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NEW GUIDELINES DEMONSTRATE GREATER ROLE FOR HPV TESTING IN CERVICAL CANCER SCREENING

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In May of 2012, the American Cancer Society (ACS), American Society for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology (ASCP) published joint consensus guidelines for the Prevention and Early Detection of Cervical Cancer[1]. In November of 2012, the American College of Obstetricians and Gynecologists (ACOG) also released new guidelines for cervical cancer screening[2]. These guidelines are primarily in agreement with the U.S. Preventive Services Task Force's (USPSTF) most current recommendations for cervical cancer screening[3]. These recommendations are for *screening only* and do not relate to other uses of cytology and human papillomavirus (HPV) testing such as follow-up of patients with untreated disease, post-colposcopic or immediate post-treatment follow-up, or surveillance. Testing at more frequent intervals may be appropriate under such circumstances.

In general, newly recommended screening intervals in general extend the recommended testing interval to every 3 years (cytology alone) for women 21-29 years of age, and every 5 years (HPV and cytology "co-testing") for women 30-65 years old. The use of co-testing in women age 30-65 allows for an extended test interval (5 years) and provides better sensitivity for ≥CIN 3 (cervical intraepithelial neoplasia grade 3) than screening by cytology alone[4-6]. Co-testing is not recommended for women 21-29 years old because of the high prevalence of HPV in this age group. However HPV testing can be useful for these patients if the cytology results identify atypical cells of undetermined significance (ASC-US).

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Screening more frequently than these recommendations not only offers no benefit but has significant risks. Both the USPSTF and ACS/ASCCP/ASCP state that screening more often than every 3 years causes significant harm in terms of potential short-term psychological stress, additional procedures, assessment and treatment of transient lesions, vaginal bleeding and infection, and potential adverse pregnancy outcomes.

The new guidelines also recommend discontinuation of screening in women >65 years if there is a documented negative screening history. Adequate negative screening results are defined as three consecutive negative cytology results or two consecutive negative co-test results within the past 10 years, and the most recent test performed within 5 years. Women with a history of ≥CIN 2 or adenocarcinoma in situ should continue screening for 20 years after spontaneous regression or appropriate management even if it extends the screening past age 65 years.

Future evidence may show that less frequent screening is appropriate for women who have received the HPV vaccine, but given the limitations of current research and the low vaccination coverage among U.S. adolescents prior to first intercourse, the screening protocol is now the same for both vaccinated and unvaccinated women.

Summary of 2012 ACOG, ASCP, ACS and ASCCP Cervical Cancer Screening Guidelines

Population Age	Screening Recommendation	Comments
Less than 21 years	No cervical cancer screening of any kind	HPV testing should not be used for screening or ASC-US reflex in this age group
21-29 years	Thin Prep primary screening cytology alone every 3 years (acceptable)	Routine HPV "co-testing" is not recommended in this age group; in cases of ASC-US cytology, HPV testing is recommended
30-65 years	HPV and cytology "co-testing" every 5 years (preferred); cytology alone every 3 years (acceptable)	Screening by HPV testing alone is not recommended for most clinical settings
Over 65 years	No screening following adequate history of negative prior screening	Women with history of ≥CIN 2 should continue screening for at least 20 years
After hysterectomy	No screening if no previous history of ≥CIN 2	Continue screening (cytology) if there is history of ≥CIN 2 in the past 20 years or cervical cancer ever
HPV vaccinated	Follow age-specific recommendations (same as unvaccinated women)	

BACKGROUND

The widespread use of high-quality screening with cytology (Papanicolaou [Pap] testing) in the U.S. has markedly reduced mortality from squamous cell cervical cancer, which comprises 80% to 90% of

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cervical cancers. Cervical cancer, once the most frequent cause of cancer death in women, now ranks 14th for cancer deaths in the U.S.[7]. Still, in 2012, it was estimated that 12,170 cases of invasive cervical cancer would be diagnosed and an estimated 4,220 women would die[7]. It is now understood that a persistent cervical infection with a high-risk HPV genotype is necessary for the development of cervical cancer and its immediate precursor lesion, CIN 3. Significant evidence exists to support the causal relationship between the duration of HPV infection and a woman's risk of cervical cancer[8].

Figure 1 depicts the progression of cervical neoplasia to cancer with HPV persistence. Early HPV infections can be recognized as cytologic or histologic abnormalities, most often CIN 1[9]. Most of these infections will spontaneously resolve by host immunity and their corresponding cellular abnormalities will revert back to normal. When HPV infections persist, cervical precancers, such as CIN 3, can arise from genetic instability and ultimately clonal expansion of highly transformed cells. Regression is much less likely to occur among these later stage lesions. Factors leading to HPV persistence include: HPV genotype (greatest risk HPV 16), increasing age, smoking, mutagens, immunosuppression, inflammation, hormones, and genetic factors.

