spectroscopy emits light from a probe onto the cervix, allowing the examiner to objectively categorize tissues as either normal or diseased. Spectroscopy is based on the principle that epithelial tissues that are abnormal have different optical properties than normal tissues and that these optical differences can be used to determine whether a tissue is normal or abnormal. Devices that are currently under various stages of research and development for diagnostic purposes use various approaches, including: fluorescence spectroscopy, image analysis of visible images, infrared spectroscopy, Raman spectroscopy, white light elastic backscatter spectroscopy, or combinations of the different methods (Wright et al, 2002).

Wright et al (2002) stated that visual screening techniques include both low-technology approaches, such as direct visual inspection (DVI), and high-technology approaches, such as those that utilize electro-optical detectors to identify cervical cancer precursors and invasive cervical cancer. Simple visual screening techniques, such as DVI, consist of washing the cervix with a solution of 5 % acetic acid (e.g., vinegar) and then inspecting it using either the naked eye or with a low-power magnifying device to identify areas of aceto-whitening, which frequently correspond to cervical squamous intraepithelial lesions (SILs). The simple visual screening methods are being evaluated as an alternative to cytology in low-resource settings where screening using cervical cytology is not feasible. Multiple studies have shown DVI to have sensitivity similar to that of cervical cytology for identifying women with high-grade SIL but much lower specificity. The novel high-technology visual screening methods that utilize electro-optical sensors to identify cervical abnormalities are still in the developmental phases but offer considerable potential.

In a prospective observational study, Brown et al (2005) compared cervical impedance spectroscopy in the cervical epithelium of women with CIN and normal epithelium. A total of 87 women referred to colposcopy with a moderate or severely dyskaryotic smear were included in this study. A pencil probe incorporating 4 gold electrodes was used to measure an electrical impedance spectrum from cervical epithelium. Colposcopy examinations, including probe positioning, were recorded by video to allow for correlation between results obtained from colposcopic impression, histopathological examination of colposcopically directed punch biopsies and the impedance measurements. Cervical impedance derived parameters R, S and C were assessed to see if there was a significant difference in values obtained in CIN and normal squamous epithelium. Analysis was based upon matching the electrical components measured to those identified by cellular modeling as being most sensitive for pre-malignancy. From normal epithelium through CIN 1 to CIN 2/3, R decreased by a factor of 4.5, S increased by a factor of 2.5, but C remained unchanged. The authors concluded that cervical impedance spectroscopy provided a potentially promising screening tool with similar sensitivity and specificity to currently used screening tests, but with the potential advantage of providing instant results. Moreover, they stated
that further work is currently being undertaken to improve the probe in its clinical use.

In a prospective, comparative, multi-center clinical study, Tidy et al (2013) examined if electrical impedance spectroscopy (EIS) improves the diagnostic accuracy of colposcopy when used as an adjunct. In phase-1, EIS was assessed against colposcopic impression and histopathology of the biopsies taken. In phase-2, a probability index and cut-off value for the detection of high-grade cervical intraepithelial neoplasia (HG-CIN, i.e., grade CIN2+) was derived to indicate sites for biopsy. Electrical impedance spectroscopy data collection and analyses were performed in real time and blinded to the clinician. The phase-2 data were analyzed using different cut-off values to assess performance of EIS as an adjunct. Main outcome measure was histologically confirmed HG-CIN (CIN2+). A total of 474 women were recruited: 214 were eligible for analysis in phase-1, and 215 were eligible in phase-2. The average age was 33.2 years (median age of 30.3 years, range of 20 to 64 years) and 48.5 % (208/429) had high-grade cytology. Using the cut-off from phase-1 the accuracy of colposcopic impression to detect HG-CIN when using EIS as an adjunct at the time of examination improved the PPV from 78.1 % (95 % CI: 67.5 to 86.4) to 91.5 %. Specificity was also increased from 83.5 % (95 % CI: 75.2 to 89.9) to 95.4 %, but sensitivity was significantly reduced from 73.6 % (95 % CI: 63.0 to 82.5) to 62.1 %, and the negative predictive value (NPV) was unchanged. The positive likelihood ratio for colposcopic impression alone was 4.46. This increased to 13.5 when EIS was used as an adjunct. The overall accuracy of colposcopy when used with EIS as an adjunct was assessed by varying the cut-off applied to a combined test index. Using a cut-off set to give the same sensitivity as colposcopy in phase-2, EIS increased the PPV to detect HG-CIN from 53.5 % (95 % CI: 45.0 to 61.8) to 67 %, and specificity increased from 38.5 % (95 % CI: 29.4 to 48.3) to 65.1 %. Negative predictive value was not significantly increased. Alternatively, applying a cut-off to give the same specificity as colposcopy alone increased EIS sensitivity from 88.5 % (95 % CI: 79.9 to 94.4) to 96.6 %, and NPV from 80.8 % (95 % CI: 67.5 to 90.4) to 93.3 %. Positive predictive value was not significantly increased. The receiver operator characteristic (ROC) to detect HG-CIN had an area under the curve (AUC) of 0.887 (95 % CI: 0.840 to 0.934). The authors concluded that EIS used as an adjunct to colposcopy improves colposcopic performance; and the addition of EIS could lead to more appropriate patient management with lower intervention rates.

