AACC Guidance Document on Cervical Cancer Detection: Screening, Surveillance, and Diagnosis

Authors:

*Yusheng Zhu, PhD, DABCC, FAACC
Departments of Pathology and Laboratory Medicine, Pharmacology
Pennsylvania State University College of Medicine
Hershey, PA

Sarah Feldman, MD, MPH
Departments of Obstetrics, Gynecology, and Reproductive Biology
Brigham and Women’s Hospital, Harvard Medical School
Boston, MA

Shuk On Annie Leung, MD
Department of Obstetrics and Gynecology
McGill University Health Centre, McGill University
Montreal, QC

Michael H. Creer, MD
Departments of Pathology and Laboratory Medicine
Pennsylvania State University College of Medicine
Hershey, PA

Joshua Warrick, MD
Departments of Pathology and Laboratory Medicine
Pennsylvania State University College of Medicine
Hershey, PA

Williams, Nicole, MD
Departments of Pathology and Laboratory Medicine
Pennsylvania State University College of Medicine
Hershey, PA

Stephen Mastorides, MD
Department of Pathology and Laboratory Medicine Service
James A. Haley Veterans' Hospital
Tampa, Fl

*To whom correspondence should be addressed: yzhu@pennstatehealth.psu.edu
List of abbreviations

American Cancer Society (ACS)
American Society for Colposcopy and Cervical Pathology (ASCCP)
adenoacarcinoma in situ (ACIS)
atypical glandular cells (AGC)
atypical squamous cells – cannot exclude high grade squamous intraepithelial lesion (ASC-H)
atypical squamous cells of undetermined significance (ASC-US)
Centers for Disease Control and Prevention (CDC)
Food and Drug Administration (FDA)
high-grade squamous intraepithelial lesion (HSIL)
high risk human papilloma virus (hrHPV)
human papilloma virus (HPV)
Kaiser Permanente Northern California (KPNC)
liquid-based cytology (LBC)
low-grade squamous intraepithelial lesion (LSIL)
negative for intraepithelial lesion or malignancy (NILM)
negative predictive value (NPV)
positive predictive value (PPV)
United States Preventative Services Task Force (USPSTF)
Table of Contents

Abstract ....................................................................................................................................... 5
Background ............................................................................................................................... 5
Content ...................................................................................................................................... 5
Summary ................................................................................................................................... 5

Introduction ................................................................................................................................. 7

Cervical Cancer Screening Tests ......................................................................................................... 9
HPV Test ......................................................................................................................................... 9
Cervical Cytology Test ..................................................................................................................... Error! Bookmark not defined.1
Self-collected Vaginal Specimen for HPV Screening Test ............................................................ Error! Bookmark not defined.4

Screening Strategies ....................................................................................................................... Error! Bookmark not defined.9
High-risk HPV Testing Alone .......................................................................................................... 19
High-risk HPV and Cervical Cytology Co-testing ........................................................................... Error! Bookmark not defined.0
Cervical Cytology Alone .................................................................................................................. Error! Bookmark not defined.
Comparison of Screening Strategies ............................................................................................... Error! Bookmark not defined.2

Beginning and Ending of Screening .................................................................................................. 23

Criteria for Routine Screening ........................................................................................................ 25

Surveillance Using Risk-based Guidelines ....................................................................................... 27

Diagnostic Testing/Evaluation .......................................................................................................... 29
Colposcopy ...................................................................................................................................... 29
Biopsy .............................................................................................................................................. 30

The Ideal Laboratory Report ......................................................................................................... 32
Summary of Recommendations and Future Directions .................................................................... 32

Figure Legend ................................................................................................................................. 35
Tables .............................................................................................................................................. 36
References ...................................................................................................................................... 38
Abstract

Background
Persistent genital infection with high-risk human papilloma virus (HPV) types causes the vast majority cases of cervical cancer, which is the fourth most common cancer in women. The global strategy for the elimination of cervical cancer includes prevention, screening, and treatment. Early screening, ongoing surveillance, and accurate diagnosis are crucial for the elimination of cervical cancer. New screening guidelines for testing in asymptomatic healthy populations and management guidelines for managing abnormal results have been published by professional organizations and societies.

Content
This guidance document addresses key questions related to cervical cancer screening and management such as currently available cervical cancer screening tests and the testing strategies for cervical cancer screening. The guidance document introduces the most recently updated screening guidelines regarding age to start screening, age to stop testing, and frequencies of routine screening as well as risk-based management guidelines for screening and surveillance. This guidance document also summarizes the methodologies for the diagnosis of cervical cancer including biopsy and colposcopy. In addition, we propose a report template for HPV and cervical cancer detection to facilitate interpretation of results and clinical decision-making.

Summary
Currently available cervical cancer screening tests include high-risk HPV testing and cervical cytology screening. There are five FDA approved HPV molecular assays. An emerging trend in cervical cancer screening is the use of self-collected cervical specimens for genotyping of HPV, although the United States Food and Drug Administration (FDA) has not yet approved any self-collection methods. The strategies for cervical cancer screening can be primary HPV screening, co-testing with HPV testing and
cervical cytology, and cervical cytology alone, but each strategy has different sensitivity, specificity, and costs. The age to start routine screening is 21 recommended by the United States Preventative Services Task Force (USPSTF) and 25 by the American Cancer Society (ACS) and the age to end screening is 65. The screening intervals are 3 years or 5 years based on age and screening strategies. However, the above routine screening guidelines do not apply to certain patients with special conditions. The new American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines recommend variable frequencies of screening and surveillance based on risk, which relies on the patient’s age, clinical history, and prior as well as current HPV testing and cytology results. In order to implement the above guidelines, the ideal lab report for cervical cancer testing should include the indication for the test: screening, surveillance, or diagnostic, type of test: primary HPV screening with specific assay information, co-testing, or cytology along, clinical history of the patient, and prior testing results.
Introduction

Cervical cancer is a group of invasive epithelial neoplasms of the cervix, all of which have metastatic potential. These comprise 70% squamous cell carcinoma, 25% adenocarcinoma, and the remainder are rare tumors, such as small cell carcinoma. The vast majority of cervical cancers are driven by infection with high-risk human papilloma virus (hrHPV), most notably HPV types 16 and 18, which are responsible for about 70% of cervical cancers. HPV is a double-stranded DNA virus with over 200 known genotypes. In addition to types 16 and 18, other clinically relevant high-risk types include 58, 33, 45, 31, 52, 35, 59, 39, 51, 56, 66, and 68 in order of worldwide frequency. Several biological steps must take place for infection with hrHPV to progress to cervical cancer. The earliest and most obvious is HPV acquisition, which is often spontaneously cleared. This can be seen histologically as the koilocytotic atypia characteristic of low grade squamous intraepithelial lesions. If HPV infection persists, viral DNA integrates into the host genome, inducing expression of high levels of oncogenic viral proteins, such as E6- and E7-encoded oncoproteins, which facilitate degradation of the host tumor suppressor proteins p53 and RB1, respectively. These are seen histologically in either the extensive basaloid atypical characteristic of high grade squamous intraepithelial lesions or the atypical non-invasive glands of adenocarcinoma in situ. Over time, these cells acquire somatic driver mutations and invade. The most common somatic mutations involve members of the PI3K/AKT pathway, specifically activating mutations in PIK3CA and copy number losses or inactivating mutations of PTEN, seen in both squamous cell carcinoma and adenocarcinoma. As the disease progresses, invasive cervical cancers are capable of local invasion as well as distant metastasis and patient mortality.