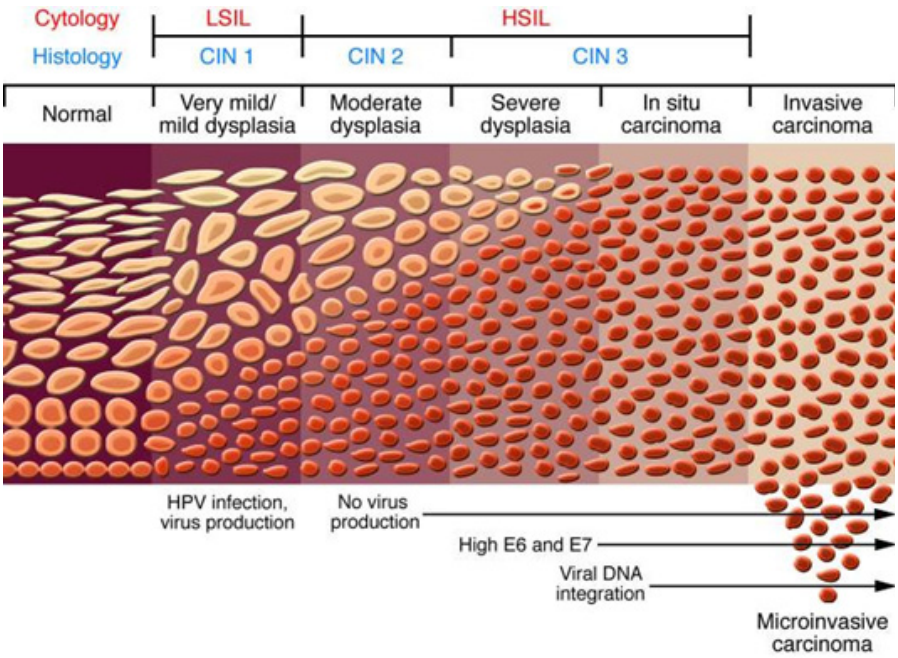

Figure 1. Stages of HPV Infection and Progression of Cervical Dysplasia to Cancer[1]

Specimen:
Obtain an adequate sampling from the ectocervix using a plastic spatula. Immediately rinse the spatula into the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula. Obtain an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate 1/4 to 1/2 turn in one direction. DO NOT OVER-ROTATE. Immediately rinse the brush in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush. Tighten the cap so that the torque line on the cap passes the torque line on the vial. Record the patient name and ID number on the vial and the patient information and medical history on the cytology requisition or enter this information when order is placed.
For HPV Orders see Information section below.

Minimum:
For HPV co-testing: The ThinPrep pap is processed first. The Thin Prep vial must contain at least 1.0 mL of residual PreservCyt solution for HPV testing.

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Rejection Criteria:
For HPV testing: Insufficient sample (less than 1mL), inadequate sample, or sample depleted without obtaining an interpretive result.

Storage:
Local - Room Temperature

Available:
Pap test is performed Monday through Friday, days only.
HPV test is batched daily, Monday through Friday. Analytical time is 24 hours.

Qualitative Interpretation:
No interpretations are available at this time.

For interpretive questions contact:
Cytology: George Rupp, MD at ext. 1-6113 or 715-221-6113
HPV: Timothy Uphoff, PhD at ext. 1-6189 or 715-221-6189

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NEW HPV TEST METHODOLOGY PROVIDES BETTER SPECIFICITY FOR CERVICAL CANCER

TIMOTHY UPHOFF, PHD, DABMG, MLS (ASCP)CM

Marshfield Labs is proud to offer a new method for HPV screening. Beginning February 18th, 2013, we will use the Aptima® HPV (AHPV) assay that detects oncogenic viral RNA. HPV DNA testing using the Digene Hybrid Capture 2 (HC2) assay will be discontinued. Detection of viral RNA using AHPV has been shown to be more specific and as sensitive for cervical intraepithelial neoplasia (CIN) grades 2 and 3 as HC2. This superior specificity should result in fewer samples being incorrectly identified as precancerous than with the HC2 assay. The new AHPV assay also requires just 1 mL of PreservCyt specimen as opposed to the 4 mL needed for the HC2 assay. The new AHPV assay will be performed daily Monday through Friday and thus provide better turnaround times for results. Test ordering will remain the same. The test comments and Test Reference Manual entries will reflect the new methodology.

HPV is a common sexually transmitted DNA virus comprised of more than 100 genotypes. Almost all cervical cancers are caused by persistent infection with one or more of 14 high-risk human papillomavirus (HR-HPV) types. The longer an infection with HR-HPV persists, the less likely it is to resolve and the higher a woman's risk of developing cervical cancer.