An UpToDate review on “Cervical cancer screening tests: Evidence of effectiveness” (Sirovich et al, 2014) does not mention the use of spectroscopy/optical detection system as a screening tool. Furthermore, the National Comprehensive Cancer Network’s clinical practice guideline on “Cervical cancer” (Version 1.2014) does not mention spectroscopy/optical detection systems as screening tools.

In summary, as a consequence of the lack of well-designed studies, there is insufficient evidence to support the use of spectroscopy/optical detection systems as a primary stand-alone or as an adjunct to standard screening techniques for the detection of cervical cancer.

Resolve™

Colposcopy is a diagnostic procedure in which a colposcope is used to provide an illuminated, magnified view of the cervix, vagina, and vulva to detect malignant and pre-malignant epithelium. Malignant and pre-malignant epithelium have specific macroscopic characteristics relating to contour, color, and vascular pattern that can be identified by the colposcopist for directed biopsy. Colposcopy is the “gold standard” diagnostic tool in the U.S. for diagnosing cervical dysplasia following abnormal cytology.

The Resolve laboratory testing kit (Gynecor, Glen Allen, VA) is a new colposcopic method
that obtains endocervical samples using cytobrushes. The kit contains 2 cytobrushes and 2 vials of fixative. The 1st cytobrush is used to clear mucus from the cervix and the 2nd cytobrush is used to abrade cells from the endocervix. The fixative enables both cytology and histology to be run on both vials. HPV is also tested from the same specimen. If HPV is detected, genotyping by PCR is also reported.

There is insufficient evidence of the effectiveness of the Resolve laboratory testing kit for cervical cancer screening or diagnosis. How this method compares with conventional colposcopy and cytology using quantified values of sensitivity and specificity awaits results from randomized controlled trials and/or recommendations from leading national medical organizations.

**Methylation Markers**

Studies of cervical cancer and its immediate precursor, cervical intra-epithelial neoplasia 3, have identified genes that often show aberrant DNA methylation and thus represent candidate early detection markers. Wentzensen et al (2009) identified the most promising methylation marker candidates for cervical cancer early detection. A systematic literature review was performed in Medline and weighted average frequencies for methylated genes stratified by tissue source and methods used were computed. A total of 51 studies were identified analyzing 68 different genes for methylation in 4,376 specimens across all stages of cervical carcinogenesis. A total of 15 genes (DAPK1, RASSF1, CDH1, CDKN2A, MGMT, RARB, APC, FHIT, MLH1, TIMP3, GSTP1, CADM1, CDH13, HIC1, and TERT) have been analyzed in 5 or more studies. The published data on these genes is highly heterogeneous; 7 genes (CDH1, FHIT, TERT, CDH13, MGMT, TIMP3, and HIC1) had a reported range of methylation frequencies in cervical cancers of greater than 60% between studies. Stratification by analysis method did not resolve the heterogeneity. Three markers (DAPK1, CADM1, and RARB) showed elevated methylation in cervical cancers consistently across studies. The authors concluded that there is currently no methylation marker that can be readily translated for use in cervical cancer screening or triage settings. They stated that large, well-conducted methylation profiling studies of cervical carcinogenesis could yield new candidates that are more specific for HPV-related carcinogenesis. New candidate markers need to be thoroughly validated in highly standardized assays.