Histopathologically, the precursor lesions of squamous cell carcinoma of the cervix are termed cervical intraepithelial neoplasia (CIN), which divides cervical cancer precursors into 3 groups: CIN 1, 2, and 3, corresponding to mild dysplasia, moderate dysplasia, and severe dysplasia/carcinoma in situ, respectively. For exfoliative cytology specimens, cervical cancer precursors are classified as "low-grade
squamous intraepithelial lesion (LSIL)” for lesions histopathologically classified as koilocytic atypia and CIN 1 and “high-grade squamous intraepithelial lesion (HSIL)” for lesions called CIN 2 and CIN3 in histopathology. For histopathological reporting, it has been suggested using LSIL (CIN1) and HSIL (CIN 2 and CIN 3) 4. Both terminology systems are currently in use. The 2019 ASCCP guidelines recommend that the histopathology report should include CIN 2 or CIN 3 qualifiers, i.e., HSIL (CIN 2) and HSIL (CIN 3) 7.

Approaches for cervical cancer screening include primary cervical hrHPV testing, co-testing of HPV and cervical cytology, and cytology screening alone. These approaches have variable sensitivity and specificity, which will be detailed in the later sections 8.

In addition to screening, cervical cancer tests are used in surveillance as well as diagnosis of cervical cancer. It is important to distinguish between screening, surveillance, and diagnostic testing. Screening refers to testing for disease among individuals who are asymptomatic and have not been tested previously or have normal prior results (i.e., low risk). Surveillance is the interval testing among individuals who had a prior abnormal result, with or without treatment. Recent evidence indicates that an individual’s risk of developing cervical precancer or cancer can be estimated using current screening test results and previous screening test and biopsy results, while considering personal factors such as age and immunosuppression 7. These data form the basis of the 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors, which will be discussed in later sections 7. When an individual’s history is unknown, that individual’s risk falls somewhere in between screening and surveillance. It is important to note that an unknown history is itself a risk factor for development of cervical precancer and cancer 9. Finally, diagnosis refers to testing when an individual presents with symptoms (e.g., bleeding, discharge, pain). The distinction between these three categories is important because although similar tests (i.e., HPV testing, cytology) might be utilized, the subsequent interpretation of risk to guide management is different.
This guidance document introduces currently available cervical cancer screening tests, testing strategies, and the most recently updated screening guidelines as well as risk-based management guidelines. In addition, we propose a report template for HPV and cervical cancer detection to facilitate interpretation of testing results and clinical decision-making.

### Cervical Cancer Screening Tests

Currently, cervical cancer screening tests include HPV testing and cervical cytology in clinical settings. Recently, it has been proposed that self-collected vaginal specimens are suitable for HPV testing, although the FDA has not yet approved any self-collection methods.

#### HPV test

There are five FDA approved HPV molecular assays.\(^1\)\(^-\)\(^4\)

1. The HPV assays with the FDA approval for primary cervical cancer screening

   a. Roche cobas Assay (Roche Molecular Systems, Inc, Roche Diagnostics, Indianapolis, Indiana, USA)

   The FDA approved the assay in 2011 for reflex HPV testing and co-testing with cytology. In 2014, it was approved for primary cervical cancer screening but on Hologic ThinPrep specimens (see the following “cervical cytology test” section) only. The DNA real-time qPCR-based assay targets L1 gene of HPV. It covers 14 high-risk types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 with genotyping of 16 and 18. The beta-globin gene serves as an internal control. Sensitivity for detecting CIN 2/3 ranges from 90.5-97% and specificity 13-67.6%.\(^5\)\(^-\)\(^7\)

   b. Becton Dickinson BD Onclarity Assay (Becton, Dickinson and Company, Sparks, Maryland, USA)
The FDA approved this assay in 2018 for reflex HPV testing and co-testing with cytology as well as primary cervical cancer screening, but on SurePath Specimens (see the following “cervical cytology test” section) only. The DNA PCR-based assay targets E6/E7 genes. It covers 14 high-risk types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 with genotyping of 16, 18, and 45. The beta-globin gene serves as an internal control. Sensitivity for CIN2/3 ranges from 94-98% and specificity 17-31%.

2. The HPV assays approved for reflex and co-testing with cytology
   a. Digene Hybrid Capture II Assay (Qiagen, Hilden, Germany)

   The FDA approved this assay in 2001 for reflex HPV testing and co-testing with cytology. The DNA signal amplification (non-PCR) assay utilizes a full genome probe. It covers 13 high-risk types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. There is no build-in internal control. Sensitivity for CIN 2/3 ranges from 80.8-98% and specificity 21-70.6%.

   b. Hologic Cervista Assay (Hologic Inc., Marlborough, Massachusetts, USA)

   The FDA approved this assay in 2009 for reflex HPV testing and co-testing with cytology. The DNA signal amplification (non-PCR) assay targets L1, E6, and E7 genes. Cervista HPV HR assay covers 14 high-risk types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The Cervista HPV 16/18 assay tests HPV 16 and 18 only. The HIST2H2BE gene serves as an internal control. Sensitivity for CIN2/3 ranges from 77-92.8% and specificity 44.2-72.7%.

   c. Hologic (Gen Probe) Aptima Assay (Hologic Inc., Marlborough, Massachusetts, USA)

   The FDA approved the assay in 2011 for reflex HPV testing and co-testing with cytology. The mRNA transcription-mediated amplification assay targets E6/E7 genes. It covers 14 high-risk types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 with separate genotyping of 16, and 18/45 by the
Aptima 16,18/45 genotype assay. The HPV16 E6/7 transcript serves as an internal control. Sensitivity for CIN2/3 ranges from 87.5-98% and specificity 30-78%\textsuperscript{10, 15, 17, 19, 24}.

**Cervical cytology test**

Cervical cytology screening, also known as the Pap smear test, involves the direct sampling of the transformation zone between the ectocervix and endocervix. The traditional Pap test involves collecting cells from the vagina or cervix, smearing them onto a slide at the patient bedside, and evaluating the slide in the laboratory under a microscope. A significant advance in cervical cancer screening is the introduction of liquid-based cytology (LBC). Currently, LBC is utilized in over 90% of Pap tests in the United States and has higher sensitivity for high-grade lesions than conventional smears with lower false negativity rate\textsuperscript{25-28}. LBC was first approved by the FDA in 1996 with the ThinPrep\textsuperscript{®} Pap test (Hologic Inc., Marlborough, Massachusetts, USA). The FDA approved a second test in 1999, the BD SurePath\textsuperscript{™} Pap test (BD Diagnostic, Burlington, North Carolina, USA).

The ThinPrep\textsuperscript{®} Pap test sample is collected by a clinician with a spatula and a brush and rinsed in a ThinPrep vial prefilled with a methanol-based fixative (PreservCyt). The vial is sent to the laboratory for processing on the ThinPrep Processor, an automated slide preparation unit that uses a liquid-based vacuum filtration method to disperse, filter, and transfer the specimen onto a slide using air pressure for adherence resulting in a uniform monolayer of cells. The residual specimen is available for other diagnostic tests, for example, HPV testing\textsuperscript{26, 27}.

A clinician may also collect the SurePath\textsuperscript{™} Pap test sample and place a broom-like device with a detachable head in a collection vial with an ethanol-based fixative (CytoRich) and send it to the laboratory for processing. The cells are centrifuged, suspended within a sucrose density gradient, and transferred to slide via gravity for adherence in a monolayer. The residual specimen is available for other diagnostic tests, for example, HPV testing\textsuperscript{26, 27}.
Both ThinPrep and SurePath Pap tests are approved for primary screening by automated imagers. The ThinPrep Imaging System (TIS, Hologic Corp., Malborough, MA) is used with ThinPrep slides and the FocalPoint GS Primary Screening System (Focal Point GS, BD Diagnostics, Burlington, NC) can be used with SurePath, Thinprep, and conventional Pap tests. The automated imagers have slightly increased sensitivity over manual screening alone; however, there is a slight decrease in specificity\textsuperscript{29-32}.

Evaluation of slides by automated screening or manual screening by cytotechnologist or cytopathologist is considered primary review. All abnormal cervical Pap smears must have a secondary review by a cytopathologist. The reporting of results follows the Bethesda System for Reporting Cervical Cytology (Table 1).