The HC2 assay detects HPV deoxyribonucleic acid (DNA) from 13 of the most common HR-HPV genotypes (16/18/31/33/35/39/45/51/52/56/58/59/68). The specificity of this test for the detection of high-grade cervical disease is influenced by the natural history of HPV infection, which is often transient in nature and can resolve spontaneously without resulting in invasive cervical disease. HPV DNA can be found in cervical samples very early during the course of infection and has been shown to decrease in cervical cancer. (Fig 1)

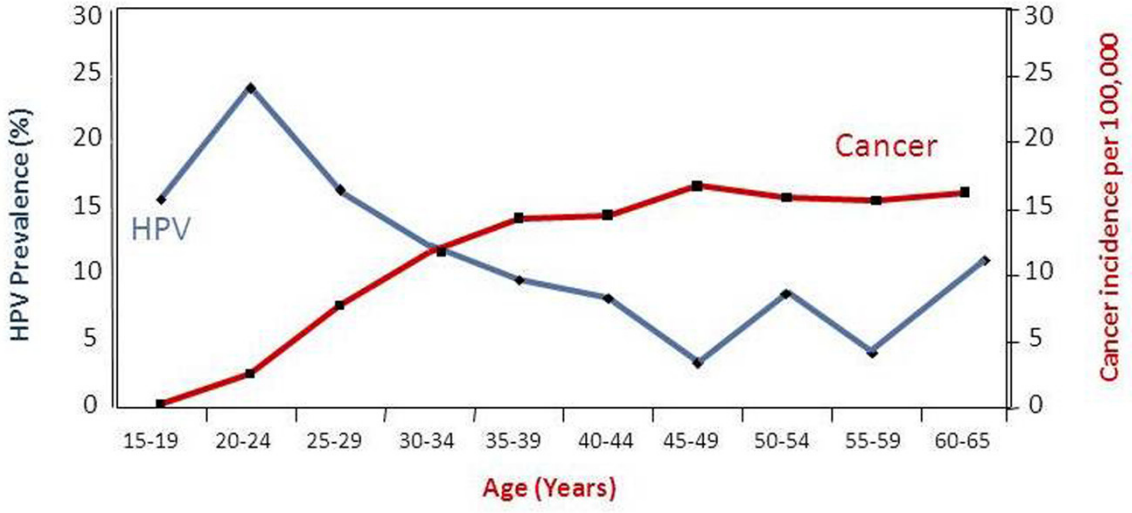

Figure 1. HPV and Cervical Cancer Prevalence
National Cancer Institute SEER data 1990-1994 and Melkert et al., 1993. Int J Canc 53:919.

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Because even HR-HPV infections are very common and because most women will clear HPV infections within 6 to 12 months, the presence of HPV DNA alone does not mean that cervical dysplasia or cervical cancer is present. Hence, many women with normal cervical cytology results are found to be positive for HR-HPV DNA and yet are HPV negative on subsequent tests due to natural resolution of their infection. This is one of the primary reasons HPV tests are not recommended as primary screening in women less than thirty years old and why DNA may not be the ideal screening target for persistent HPV infection. A more efficacious approach for detection of cervical disease is to target those oncogenic elements of HPV that foster persistent viral infection and cellular transformation.

The AHPV test detects the corresponding E6/E7 oncogenic messenger ribonucleic acids (mRNAs) from 14 HR-HPV genotypes (16/18/31/33/35/39/45/51/52/56/58/59/66/68). The E6 and E7 genes from HR-HPV genotypes are known oncogenes. Proteins expressed from E6/E7 mRNAs alter cellular p53 and retinoblastoma protein functions, leading to disruption of cell-cycle check points and cell genome instability. The E6 and E7 mRNAs are expressed at higher levels during the later stages of infection (more likely to be persistent) and in cervical cancer. Recent studies have repeatedly demonstrated that the AHPV assay is more specific than the DNA-based HC2 test among ASC-US reflex and co-testing sample types for the detection of ≥CIN 2 likely to indicate persistent infection and clinically significant cervical dysplasia[2-7].

In summary, adoption of the new AHPV technology will provide higher specificity without a loss of sensitivity for ≥CIN 2, reduce the amount of PreservCyt sample required from 4 to 1 ml, and allow detection of the additional HR-HPV genotype 66. This methodology change should result in improved turnaround times; fewer samples being rejected due to insufficient quantity; and reduce the anxiety, discomfort and costs associated with additional diagnostic and treatment procedures ensuing from the lower specificity of the old HC2 assay.

Specimen:
Cervical specimens can be stored in the PreservCyt Solution vials for 30 days at 2°C to 30°C prior to transfer to APTIMA Specimen Transfer tubes. Once the PreservCyt Solution liquid Pap specimen is transferred to the APTIMA Specimen Transfer tube, the specimen must be tested within 30 days when stored at 2°C to 8°C or 14 days at 15°C to 30°C. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after transfer.

Minimum:
Thin prep vials must contain at least 1.0 mL PreservCyt solution.

Rejection Criteria:
Insufficient sample (less than 1 mL), inadequate sample, or sample depleted without obtaining an interpretable result.

Available:
Test is batched daily, Monday through Friday. Analytical time is 24 hours.

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Qualitative Interpretation:

- Negative result
- Positive result
- Indeterminate with commentary recommending repeat testing if clinically indicated

CPT Code:

HPV: Nucleic Acid Method: 87621

For questions contact:

Timothy Uphoff, PhD at ext. 1-6189 or 715-221-6189

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