**The Ikonisys oncoFISH Cervical Test**

According to Ikonisys Clinical Laboratories, the oncoFISH® cervical test is a qualitative fluorescence in-situ hybridization (FISH) test for determining the acquisition of specific chromosomal aneuploidies within the 3q26 region in cytological specimens revealing LSIL. Until now, routine testing for 3q gain was not feasible because assessment required analysis of a large number of stained, squamous cell nuclei -- impractical for manual methods. By using the Ikoniscope Digital Microscopy System to automate analysis, the oncoFISH cervical test makes testing for 3q gain a practical reality. The test is performed on cervico-vaginal cytology specimens, identical to those used for Pap and HPV testing. It evaluates amplification of the 3q26 region by use of 2 FISH probes, one for the 3q26 locus and a control probe. Enumeration and comparison of the 3q26 and control probes, in conjunction with the nuclear morphology, result in a 3q copy number for each of the nuclei analyzed. Results of the oncoFISH cervical test are intended for use with other clinical findings for further evaluation and monitoring of cervical dysplasia in women with LSIL Pap results. The oncoFISH cervical test is a laboratory developed test and is intended to supplement, and not replace or alter the current standards of practice used for the clinical management of women undergoing evaluation for cervical dysplastic lesions. The oncoFISH cervical test results should be considered by the clinician in the context of other...
Caraway et al (2008) noted that chromosomal aberrations have been documented in cervical carcinomas, especially chromosome 3q. The human telomerase RNA gene (hTERC) is located in the chromosome 3q26 region, and its product, telomerase, is involved in the maintenance of chromosome length and stability. Up-regulation of telomerase is in general associated with tumorigenesis. In this study, cervico-vaginal specimens were analyzed by FISH for gain of chromosome 3q26 containing hTERC, and FISH findings were compared with the cytologic and histologic diagnoses. Slides prepared from 66 liquid-based preparations from cervical specimens with cytologic diagnoses of negative for SiL or malignancy (NILM, n = 4), atypical squamous cells of undetermined significance (ASC-US, n = 15), LSIL (n = 20), HSIL (n = 24), or cervical squamous cell carcinoma (SCCA, n = 3) were analyzed for aberrations of 3q26 using a commercially available 2-color FISH probe. The results of the cytologic analysis and those of concurrent or subsequent biopsies, when available, were compared with the FISH-detected 3q26 abnormalities. The Wilcoxon rank-sum test was used to assess associations between 3q26 gains and diagnoses. Gain of 3q26 was significantly associated with the cytologic diagnosis (p < 0.0001). Patients with HSIL or SCCA cytology diagnoses had significantly higher percentages of cells with 3q26 gain than did patients with NILM or ASC-US cytologic diagnoses. The authors concluded that FISH can be performed on cervico-vaginal liquid-based preparations to detect gain of 3q26; gain of 3q26 is associated with HSIL and SCCA. They stated that this test may be an adjunct to cytology screening, especially high-risk patients. This study did not have enough correlative data to be useful.

Seppo et al (2009) evaluated an automated FISH assay for detection of 3q gain in liquid cytology samples as a potential tool for risk stratification and triaging. Slides prepared from 257 liquid cytology specimens (97 negative, 135 LSIL and 25 HSIL) were hybridized with a single-copy probe for the chromosome 3q26 region and a probe for the centromeric alpha-repeat sequence of chromosome 7, using standard FISH methods. Using automated analysis, the total number of nuclei and the number of nuclei with greater than 2 signals for 3q26 were determined, using a 20x objective. The nuclei were rank ordered based on number of 3q26 FISH signals. The 800 nuclei with the highest number of signals were scored using both FISH probes and nuclei with increased numbers of 3q signals were enumerated. Analysis of 257 specimens demonstrated that a fully automated FISH scoring system can detect 3q gain in liquid cytology samples. The authors concluded that a fully automated method for determination of 3q gain in liquid cytology may be the assay necessary to implement routine testing; and they stated that additional studies to validate the utility of this technology are needed.