The spectrum of lesions in cervix caused by HPV ranges from premalignant dysplasia to invasive carcinoma. Low-grade dysplasia or LSIL in cytology may be indicative of HPV infection that can be transient with regression within two years\textsuperscript{33}. Cytomorphologic changes of LSIL in Pap test are similar to those identified as CIN 1 in cervical tissue biopsies. Changes of LSIL can range from viral cytopathic change (koilocytosis) to morphologic changes of low-grade dysplasia. Pap test with atypical changes involving squamous cells that fall short of criteria for LSIL can be reported as atypical squamous cells of undetermined significance (ASC-US). The ASC-US/LSIL ratio is a laboratory quality indicator and can highlight ASC-US overuse.

The cytomorphology of HSIL is similar to CIN 2 and CIN 3 in tissue biopsy. Squamous cells with high-grade dysplasia are smaller than those with low-grade dysplasia. They have high nuclear to cytoplasmic ratio, marked nuclear membrane irregularity, and can have nuclear hyperchromasia. Atypical changes that fall short of criteria for HSIL can be reported as atypical squamous cells – cannot exclude high grade squamous intraepithelial lesion (ASC-H).
Squamous cell carcinoma is the most common malignancy of the cervix. Tissue architecture is not present in a cytology sample; however, other malignant features are present. Tumor cells can have similar cytomorphology as those seen in HSIL; however, these cells also may have increased pleomorphism and dense eosinophilic cytoplasm in keratinizing squamous cell carcinoma. Additionally, an associated tumor diathesis comprised of necrotic debris and degenerated blood clings to cells in liquid-based cytology.

Cervical cytology is a screening test for squamous lesions; however, atypical glandular cells (AGC) and changes suggestive of glandular malignancies can also be identified. There is lower sensitivity for glandular lesion detection by cytology due to several issues, including cellular degeneration, interpretation, and sampling. AGC can be endocervical or endometrial; however, it may not be possible to identify the origin based on cytology alone. AGC in Pap test samples may correlate to reactive inflammatory lesions, extension of squamous dysplasia into endocervical glands, in situ or invasive adenocarcinoma in tissue biopsy specimens. Cytomorphologic changes of atypia include nuclear enlargement with overlapping, increased nuclear to cytoplasmic ratio, nucleoli, and mild hyperchromasia. These changes are beyond those seen in reactive glandular epithelium; however, they fall short of the criteria for malignancy.

Changes suggestive of endocervical adenocarcinoma in situ (ACIS) include crowded hyperchromatic glandular cells in pseudostratified strips with occasional gland-like architecture or rosettes. Additionally, there can be peripheral feathering and prominent nucleoli. These features may be subtle, and the interpretation of ACIS can be difficult. Challenging cases can be interpreted as atypical endocervical cells, favor neoplastic. Adenocarcinoma has more prominent malignant cytomorphic features and commonly associated degenerated blood and necrosis. Adenocarcinoma can be endocervical, endometrial, or rarely metastatic in confirmatory tissue biopsy sections. Glandular and squamous abnormalities may be present in a single Pap test, and each interpretation should be reported.
Publication of the 2019 ASCCP consensus guidelines in April 2020 introduced a change from test-result management to risk-based guidelines. The new guidelines change one management for all with similar diagnoses and varied risk levels to patient management based on a combination of the patient’s level of risk, previous clinical history, and current screening test results. Risk levels from tables of risk variables from the fifteen-year Kaiser Permanente Northern California cervical cancer screening study were utilized for comparison to identify a clinical action threshold for patient management decisions. Generally, patients at higher risk will undergo more frequent cervical carcinoma screening, followed by colposcopy and treatment as needed while those at lower risk will have less frequent surveillance. Therefore, patients with similar Pap test results may be managed differently based on their risk for developing high-grade dysplasia.

In summary, the Pap test is a screening test for precancerous changes of the cervix. Screening intervals, management, and treatment are risk-based, taking into consideration the age of the patient, current cytology, pathology and HPV results, previous test results, age and immune status, which will be discussed later.

**Self-collected vaginal specimens for HPV Screening Test**

An emerging trend in cervical cancer screening is the use of self-collected vaginal specimens for genotyping of HPV.

1. Stability of self-collected vaginal specimens for HPV Genotyping

Self-collected vaginal specimens for HPV genotyping are generally collected using a “dry” or lavage-based HPV self-sampling approach, most commonly using a brush/broom. A number of studies have demonstrated excellent recovery and stability of HPV DNA from exfoliated cervical cells attached to the hydrophobic material used for manufacture of the collecting brush/broom. In one study, HPV DNA stability was evaluated with exfoliated cells remaining on the brush/broom in a “dry” state with
specimens stored at different temperatures ranging from 4 - 30 °C for up to 32 weeks. At various time points, HPV genotyping was performed along with an assessment of the degree of DNA fragmentation in the combined extracted HPV and human genomic material. DNA fragmentation was modestly and progressively increased over time at all temperatures, however, HPV genotyping utilizing PCR demonstrated minimal increases in cycle threshold for oncogenic HPV genotypes. The authors concluded that HPV genotype results are stable for an extended period of time for specimens stored at 4 - 30 °C after collection by the “dry” method. The “dry” HPV-sampling approach is preferred for collection of self-collected vaginal specimens for HPV genotyping. After collection, the manner of specimen transport and conditions for storage should be selected in order to maintain specimen temperature between 4 °C and 30 °C. Although stable for extended periods of time, Specimens should be processed for HPV genotyping as soon as possible following collection.

2. Comparison of results for clinician collected specimens and self-collected vaginal specimens

The “gold standard” for evaluating the success of self-collected vaginal specimens for HPV screening is based on the correlation of HPV genotyping results obtained from self-collected specimens with those obtained from specimens collected by a trained clinician using the conventional approach employing a speculum, which allows direct visualization of the cervix so that cells from both ectocervical and endocervical areas are collected.

In the United States, utilization of self-collected vaginal specimens for HPV screening has thus far been relatively limited. Accordingly, the great majority of published studies using self-collected vaginal specimens have been conducted in foreign countries where most of the experience using this approach has been obtained39-44. Two studies to examine the correlation between self-collected vaginal specimens and clinician-collected specimens for HPV screening were conducted in the Netherlands (16,410 total randomized patients) and in Mexico (25,061 total randomized patients).
In the Dutch study\textsuperscript{39}, 8,212 participants were randomly allocated to the self-sampling group and 8,198 to the clinician-based sampling group. 569 (7.4\%) self-collected samples and 451 (7.2\%) clinician-collected samples tested positive for HPV based on genotype analysis (relative risk 1.04 [95\% CI 0.92–1.17]). After a median follow-up duration for HPV-positive women of 20 months, the sensitivity and specificity of HPV testing did not differ between self-sampling and clinician-based sampling in terms of the detection of CIN 2+ or CIN 3+ lesions in the follow-up cytology testing. The authors concluded self-collected vaginal specimens for HPV genotyping could be used as a primary screening method in routine cervical cancer screening.

In the study from Mexico\textsuperscript{40}, 12,330 women were randomly assigned to the self-collected vaginal specimen arm and underwent HPV genotyping, with follow-up colposcopy on patients testing positive. An additional 12,731 patients were randomly assigned to undergo cervical cytology only. The goal was to determine whether self-collected vaginal specimen could identify patients with CIN 2 or worse as well as conventional cytology. HPV testing identified 117.4 women with CIN 2 or worse per 10,000 (95.2-139.5) compared with 34.4 women with CIN 2 or worse per 10,000 (23.4-45.3) identified by cytology. The relative sensitivity of self-collected vaginal specimens to identify CIN 2 or worse cervical cancer using HPV testing was 3.4 times greater (2.4-4.9) than cervical cytology alone. On the other hand, the positive predictive value (PPV) of HPV testing for CIN 2 or worse was 12.2\% (9.9-14.5) compared with 90.5\% (61.7-100) for cytology alone. The authors concluded that despite the much lower PPV for HPV testing of self-collected vaginal specimens compared with cytology, such testing might be preferred for detecting CIN 2 or worse in low-resource settings where restricted infrastructure reduces the effectiveness of cytology-based screening programs.