Policht et al (2010) evaluated 35 genomic regions associated with cervical disease and selected those which were found to have the highest frequency of aberration for use as probes in FISH. The frequency of gains and losses using FISH were assessed in these 35 regions on 30 paraffin-embedded cervical biopsy specimens. Based on this assessment, 6 candidate fluorescently labeled probes (8q24, Xp22, 20q13, 3p14, 3q26, CEP15) were selected for additional testing on a set of 106 cervical biopsy specimens diagnosed as normal, CIN1, CIN2, CIN3, and SCC. The data were analyzed on the basis of signal mean, % change of signal mean between histological categories, and % positivity. The study revealed that the chromosomal regions with the highest frequency of copy number gains and highest combined sensitivity and specificity in high-grade cervical disease were 8q24 and 3q26. The cytological application of these 2 probes was then evaluated on 118 ThinPrep samples diagnosed as normal, ASCUS, LSIL, HSIL and cancer to determine utility as a tool for less invasive screening. Using gains of either 8q24 or 3q26 as a positivity criterion yielded specificity (normal + LSIL + ASCUS) of 81.0 % and sensitivity
(HSIL + cancer) of 92.3% based on a threshold of 4 positive cells. The authors concluded that the application of a FISH assay comprised of chromosomal probes 8q24 and 3q26 to cervical cytology specimens confirmed the positive correlation between increasing dysplasia and copy gains and showed promise as a marker in cervical disease progression.

Verri and colleagues (2011) stated that studies have demonstrated a correlation between the gain in 3q26 copy number and the severity and stage of cervical disease progression. A recent study has examined the potential of using a measure of 3q26 gain as a predictor of regression, persistence, or progression of LSIL of the cervix. The case described in this report documented a marked progression in both the severity and extent of this patient’s lesion from atypical squamous cells on cytology to biopsy established CIN2-CIN3 over the span of 1 year. The initial gain of at least 5 copies of 3q26 in only 3 nuclei in this patient’s first cervical smear may be an indication of the significance and sensitivity of this degree of gain, even in a small number of cells at a low level of disease, and may suggest the potential of predicting the progression of the lesion. The subsequent gain of at least 5 copies of 3q26 1 year later in the very large number of 264 nuclei may reflect both the severity and extent of disease progression. Nevertheless, since it is not possible to exclude the possibility that a high-grade CIN already existed at the time of the initial cytology, the presence of the 3q26 gain, even in a small number of cells, may also serve as an indicator of the possible presence of a high-grade lesion in those cervical specimens in which a definitive cytological diagnosis is not or cannot be made. The authors concluded that this case report supported the findings of other investigators on the potential utility of using 3q26 gain in predicting, at an early stage, the progression or non-progression of low-grade pre-neoplastic lesions of the cervix.

Rodolakis and associates (2012) examined if 3q26 gain can predict which LSILs and ASC-USs will progress to HSIL. Liquid cytology specimens of LSIL and ASC-US from 73 women were examined using FISH for the detection of 3q26 gain. All women underwent colposcopy and biopsy at the initial visit and 40 of them with histology showing CIN 1 or HPV infection (koilocytosis) were included in the study. They were re-evaluated with liquid cytology, colposcopy, and biopsy after a median follow-up of 17.5 months. A total of 40 cases were analyzed (31 LSILs and 9 ASCUSs). Of these cases, 8 (20%: 6 LSILs and 2 ASCUSs) were positive and 32 (80%) were negative for 3q26 gain according to FISH. Three of the 8 positive women (38%) progressed to HSIL/CIN 2 or worse, whereas none of the 32 negative women did so. 3q26 gain could predict progression with a negative-predictive value of 100% (95% confidence interval: 89.1% to 100%). In addition, women positive for 3q26 gain had a significantly lower regression rate compared with negative women (p = 0.009). The authors concluded that in this first prospective study, 3q26 gain in LSIL/ASCUS cytology exhibited an impressive negative predictive value for progression to HSIL/CIN 2 or worse. They stated that 3q26 gain may be useful in stratifying patients' risk for progression and possibly alter management and reduce cost of follow-up.

Currently, there is insufficient evidence regarding the clinical value of the oncoFISH cervical test. Furthermore, there are no guidelines from leading medical professional organizations or public health agencies that recommend FISH measurement of 3q26 in cervical cancer screening.

Fluorescence In-Situ Hybridization (FISH) Testing:

Earley et al (2014) examined the diagnostic performance of fluorescence in-situ hybridization (FISH) tests on cervical cytology for precancerous lesions or cancer on cervical histology. These researchers performed a search in MEDLINE, the Cochrane Central Register of Controlled Trials, and Scopus through September 3, 2013. A total of 11
studies examined FISH tests for telomerase RNA component gene (TERC), myelocytomatosis oncogene (MYC), or HPV type 16 or 18 in samples exhibiting ASC-US or LSIL. None examined HPV-positive, cytologically normal samples. These investigators extracted data on the sensitivity and specificity for high-grade cervical intraepithelial neoplasia (CIN 2+ or CIN 3+). Fluorescence in-situ hybridization test probes and thresholds varied across studies. Included populations were convenience samples. Only 1 study testing for TERC specified HPV status. In meta-analysis, FISH for TERC in LSIL (9 studies, 1,082 cases) had a summary sensitivity of 0.76 (95 % CI: 0.63 to 0.85) and a summary specificity of 0.78 (95 % CI: 0.57 to 0.91) for CIN 2+. Fluorescence in-situ hybridization for TERC in ASC-US (3 studies, 839 cases) showed sensitivities ranging from 0.75 to 1.00 and specificities from 0.87 to 0.93 for CIN 2+. For CIN 3+, sensitivity and specificity appeared similar, although a small number of studies preclude firm conclusions. For FISH tests for HPV, these researchers found only few studies with small sample sizes. The authors concluded that the evidence on FISH testing is limited given the small number of studies for each cytology subgroup and the lack of studies in well-defined screening contexts stratifying participants by HPV status.

An AHRQ assessment on fluorescent in situ hybridization or other in situ hybridization of uterine cervical cells to predict precancer and cancer (Uhlig, et al., 2013) concluded: “Overall, the evidence of the analytic and clinical validity of ISH tests in screening for cervical cancer was limited. Further research is needed to standardize techniques, compare clinical validity, thresholds, and combinations across different ISH tests, and compare the clinical utility of combinations of probes as add-on tests to HPV and cytology tests.”

Furthermore, an UpToDate review on “Invasive cervical cancer: Epidemiology, risk factors, clinical manifestations, and diagnosis” (Frumovitz, 2015) and National Comprehensive Cancer Network’s clinical practice guideline on “Cervical cancer” (Version 2.2015) do not mention fluorescence in-situ hybridization/FISH as a diagnostic tool.

CPT Codes / HCPCS Codes / ICD-9 Codes

Annual cervical cancer screening with Papanicolaou (Pap) smears (21 years of age and older):

CPT codes covered if selection criteria are met:

88141 Cytopathology, cervical or vaginal (any reporting system), requiring interpretation by physician

88142 Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; manual screening under physician supervision

88143 with manual screening and rescreening under physician supervision

88147 Cytopathology smears, cervical or vaginal; screening by automated system under physician supervision

88148 screening by automated system with manual rescreening under physician supervision
88150  Cytopathology, slides, cervical or vaginal; manual screening under physician supervision
88152  with manual screening and computer-assisted rescreening under physician supervision
88153  with manual screening and rescreening under physician supervision
88154  with manual screening and computer-assisted rescreening using cell selection and review under physician supervision
+ 88155  Cytopathology, slides, cervical or vaginal, definitive hormonal evaluation (e.g., maturation index, karyopyknotic index, estrogenic index) (List separately in addition to code(s) for other technical and interpretation services)
88164  Cytopathology, slides, cervical or vaginal (the Bethesda System); manual screening under physician supervision
88165  with manual screening and rescreening under physician supervision
88166  with manual screening and computer-assisted rescreening under physician supervision
88167  with manual screening and computer-assisted rescreening using cell selection and review under physician supervision
88174  Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; screening by automated system, under physician supervision
88175  with screening by automated system and manual rescreening or review, under physician supervision

HCPCS codes covered if selection criteria are met:
G0101  Cervical or vaginal cancer screening; pelvic and clinical breast examination
G0123  Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; screening by cytotechnologist under physician supervision
G0124  requiring interpretation by physician
G0141  Screening cytopathology smears, cervical or vaginal, performed by automated system, with manual rescreening, requiring interpretation by physician
G0143  Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; with manual screening and rescreening by cytotechnologist under physician supervision,
G0144  with screening by automated system, under physician supervision
Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with screening by automated system and manual rescreening under physician supervision

Screening cytopathology smears, cervical or vaginal; performed by automated system under physician supervision

Performed by automated system with manual rescreening

Screening papanicolaou smear, cervical or vaginal, up to three smears; by technician under physician supervision

Requiring interpretation by physician

Screening papanicolaou smear; obtaining, preparing and conveyance of cervical or vaginal smear to laboratory