Additional, smaller-scale studies have largely supported the conclusions from these 2 pivotal studies\textsuperscript{41, 42} and a detailed meta-analysis of self-collected versus clinician-collected samples was published for studies performed prior to 2014\textsuperscript{43}. In addition, one study addressed self- versus clinician-collected
specimens for cervical cancer screening in post-menopausal women and demonstrated that even in this population, there was no significant difference between the two sampling methods for extended HPV genotyping (P=0.827)\(^4\). Self-collected vaginal specimens have equal sensitivity compared to clinician-collected specimens for detection of CIN 2+ or CIN 3+ lesions based on HPV genotyping only\(^3\) and significantly greater sensitivity compared to clinician-collected cytology only\(^4\). Self-collected vaginal specimen results for HPV genotyping have significantly lower specificity compared to conventional cytology for detection of CIN 2 or worse lesions\(^4\). Thus, HPV genotyping using self-collected vaginal specimens is a highly effective screening approach for cervical cancer. The importance of appropriate follow-up of positive results by conventional cytology or imaging studies cannot be overemphasized.

3. Advantages and potential concerns associated with self-collected vaginal specimens

The benefits and potential drawbacks of self-collected vaginal specimens for HPV genotyping are summarized below:

Advantages\(^4\)\(^5\)\(^6\)\(^7\)

a. patient preference  
b. convenience which helps reduce frequency of missed appointments or failure to make appointments  
c. increased availability to cervical cancer screening in remote areas with limited access to healthcare providers  
d. wider availability for underserved (uninsured) populations with high HPV exposure risk  
e. comparable performance compared to clinician-collected specimens  
f. reduced procedure costs and eliminated travel costs to a clinical site as the patient performs the procedure
Potential concerns

a. increased frequency of specimen rejection (inadequate specimens) and decreased overall screening performance compared to clinician-collected specimens. With self-collected vaginal specimens, there is no direct visualization of the cervix so sampling errors may arise due to inadequate sampling of the squamocolumnar junction.

b. significant differences in screening performance of different self-collected vaginal specimen collection methods

c. potential lack of appropriate follow-up

d. challenges with interpretation of results if not directly communicated to a professional care provider

Several published studies have asserted the advantages listed above and demonstrated that concerns related to increased specimen rejection rate or poor performance with self-collected vaginal specimen compared to clinician-collected specimens for HPV screening are unwarranted. With regard to self-collection methods, there is no significant performance difference between brush and broom-based “dry” sampling or lavage-based self-sampling devices. Of particular significance is the observation that most women prefer self-sampling to a clinician-performed procedure, which has the added benefit of increasing participation in routine cervical cancer screening programs in asymptomatic women. Increased access to HPV-based cervical cancer screening in remote areas is also a notable advantage of self-collected vaginal specimens and there is good reason to believe that this approach is more cost-effective, although large-scale studies to demonstrate this are lacking. Current clinical practice recommendations in the United States already acknowledge the value of self-collected vaginal sampling methods in specific settings; however, self-sampling is not yet FDA approved currently.
Screening Strategies

The availability of screening, along with vaccination programs, have decreased the incidence and mortality rates of cervical cancer. Screening can detect precursors and early-stage disease of squamous cell carcinoma and adenocarcinoma. Treatment of precursors and early-stage disease can prevent the development of invasive cervical cancer and reduce cervical cancer mortality. The three available cervical screening strategies in the United States are primary HPV screening, co-testing with HPV testing and cervical cytology, and cervical cytology alone. Recommendations for screening aim to balance benefits of early detection of treatable lesions and reduction in incidence and mortality of cervical cancer with potential risks for false positives, unnecessary procedures, and potential harms (e.g., patient discomfort, healthcare costs, and risks of treatment on future pregnancies). The most recent screening recommendations from the 2018 USPSTF and the 2020 ACS will be reviewed here. The recommendations are summarized in Table 2. The main differences between the two guidelines relate to age to initiate screening and the test used in individuals aged 21 to 29 years old.

High-risk HPV testing alone

The FDA approved the cobas HPV test in March 2014 and the Onclarity HPV test in April 2018, for primary HPV testing for screening in individuals 25 years or older. Both these tests offer and are approved for partial HPV genotyping. It has been demonstrated that primary HPV screening is more effective than screening with cytology alone and performs similarly to and with lower costs than screening with co-testing. The two FDA approved tests for primary HPV screening are not available at all institutions. In many settings, co-testing will be ordered in lieu of primary testing, until an FDA approved primary test is available.

The USPSTF recommends that primary HPV testing should not be used to screen individuals 21 to 29 years old as a stand-alone test. This is due to the high prevalence of HPV in those under the age of 30, although this may change as increasing number of people are vaccinated. In one study, primary HPV
screening starting at 25 years of age doubled the number of colposcopies but resulted in a 54% greater
detection of CIN 3+ when compared to the same strategy starting at 30 years of age. However, despite
the increased detection of CIN 3+, quick progression to cancer is uncommon, and so on balance,
cytology screening is felt to be adequate for detection of serious disease, while avoiding the potential
for over-evaluation associated with the highly sensitive HPV test in patients younger than 30 years old.
Based on this data, the USPSTF recommends that primary HPV screening should only be used for
patients 30 years and older. An important difference in the ACS guideline is the recommendation for
the use of primary HPV testing starting at 25 years old. Although based on the same data, the
difference in interpretation reflects the balance of increased intervention (i.e., colposcopies) with
increased number of precancerous lesions detected.

With regards to interval of screening, both organizations recommend screening with primary HPV
should not occur at intervals shorter than 3 years and not beyond 5 years among patients with negative
screening results. Analysis by Ronco et al. concluded that a screening interval of at least 5 years for HPV
screening is safer than cytology every 3 years.

**High-risk HPV and cervical cytology co-testing**

In co-testing, cytology and HPV testing are collected and reported together. In addition to the two FDA-
approved tests for primary HPV screening, Digene HC2 Assay, Cervista HPV HR, Cervista HPV 16/18,
Aptima HPV Assay, and Aptima HPV 16,18/45 are all approved by the FDA as of March 2019 for co-
testing and these are available at most institutions. As not all institutions currently have access to
FDA-approved assays for primary HPV testing, providers may order co-testing when HPV-based testing is
recommended. As laboratories increase in capacity and access to FDA-approved tests, either through
new FDA-approvals or through switching to approved platforms, providers would ideally switch to
primary HPV testing.
The USPSTF recommends that co-testing may be offered to patients 30 years and older with retesting in 5 years recommended after a negative screen \(^7\). Similar to primary HPV testing, the ACS recommendation differs slightly in that co-testing is also acceptable among those older than 25 years old \(^6^4\). The addition of HPV testing to cytology increases the detection of prevalent CIN 3 with a concomitant decrease in CIN3 + or cancer detected in subsequent rounds of screening \(^6^7\)\(^-\)\(^6^9\). The increase in diagnostic lead-time with co-testing translates into lower risk following a negative screen, which allows for an interval of 5 years between screens with incident cancer rates similar to or lower than screening with cytology alone at 3-year intervals \(^7^0\)\(^,\) \(^7^1\). The addition of HPV testing to cytology also enhances the identification of women with adenocarcinoma of the cervix and its precursors \(^7^1\)\(^,\) \(^7^2\). Compared to squamous cell cancers, cytology has been relatively ineffective in decreasing the incidence of invasive adenocarcinoma of the cervix \(^7^3\).