ICD-9 codes covered if selection criteria are met:

042 Human immunodeficiency virus [HIV] disease
079.4 Human papillomavirus [HPV]
090.0 - 099.9 Syphilis and other venereal diseases
131.00 - 131.09 Other urogenital trichomoniasis
179 - 184.9 Malignant neoplasm of female genital organs
198.82 Secondary malignant neoplasm of genital organs
198.6 Secondary malignant neoplasm of ovary
233.1 - 233.39 Carcinoma in situ of female genital organs
236.3 Neoplasm of uncertain behavior of female genital organs
279.00 - 279.9 Disorders involving the immune mechanism [immunosuppression]
616.0 Cervicitis and endocervicitis
616.81 Mucositis (ulcerative) of cervix, vagina, and vulva
622.10 - 622.12 Dysplasia of cervix (uteri)
623.5 Leukorrhea, not specified as infective [abnormal discharge]
623.8 Other specified noninflammatory disorders of vagina [abnormal bleeding]
626.8 Other disorders of menstruation and other abnormal bleeding from female genital tract
795.00 - 795.09 Abnormal Papanicolaou smear of cervix and cervical HPV
E932.2 Adverse effects of ovarian hormones and synthetic substitutes [exposure to DES]
V08  Asymptomatic human immunodeficiency virus [HIV] infection status
V10.40 - V10.44  Personal history of malignant neoplasm of female genital organs
V13.29  Personal history of other genital system and obstetric disorders
V69.2  High-risk sexual behavior [multiple sexual partners]
V71.1  Observation for suspected malignant neoplasm
V72.31  Routine gynecological examination
V76.2  Special screening for malignant neoplasm of cervix

**ICD-9 codes not covered for indications listed in the CPB:**

752.49  Other anomalies of cervix, vagina, and external female genitalia [congenital absence of cervix]
V88.01  Acquired absence of both cervix and uterus
V88.03  Acquired absence of cervix with remaining uterus

*Annual cervical cancer screening with Papanicolaou (Pap) smears (under 21 years of age):*

**CPT codes covered if selection criteria are met:**

88141  Cytopathology, cervical or vaginal (any reporting system), requiring interpretation by physician
88142  Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; manual screening under physician supervision
88143   with manual screening and rescreening under physician supervision
88147  Cytopathology smears, cervical or vaginal; screening by automated system under physician supervision
88148   screening by automated system with manual rescreening under physician supervision
88150  Cytopathology, slides, cervical or vaginal; manual screening under physician supervision
88152   with manual screening and computer-assisted rescreening under physician supervision
88153   with manual screening and rescreening under physician supervision
88154   with manual screening and computer-assisted rescreening using cell selection and review under physician supervision
Cytopathology, slides, cervical or vaginal, definitive hormonal evaluation (e.g., maturation index, karyopyknotic index, estrogenic index) (List separately in addition to code(s) for other technical and interpretation services)

88164  Cytopathology, slides, cervical or vaginal (the Bethesda System); manual screening under physician supervision

88165  with manual screening and rescreening under physician supervision

88166  with manual screening and computer-assisted rescreening under physician supervision

88167  with manual screening and computer-assisted rescreening using cell selection and review under physician supervision

88174  Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; screening by automated system, under physician supervision

88175  with screening by automated system and manual rescreening or review, under physician supervision

HCPCS codes covered if selection criteria are met:

P3000  Screening papanicolaou smear, cervical or vaginal, up to three smears; by technician under physician supervision

P3001  requiring interpretation by physician

HCPCS codes not covered for indications listed in the CPB (routine/preventive):

G0123  Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; screening by cytotechnologist under physician supervision

G0124  requiring interpretation by physician

G0141  Screening cytopathology smears, cervical or vaginal, performed by automated system, with manual rescreening, requiring interpretation by physician

G0143  Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; with manual screening and rescreening by cytotechnologist under physician supervision

G0144  with screening by automated system, under physician supervision

G0145  Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with screening by automated system and manual rescreening under physician supervision

G0147  Screening cytopathology smears, cervical or vaginal; performed by automated system under physician supervision
Cervical Cancer Screening and Diagnosis

G0148 performed by automated system with manual rescreening
Q0091 Screening papanicolaou smear; obtaining, preparing and conveyance of cervical or vaginal smear to laboratory