**Cervical cytology alone**

When cervical cytology alone is used, the cervical sample is analyzed for cellular abnormalities. After cytology is performed, there is an option to perform reflex HPV testing when the cytology result returns positive for ASC-US. The USPSTF recommends screening for cervical cancer every 3 years with cervical cytology alone with reflex to HPV for ASC-US in women aged 21 to 29 years. Given the high prevalence of transient HPV infection among adolescents and young adults, initial screening at age 21 years should be with cytology alone. If cytology alone is used, the ACS recommends that the screening interval should be every 3 years \(^6^4\). Studies of screening intervals in women with a history of negative cytology results report an increased risk of cancer after 3 years even after controlling for prior number of negative cytology tests \(^7^4\). Conversely, the incidence of high-grade cytology within three years of a normal cytology is low (10 to 66 per 10,000) \(^7^5\) and modeling studies demonstrating that detection was similar with annual or triennial screening, but annual screening resulted in increased number of interventions (i.e., colposcopies) \(^7^6\)\(^,\) \(^7^7\).
Comparison of screening strategies

There are no randomized trials comparing mortality rates among the various screening strategies. One modeling study found that HPV-based screening strategies (i.e., primary HPV testing or co-testing) were associated with fewer cervical cancer deaths (0.23 to 0.29 per 1,000) compared with screening strategies that included cervical cytology (i.e., cytology alone or reflex HPV testing, 0.30 to 0.76 per 1,000).76

With respect to detection, a systematic review found that primary HPV testing among individuals 25 to 65 years compared with cytology alone was associated with increased detection of CIN 3+ in the initial round of screening (RR range, 1.61 [95% CI, 1.09-2.37]) to 7.46 [95% CI, 1.02-54.66]).8 Colposcopy rates were higher for primary HPV testing than for cytology alone in 1 of 3 trials (NTCC Phase II 69, 78) and similar in 2 trials (FINNISH79 and HPV FOCA80). False-positive rates for CIN 2+ were higher for primary HPV testing alone than for cytology alone in 1 trial (NTCC Phase II) and similar in another trial (FINNISH).

In comparing detection of CIN 3+ using co-testing versus cytology alone, randomized control trials (NTCC Phase I69, 78, SWEDESCREEN67, POBASCAM68, ARTISTIC81) have found that including HPV testing leads to earlier detection, but not reduced incidence, of high-grade cervical dysplasia and cancer. In all four trials, HPV testing in the first screening round detected cases of CIN 3+ that were missed by cytology, but there were fewer cases in the combined HPV testing plus cytology group at round 2, and over both screening rounds there were no significant differences. In contrast, the HPV FOCAL study found a lower incidence of CIN 3+ associated with initial HPV testing (incidence ratio (IR) 2.3 per 1,000 [95% CI 1.5-3.5]) compared with initial Pap testing (IR 5.5 per 1,000 [95% CI 4.2-7.2]; RR 0.42 [95% CI, 0.25-0.69])80.

Colposcopy rates were higher for screening with co-testing than for cytology alone in two trials (ARTISTIC and NTCC Phase I) and not reported in the other two trials (SWEDESCREEN and POBASCAM). False-positive rates were higher for screening with co-testing in 3 of 4 trials (SWEDESCREEN did not report the false positive rate for the intervention group).
A benefit of co-testing is that among individuals with a negative co-test, the risk of developing CIN 3+ was less than 1% in the next 5 to 10 years. Meta-analysis indicated that compared with cytology-based testing, screening with HPV testing (mainly with co-testing) was associated with a lower incident of cervical cancer at a median follow up of 6.5 years (rate ratio 0.60, 95%CI 0.40-0.89).

Consistent with the low-risk associated with negative co-testing, modeling studies found that co-testing every five years was as effective as screening with cytology alone every three years and was associated with decreased colposcopies compared with co-testing every three years, with only a minimal change in lifetime cancer risk (0.39% vs. 0.61%).

**Beginning and Ending of Screening**

Screening for cervical cancer in asymptomatic, immunocompetent patients, regardless of the age of sexual debut should not be performed in individuals younger than 21 years old. Cervical cancer rates have been reported to be 0.15% in females 15 to 19 years old and 1.4% in women 20 to 24 years old. The prevalence of CIN 3 in women under 21 is estimated at 0.2 % while the false-positive cytology rate is reported at 3.1% again emphasizing the potential harm of early screening. This is because exposure of cervical cells to HPV during vaginal intercourse may lead to cervical precancers but regression is common and is generally not a rapid process. Furthermore, screening initiation is not tied to sexual debut since the incidence of HPV infection is highest following the initiation of sexual intercourse, but usually clears spontaneously in 90 % within 2 years. In counseling patients, it is important to emphasize the need for screening even after vaccination.

As noted above, the USPSTF recommends screening at 21 years and older with cytology every 3 years based on a meta-analysis of randomized trials and observational studies that demonstrated higher false-positive rates with HPV testing because of the higher rates of transient infection in this age group. Alternatively, the ACS recommends that screening should begin at age 25 with primary HPV testing every 5 years. The higher age of screening initiation is based on the low incidence of cervical cancer.
(0.8%) due to high rates of spontaneous regression of HPV infection. The ACS favors primary HPV testing based on randomized controlled trial showing higher specificity of HPV-based testing than cytology alone, which is important in the context of increased vaccination rates. This will become increasingly relevant as a greater number of women are vaccinated prior to exposure to HPV.

The timing to discontinue screening depends on adequacy of screening, prior results, life expectancy, and patient preferences. Adequate screening is defined by either: 1) two consecutive negative HPV tests within the past 10 years (with the most recent within the previous 5 years); or 2) two consecutive negative co-tests within the past 10 years (with the most recent within the previous 5 years); or 3) three consecutive negative Pap tests within the past 10 years (with the most recent test within the previous 3 years). If results for the past 10 years are unknown, screening would be considered inadequate. In addition to adequate screening, the patient should not have had CIN 2 or worse for the past 25 years.

The ACS and USPSTF both recommend that those over age 65 who have had regular screening in the past 10 years with normal results and no history of CIN2+ within the past 25 years can discontinue screening. Those with a history of precancer or cancer should continue to have testing for at least 25 years after diagnosis even if the testing goes past age 65. The evidence for discontinuation of screening is based primarily on a single modeling study with a model of continued screening up to age 90. A prolonged screening model only resulted in the reduction of 1.6 cancer cases and 0.5 cancer deaths per 1,000 women compared to an additional 127 colposcopies per 1,000 women. However, it is important to note that approximately 20% of cervical cancers occur in patients older than 65 years and evidence indicates that screening in those 65 years and older is associated with a lower risk of subsequent development of cervical cancer. In patients with inadequate prior screening or unknown screening history the high incidence of mortality from cervical cancer and modeling studies suggest that screening older patients who have never been screened with cytology could reduce mortality by 74%. Based on this data, the USPSTF suggests in those with inadequate or unknown prior screening, screening
should be continued until age 70 or 75 years old. Overall, data regarding the stopping age for screening are limited and should be based on an informed decision-making discussion with the patient.

Criteria for Routine Screening

Despite the somewhat nuanced differences between the ACS and USPSTF guidelines, two key concepts to the implementation of screening are: 1) correctly identifying those who meet criteria for routine screening and 2) ensuring that patients who have abnormal Pap and/or HPV testing results are evaluated, usually by colposcopy with biopsy, undergo treatment if appropriate, and finally adhere to follow-up. Figure 1 includes these concepts and is adapted from the 2019 ASCCP guideline to demonstrate how a patient’s risk is evaluated, irrespective of which of the three screening strategies is used.

To determine if an individual meets criteria for routine screening, the following should be elicited from clinical history:

- **History of immunosuppression:** Patients with HIV as well as solid organ transplant, allogeneic hematopoietic stem cell transplant, inflammatory bowel disease, lupus, and rheumatoid arthritis on immunosuppressants have a compromised immune system. Guidelines on the screening and management in patients with immunosuppression account for the higher risk of cervical cancer in this group. Screening should begin within 1 year of first penetrative sexual activity and continue throughout a patient's lifetime: annually for 3 years if all results are normal, then every 3 years (cytology only) until the age of 30 years, and then either continuing with cytology alone or co-testing every 3 years after the age of 30 years. All abnormal results need to be evaluated.

- **History of vulvar or vaginal dysplasia:** Vulvar and vaginal dysplasia share similar risk factors to cervical dysplasia. It has been reported that the rate of concurrent disease is approximately 3% and those who are immunosuppressed carries the highest likelihood (OR 20.1; 95% CI 11.33-51.82) followed by those with HIV/AIDS (OR 17.4; 95%CI 8.73-41.69). There are no guidelines available
to guide follow-up of patients with vulvar and vaginal dysplasia. However, the increased risk of concurrent cervical disease raises the importance of modified surveillance in this group.

• History of hysterectomy with removal of cervix: If a patient underwent hysterectomy with removal of the cervix and either has no previous diagnosis of CIN 2+ within the previous 25 years or has completed 25 years of surveillance, continued testing is generally not recommended. However, if testing is performed, abnormal vaginal sample results should be managed according to published guidelines. Alternatively, if hysterectomy was performed for treatment of any cervical abnormality, patients should have 3 consecutive annual HPV-based tests before entering long-term surveillance (i.e., annual cytology or every 3-year co-testing).

• Clinical signs or symptoms of bleeding, discharge, and/or pain: It is important to note that symptomatic patients of any age should undergo diagnostic evaluation regardless of prior or current screening results. Signs and symptoms of cervical disease could include abnormal discharge, abnormal bleeding, post-coital bleeding, pelvic pain, change in bladder or bowel function, and abnormality seen on visualization or palpation of the cervix. Diagnostic evaluation here may include cytology, HPV testing, colposcopic evaluation, diagnostic imaging, and cervical, endocervical, or endometrial biopsy. The results of the associated Pap test and HPV testing should be interpreted in conjunction with colposcopic evaluation and to complement biopsy results rather than used in a screening or surveillance algorithm.

• Prior abnormal results and recent testing: Patients with any prior abnormal results, with or without treatment, are at increased risk and should be managed based on the ASCCP guidelines.

In summary, anyone with a history of immunosuppression, vulvar or vaginal dysplasia, hysterectomy with removal of cervix, clinical signs and symptoms, or prior abnormal results does not meet criteria for
routine screening per the ACS or USPSTF guidelines. For those with abnormal prior results without recent testing, patients should be triaged based on the ASCCP guidelines described below and illustrated in Figure 1.

### Surveillance Using Risk-based Guidelines

The 2019 ASCCP risk-based management guidelines incorporate HPV testing and cytology results with prior test results to estimate an individual’s 5-year risk of CIN 3+. The minimum amount of data required to generate a clinical action recommendation include the patient’s age and current test results, recognizing that prior screening history might not be available. However, ideally, prior cytology, HPV and pathology data are entered into the risk calculator in order to create a personal risk score for the patient, which determines management. Data tables of risk estimates are to guide management clinical action thresholds under the principle of “equal management for equal risk”. The estimates are based on data from Kaiser Permanente Northern California (KPNC), the BD Onclarity registrational trials, the New Mexico HPV Pap Registry, and the Centers for Disease Control and Prevention’s (CDC’s) National Breast and Cervical Cancer Early Detection Program. Patients with an immediate risk of CIN 3+ that is less than 4% undergo surveillance, and based on their 5-year risk of CIN 3+, the interval may be 1, 3 or 5 years. Those with an immediate CIN 3+ risk of greater than 4% are referred to diagnostic evaluation, which may include colposcopic evaluation and/or excisional procedure.

Surveillance is defined as follow-up testing at a shorter interval than that currently recommended for routine screening with either HPV primary testing or co-testing (i.e., sooner than 5 years). Surveillance is recommended for patients whose risk of CIN 3+ based on current test results and screening history is higher than the risk for the general screening population, but lower than the risk at which colposcopy is recommended. For patients with an estimated 5-year CIN 3+ risk of less than 0.15%, return to routine screening at 5-year intervals using HPV-based testing is recommended. This is based on the estimated 5-
year CIN 3+ risks after a negative HPV test (0.14%; 95% CI 0.13%-0.15%) and co-test (0.12%; 95% CI 0.12%-0.13%). Cytology alone is never recommended at 5-year intervals. For patients who have an estimated 5-year CIN 3+ risk of 0.15% or greater but less than 0.55%, repeat testing in 3 years with HPV-based testing is recommended. Finally, those with an estimated risk of greater than 0.55% but less than 4% (threshold for immediate colposcopy), repeat testing in 1 year with HPV-based testing is recommended. For example, follow-up at 1 year is recommended after a screening test showing minimal abnormalities: HPV-positive/ negative for intraepithelial lesion or malignancy (NILM) or HPV-negative/LSIL with unknown previous screening history (immediate risks 2.1% and 1.1% respectively). Surveillance also applies to patients who are referred for colposcopic evaluation and/or treatment and are found to have CIN 1 or normal results. The 5-year CIN3+ risks for various clinical scenarios are available based on publicly available risk tables (https://CervixCa.nlm.nih.gov/RiskTables). For individuals diagnosed with high-grade abnormalities and who are treated, more frequent surveillance with HPV-based testing at 6 months is preferred and if positive, colposcopy with biopsies should be performed. Individuals treated for histologic HSIL with a subsequent abnormal screening test result have an elevated risk of cervical precancer warranting close follow-up. HPV testing and co-testing are more sensitive than cytology alone in detecting CIN 2+ in both the post-colposcopy and post-treatment settings.

The ASCCP guideline also addresses the issue of long-term follow-up surveillance after treatment for both high-grade and low grade abnormalities. For those with a history of treated high-grade histology or cytology, after initial intensive surveillance period, the ASCCP recommends surveillance at 3-year intervals for at least 25 years, and may continue as long as the patient is in reasonably good health. This is based on data from long-term population studies, which demonstrate a persistent twofold increase in cervical cancer risk after treatment of high-grade lesions. For those with history of low-grade cytology (HPV-positive NILM, ASC-US, or LSIL) or histologic LSIL abnormalities without evidence of histologic or
cytologic high-grade, co-test in one year is advised, and if results are all normal is followed by continued
surveillance at 3-year intervals.

**Diagnostic Testing/Evaluation**

**Colposcopy**

Colposcopy standards have been outlined by the ASCCP \(^{116,117}\). It is recommended that practitioners
follow the standardized terminology which captures 6 major areas: 1) general assessment, 2) evaluation
for presence of any acetowhite lesions, 3) description of normal colposcopic findings, 4) description of
abnormal colposcopic findings, 5) description of other/miscellaneous findings, and 6) reporting of the
colposcopic impression, defined as the highest-grade impression of any visible lesion on the cervix. A
comprehensive colposcopic examination should include description of the cervix visibility,
squamocolumnar junction visibility, presence of acetowhitening, presence and visualization of a lesion,
color/contours/borders/vascular changes of lesions, the location and size(s) of lesion(s), other features,
and the colposcopic impression. A diagram or marked image annotating the findings should also be
included. Minimum criteria for reporting findings at colposcopic examination should include the
following: squamocolumnar junction visibility (fully/not fully), acetowhitening (yes/no), lesion (s)
present (acetowhite or other) (yes/no), and colposcopic impression (normal/benign, low-grade, high-
grade, cancer). Colposcopy training is currently not regulated in United States and there is no
certification \(^{118}\). Standards in many other countries do include training and generally stipulate that all
clinicians who perform colposcopic examinations should have completed a formal colposcopic training
program conducted by expert trained personnel whose clinical competence and teaching abilities are
well documented \(^{119}\).