ICD-9 codes covered if selection criteria are met:

042 Human immunodeficiency virus (HIV) disease
079.53 Human immunodeficiency virus type 2 [HIV-2]
180.0 - 180.9 Malignant neoplasm of cervix uteri
233.1 Carcinoma in situ of cervix uteri
622.10 - 622.12 Dysplasia of cervix uteri
647.60 - 647.64 Other viral diseases complicating pregnancy, childbirth, or the puerperium
795.00 Abnormal glandular Papanicolaou smear of cervix
795.01 Papanicolaou smear of cervix with atypical squamous cells of undetermined significance (ASC-US)
795.02 Papanicolaou smear of cervix with atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H)
795.03 Papanicolaou smear of cervix with low grade squamous intraepithelial lesion (LGSIL)
795.04 Papanicolaou smear of cervix with high grade squamous intraepithelial lesion (HGSIL)
795.06 Papanicolaou smear of cervix with cytologic evidence of malignancy
795.71 Nonspecific serologic evidence of human immunodeficiency virus (HIV)
V01.79 Contact with or exposure to other viral diseases
V08 Asymptomatic human immunodeficiency virus (HIV) infection status
V10.41 Personal history of malignant neoplasm of cervix uteri
V13.22 Personal history of cervical dysplasia
V42.0 - V42.9 Organ or tissue replaced by transplant

ICD-9 codes not covered for indications listed in the CPB:

V72.31 Routine gynecological examination [pap smear screening]
V76.2 Special screening for malignant neoplasm of cervix [pap smear screening]

HPV testing in women 21 to 30 years of age (not covered under 21 years of age):

CPT codes not covered for indications listed in the CPB:
Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), low-risk types (eg, 6, 11, 42, 43, 44) [not covered for any age]

**ICD-9 codes covered if selection criteria are met (all-inclusive):**

- **795.00** Abnormal glandular Papanicolaou smear of cervix [atypical cervical glandular cells]
- **795.01** Papanicolaou smear of cervix with atypical squamous cells of undetermined significance (ASC-US)
- **795.02** Papanicolaou smear of cervix with atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H)
- **795.03** Papanicolaou smear of cervix with low grade squamous intraepithelial lesion (LGSIL)
- **795.04** Papanicolaou smear of cervix with high grade squamous intraepithelial lesion (HGSIL)
- **795.05** Cervical high risk human papillomavirus (HPV) DNA positive
- **V72.32** Encounter for Papanicolaou cervical smear to confirm findings of recent normal smear following initial abnormal smear

**Cervicography or speculoscopy (Pap-Sure):**

*There is no specific code for cervicography or speculoscopy:*

**ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):**

- 180.0 - 180.9 Malignant neoplasm of cervix uteri
- 233.1 Carcinoma in situ of cervix uteri
- **V76.2** Special screening for malignant neoplasm of cervix

**Methylation markers for cervical cancer screening:**

**ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):**

- 180.0 - 180.9 Malignant neoplasm of cervix uteri
- 233.1 Carcinoma in situ of cervix uteri
- **V76.2** Special screening for malignant neoplasm of cervix

**Ikonisys oncoFISH cervical test:**

**CPT codes not covered for indications listed in the CPB:**

- 88275 Molecular cytogenetics; interphase in situ hybridization, analyze 100-300 cells
- 88291 Cytogenetics and molecular cytogenetics, interpretation and report
- 88367 - 88377 Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen
ICD-9 codes not covered for indications listed in the CPB:

180.0 - 180.9  Malignant neoplasm of cervix uteri
233.1  Carcinoma in situ of cervix uteri
V76.2  Special screening for malignant neoplasm of cervix

The above policy is based on the following references:

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16. Centers for Disease Control and Prevention (CDC), National Center for Chronic Disease Prevention and Health Promotion. Cervical cancer and Pap test


34. American College of Obstetricians and Gynecologists (ACOG), Committee on Gynecologic Practice. Cervical cytology screening. ACOG Technology Assessment


74. Danish Centre for Evaluation and Health Technology Assessment (DACEHTA). The use of liquid based cytology (LBC) and conventional Pap smear (CPS) for cervical screening in Denmark. A health technology assessment - summary. Copenhagen, Denmark: DACEHTA; 2005.


96. Davis AJ. Clinical usefulness of HPV testing and genotyping. Journal Watch Women's Health, April 1, 2009.


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