For those at lowest risk (i.e., less than HSIL cytology, no evidence of HPV 16/18 infection) with a
completely normal colposcopic impression, random biopsies are not recommended. This is based on
KPNC data which demonstrated that the risk of occult CIN 2+ was 1% to 7% and CIN 3+ was less than 1% in the above described low risk group which underwent 4-quadrant biopsies and endocervical curettage in that cohort. If the above criteria are not met, multiple targeted biopsies (at least 2 and up to 4), are recommended targeting all acetowhite areas to improve detection of precancers. Moreover, biopsies are needed for any degree of acetowhiteness, metaplasia, or abnormalities.

In non-pregnant women 25 years and older with a very high risk of precancer, either immediate excisional treatment without biopsy confirmation or colposcopy with multiple targeted biopsies is acceptable. High-risk in this context is defined as at least two of the following: HSIL cytology, HPV16 and/or HPV 18 positive, high-grade colposcopy impression. This is based on systematic review of see-and-treat management strategies for patients meeting the high-risk criteria, which found that 73% to 86% of all women with had CIN 2+.

With respect to endocervical curettage, it is preferred for non-pregnant patients when colposcopy is inadequate and in those not at lowest risk and no lesion is identified. It can also be considered when a lesion is seen.

**Biopsy**

HPV induces histologic changes in the squamous epithelium of the uterine cervix, particularly at the transformation zone. These changes comprise a diverse spectrum of alterations (Figure 2). On one end of the spectrum are mild koilocytic changes, which have a degree of overlap with reactive atypia. On the other end of the spectrum are atypical basaloid epithelial cells involving the full thickness of a markedly thickened squamous epithelium. Lesions along this spectrum must be classified into discrete categories to guide clinical management. Two schemata are currently recognized to do this: the three-tier cervical intraepithelial neoplasia (CIN) system, and the two-tier squamous intraepithelial (SIL) system. The CIN system classifies lesions as CIN 1, 2, or 3, ranging lowest to highest grade. CIN 1 includes lesions with koilocytic
changes and basal atypia confined to the lower one-third of the epithelial thickness. CIN 2 includes lesions with basal atypia involving the lower and middle thirds of the epithelial thickness. CIN 3 includes those with full-thickness basal atypia. The SIL system classifies lesions as either high grade (HSIL) or low grade (LSIL). LSIL includes CIN 1. HSIL includes CIN2 and CIN3. While the latter category has the benefit of simplicity, it loses the informative distinction between CIN2 and CIN3.

There is only moderate reproducibility among pathologist in classifying HPV induced squamous lesions, using both the CIN and SIL systems. This is largely a consequence of the great diversity in the histomorphology of these lesions, and the substantial fraction of cases with features that are not clearly high or low grade. For example, while reproducibility is good for the distinction between CIN 1 and CIN 3, reproducibility is poor for the diagnosis of CIN 2. These are often difficult to distinguish from CIN 1 and CIN 3. Consistency in diagnosis has been aided by the addition of immunohistochemistry for p16, a protein product of the cell cycle gene CDKN2A. This marker is sensitive for high-grade lesions, but is also expressed in a substantial subset of low-grade lesions. Expression of p16 is particularly high in low-grade lesions driven by high-risk HPV types, with diffuse expression of p16 seen in nearly 90% of hrHPV-positive LSIL in one study. CIN 1 lesions that are p16-positive progress to CIN 2 or higher in 10-35% of cases, while those that are p16-negative progress in <5% of cases. The negative predictive value (NPV) of p16 is thus high for predicting progression to a high-grade squamous lesion.

Recent guidelines by the College of American Pathologists and the ASCCP state p16 may be used to distinguish LSIL from HSIL. Positivity for p16 is defined specifically as continuous strong nuclear or nuclear plus cytoplasmic staining of the basal cell layer with extension upward involving at least one third of the epithelial thickness (Figure 2C). Guidelines state it should only be ordered in specific circumstances, such as distinguishing CIN 2 from CIN 1 in lesions that have equivocal features. This approach depends on the threshold of the individual pathologist for the distinction between these
lesions, the major weakness in the approach. Nonetheless, it removes women with p16-negative lesions from the HSIL designation, appropriate for the high NPV of p16.

Despite issues with reproducibility, the CIN 2 category is clinically valuable, because it may prevent overtreatment of women with squamous intraepithelial lesions that are not fully developed CIN 3. We recommend a three-tier approach to classifying squamous intraepithelial lesions, and support the judicious use of p16 in cases that are truly equivocal between CIN 1 and CIN 2.

The Ideal Laboratory Report

Based on the above discussion of the importance of specifying the indication for testing (i.e., screening surveillance, or diagnosis) and the test used, we propose the following report template (Table 3) to facilitate results interpretation and clinical decision-making. While this template can be modified for local needs, we believe it incorporates the most important components. It is important to allow for all available (or most recent) prior results to be summarized in the current report to facilitate risk-based decision making. Furthermore, the specific HPV test used by the laboratory should be specified. Please note that p16/Ki67 dual-stain may be performed in cases where cytology results are abnormal (LSIL or ASCUS) and/or hrHPV-positive, but it has not been included in the current guidelines and it is optional128.

Summary of Recommendations and Future Directions

The goal of a screening protocol is to optimize the detection of precancerous lesions at a time when they are treatable while limiting the harm of overtreating benign disease. This begins with correctly identifying those patients suitable for routine screening versus those who require surveillance and/or diagnosis. The introduction of risk-based management considers factors that influence clinical action thresholds allowing for greater tailoring of screening strategy for patients. The most recent ASCCP guideline highlights that prior history profoundly influence risk estimates, specifically current HPV and
cytology test results, previous HPV test results, and history of histologic HSIL \(^9\). The estimated risk guides decisions regarding surveillance interval, colposcopic referral, and treatment.

In all three recommendations, the concepts of screening, surveillance, and diagnosis are important in framing the clinical situation at hand and the appropriate use and interpretation of tests. For example, the intervals of 1-, 3-, and 5-year discussed within the ASCCP guidelines are surveillance intervals whereas the 3- and 5-year intervals discussed in the ACS and USPSTF guidelines refer to screening intervals. Furthermore, the ACS and USPSTF guidelines were developed prior to the ASCCP guidelines and nuanced differences may be noted, specifically with updates to the use of primary HPV testing. For example, the ASCCP guidelines recommend that when primary HPV screening is used as the initial test alone, additional reflex triage test (e.g., reflex cytology) for all positive HPV tests should be performed regardless of genotype \(^7\); this is a change from the 2015 interim guidelines \(^63\). However, if primary HPV screening test genotyping results are HPV 16 or HPV 18 positive and reflex triage testing from the same laboratory specimen is not feasible, patients should proceed directly to colposcopy \(^63\). The perspective of the ASCCP guidelines is to use surveillance to address potential clinical situations involving abnormal results (e.g., HPV positive) whereas the ACS and USPSTF guidelines target routine screening in low-risk patients. Lastly, once an individual has an abnormal test result, depending on subsequent findings and estimated risk, the majority will remain in surveillance with a small subset who would qualify to return to routine screening.

Moving forward, several future directions in research and implementation have the potential to improve access and implementation of these guidelines. Given that the risk estimates are based on both current and prior testing results, automated extraction from medical records and laboratory reports would simplify risk-estimate calculation. Ideally, standardized reports would include HPV test used, genotype information, cytology, and histology using common terminology (e.g., Lower Anogenital Squamous Terminology) integrated with other clinical information from a patient’s electronic health record. This
would not only allow for accurate risk-estimates, but also establish a reliable tracking and reminder system to facilitate communication, improve patient safety and quality of care, and minimize missed or delayed diagnoses. Secondly, as additional HPV tests and data from studies become available, the FDA assessment of HPV assays may potentially increase the number of tests approved for primary HPV testing. Primary HPV testing is attractive as it has been demonstrated to be more effective than screening with cytology alone and performs similarly to and with lower costs than screening with co-testing. In addition, HPV testing is also more amenable to self-collection, which opens new opportunities to screen difficult to reach and underscreened populations at high risk of cervical cancer 129-131.

Ultimately, the key message to patients, and providers alike, is stated by the ACS, “The most important thing to remember is to get screened regularly, no matter which test you get.”
Figure legend

Figure 1. Triage algorithm for cervical cancer screening, surveillance, and diagnosis. This flow diagram incorporates the ACS and USPSTF recommendations for those who meet criteria for routine screening as well as risk-based management guidelines from ASCCP. References for the following special populations and who do not qualify for routine screening are provided: (A) history of immunosuppression, (B) history of vulvar or vaginal dysplasia, (C) history of hysterectomy with removal of cervix, (D) patients with any signs and/or symptoms should undergo further evaluation, (E) for those with prior abnormal results and recent testing results is not available, surveillance based on risk-based estimates provided by ASCCP is recommended.

Figure 2. Histology of squamous intraepithelial lesion (SIL)/cervical intraepithelial neoplasia (CIN) (all H&E stained sections except (C), all 200X magnification). Sections of various CIN lesions demonstrate the diversity in histology seen within categories. A classic CIN-1 with koilocytic change and virtually no basaloid atypia (A) contrasts with a CIN-1 lesion with basaloid atypia involve the lower 1/3 of epithelial thickness (B). The latter was positive for p16 immunohistochemistry, with nuclear and cytoplasmic expression continuously involving the lower 1/3 of the epithelium (C). CIN-2 is similarly diverse. Some cases demonstrate considerable koilocytic change and abundant cytoplasm (D). Others demonstrate less of this feature (E). Some lesions fall on the border between CIN-2 and CIN-3, lacking full thickness basal atypia but having a degree of surface maturation (F). There is also variability in CIN-3. Some cases demonstrate marked nuclear atypia and modest cytoplasm (G). Others demonstrate comparatively modest nuclear atypia, scant cytoplasm, and relatively thin epithelial thickness (H). Still others have modest nuclear atypia, scant cytoplasm, and dramatically thickened epithelial thickness (I).
### Table 1. The Bethesda System for Reporting Cervical Cytology Diagnostic Categories

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>Inadequate Cellularity</td>
</tr>
<tr>
<td></td>
<td>Obscuring Inflammation or Blood</td>
</tr>
<tr>
<td>Negative for intraepithelial lesion or malignancy (NILM)</td>
<td>Non-neoplastic (tubal metaplasia, pregnancy changes, atrophy)</td>
</tr>
<tr>
<td></td>
<td>Reactive changes</td>
</tr>
<tr>
<td></td>
<td>Organisms/Viral Cytopathic Changes</td>
</tr>
<tr>
<td>Atypical squamous cells</td>
<td>HPV cytopathic changes</td>
</tr>
<tr>
<td>- undetermined significance (ASC-US)</td>
<td></td>
</tr>
<tr>
<td>- cannot exclude a high grade squamous intraepithelial lesion (ASC-H)</td>
<td></td>
</tr>
<tr>
<td>Squamous intraepithelial lesion</td>
<td></td>
</tr>
<tr>
<td>- low grade (LSIL)</td>
<td></td>
</tr>
<tr>
<td>- high grade (HSIL)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>Atypical glandular cells (AGUS)</td>
<td>Atypical endocervical cells, NOS</td>
</tr>
<tr>
<td>Adenocarcinoma in situ</td>
<td>Atypical endometrial cells, NOS</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Atypical endocervical cells, favor neoplastic</td>
</tr>
<tr>
<td>Other malignancy</td>
<td>Metastatic tumors, Sarcoma, Neuroendocrine tumors, etc</td>
</tr>
</tbody>
</table>

### Table 2. Summary of Screening Recommendations*

<table>
<thead>
<tr>
<th></th>
<th>US Preventive Services Task Force (USPSTF), 2018</th>
<th>American Cancer Society (ACS), 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age to start screening</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>Age to end screening*</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>Screening test options and intervals</td>
<td>Ages 21-65: Cytology alone every 3 years or Ages 21-29: Cytology alone every 3 years or Ages 30-65: Cytology plus HPV testing every 5 years or</td>
<td>HPV testing alone every 5 years or Cytology plus HPV testing every 5 years or Cytology alone every 3 years</td>
</tr>
<tr>
<td></td>
<td>Ages 21-29: Cytology alone every 3 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ages 30-65: HPV testing alone every 5 years</td>
<td></td>
</tr>
<tr>
<td>Preferred strategies</td>
<td>Cytology alone every 3 years and HPV testing alone every 5 years (equally preferred)</td>
<td>HPV testing alone every 5 years</td>
</tr>
</tbody>
</table>

*Applies to women with all prior normal results and no symptoms. Patients with prior abnormal results will follow 2019 ASCCP management guidelines.
Table 3. HPV and Cervical Cancer Testing Report Template

<table>
<thead>
<tr>
<th>Identification</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Birth</td>
<td></td>
</tr>
<tr>
<td>Medical File Identifier</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
</tbody>
</table>

| Indication | □ Screening |
|           | □ Surveillance |
|           | □ Diagnosis |

| Clinical History | Provider description |
|                 | Pregnant? □ Yes □ No |
|                 | Immunosuppressed? □ Yes □ No |

<table>
<thead>
<tr>
<th>Prior results</th>
<th>Date</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Current Testing | □ Cytology Alone |
|                | □ Primary HPV (with reflex testing) |
|                | □ Co-testing |

| Current Results | HPV | □ Positive |
|                |     | □ Negative |

| HPV test used | □ 16 |
|               | □ 18 |
|               | □ Other high-risk subtypes |
|               | □ Unknown |

| HPV Genotype (if positive) | □ 16 |
|                           | □ 18 |
|                           | □ Other high-risk subtypes |
|                           | □ Unknown |

<table>
<thead>
<tr>
<th>Terminology per Last guidelines</th>
<th>p16/Ki67 Dual-Stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Positive</td>
<td>□ Negative</td>
</tr>
<tr>
<td>□ Negative</td>
<td>□ Not performed</td>
</tr>
</tbody>
</table>

| Other adjunctive tests (please specify) | |

Confidential
References


44.


102. UDoHaHS. Guidelines for the prevention and treatment of opportunistic infections in adults and adolescents with HIV. 2020.


123. Carreon JD, Sherman ME, Guillen D, Solomon D, Herrero R, Jeronimo J, Wacholder S, Rodriguez AC, Morales J, Hutchinson M, Burk RD and Schiffman M. CIN2 is a much less reproducible and less valid


Does the patient have one of the following?

- History of immunosuppression (follow Ref A)
- History of vulvar or vaginal dysplasia (follow Ref B)
- Prior hysterectomy with removal of cervix (follow Ref C)
- Clinical signs or symptoms of bleeding, discharge, and/or pain (follow Ref D)

---

Does the patient have all normal PAP and negative HPV within the past 25 years?

**NO**

**SCREENING**

Routine Screening per ACS and USPSTF (Refer to Table 1)

---

**SURVEILLANCE**

Surveillance per ASCCP guidelines

- Primary HPV testing, Co-testing (HPV and cytology), Cytology alone
- Use of Risk-estimate calculator incorporating current and past results (Ref E)

---

Is immediate CIN3+ risk ≥4%

**NO**

- 5-year CIN3+ risk <0.15%
  - Return in 5 years

- 5-year CIN3+ risk 0.15-0.54%
  - Return in 3 years

- 5-year CIN3+ risk ≥0.55%
  - Return in 1 year

---

**YES**

**DIAGNOSIS**

- Immediate CIN3+ risk 4-24%
  - Colposcopy recommended
- Immediate CIN3+ risk 25-59%
  - Expedited treatment or colposcopy acceptable
- Immediate CIN3+ risk 60-100%
  - Expedited treatment preferred

---

Colposcopy adequate and low-grade abnormalities

High-grade abnormalities (HSIL, ACIS, Cancer)

Treatment and Surveillance per ASCCP Guidelines