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REVIEW ARTICLE

Extended HPV genotyping and dual stain for the triage of primary HPV screen-positive cases: Practical guidance for the cytopathology laboratory

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Abstract

Because of many factors, the landscape of cervical cancer prevention is again at a pivot point within the United States. Primary human papillomavirus (HPV) screening has been recommended as the preferred testing method by the American Cancer Society since 2020. Although primary HPV testing provides high negative predictive value in screening, women who screen positive for HPV need triage using methods that have an optimal balance between sensitivity for precancer and the number of colposcopies required for detection. The triage test ideally should maximize specificity while also reassuring patients who test negative, although it should be acknowledged that no screening or triage test can entirely exclude disease in a screen-positive patient. While cervical cytology (the Papanicolaou test) triage of primary HPV screen-positive patients is currently recommended by most screening strategies, additional triage tests, specifically extended HPV genotyping and combined p16/Ki-67 dual-stain immunocytochemistry, are now approved by the US Food and Drug Administration and incorporated into cervical cancer screening and management guidelines. Incorporating these triage methods into practice should be achieved by using appropriate validation/verification and implementation steps and, in the case of dual-stain immunocytochemistry, appropriate cytologist/cytopathologist training. The US Food and Drug Administration approval of vaginal self-collection in May 2024 is another significant advance for increasing access to screening. These samples can only be tested using primary HPV screening platforms, and guidance for management has been endorsed by the ASCCP's enduring guidelines process. This review discusses issues that warrant consideration before implementation and provides practical guidance for the incorporation of self-collected specimens and extended genotyping/dual-stain tests into the workflow of the cytopathology laboratory.

KEYWORDS

cervical cancer screening, dual stain, extended human papillomavirus genotyping, self-collection

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INTRODUCTION

Cervical cancer prevention: Transitions over time

Over the 75-year history of cervical cancer screening, major transitions in clinical laboratory practice have occurred periodically, mostly driven by technological advances and clinical trial data. In the 1990s, the United States converted to liquid-based samples[1,2]; and, in the late 1990s and early 2000s, the first clinically validated human papillomavirus (HPV) test for the triage of atypical squamous cells of undetermined significance (ASC-US) was introduced after the National Cancer Institute (NCI)-sponsored ASC-US /Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (the ALTS trial) was published.[3] Shortly thereafter, recognition of the limitation of the sensitivity of cervical cytology led to the incorporation of HPV testing into screening through co-testing.[4] In 2014, the ATHENA (Addressing The Need for Advanced HPV Diagnostics) trial (ClinicalTrials.gov identifier NCT03522246) formed the basis of the US Food and Drug Administration's (FDA's) approval of the first primary HPV screening platform.[5] As of 2024, we have three FDA-approved primary HPV screening options available in the United States and are once again at a significant pivot point.

The current transition is attributed to a host of factors, including the effect of HPV vaccination on screening; the incorporation of primary HPV testing into updated American Cancer Society (ACS) and US Preventive Services Task Force (USPSTF) screening guidelines and of HPV testing as the basis of risk assessment for the ASCCP management guidelines; the availability and use of biomarkers for the triage of primary HPV screen-positive patients to risk-based management; and the approval of self-collection of vaginal samples for primary HPV screening.

The reality of a growing workforce shortage in the United States, which includes both cytologists and pathologists, must also be acknowledged as we weigh the merits of future laboratory testing for cervical cancer prevention.[6,7] Furthermore, to assist with the growing complexity and needs within both cytopathology laboratories and health care systems nationally, the role of the cytologist (formerly known as cytotechnologist) continues to evolve, with an educational infrastructure that is expected to fully transition to a Master's degree training program by 2030. The 2-decade long discussion and debate by a wide group of cytology stakeholders regarding the changing role of the cytologist culminated with the board of directors of the Commission on Accreditation of Allied Health Education Programs approving the entry-level competencies and standards and guidelines for all cytology (formerly known as cytotechnology) educational programs. The updated competencies and standards went into effect January 1, 2025.[8] In addition to evolution in the training of cytology laboratory personnel, technological advances are being realized with the goal of improving the efficacy of cervical cancer screening. Artificial intelligence (AI) systems that use digital imaging continue to be deeply integrated into cervical cancer screening and routine cytopathology practice. Data from large, prospective clinical trials have increased our knowledge regarding the clinically validated application of HPV genotyping and dual-stain (DS) immunocytochemistry to triage HPV-positive patients. Each of these processes must be viewed through the lens of a screened patient population that is increasingly HPV vaccinated. The changes forthcoming are a continuation of the historical and ongoing evolution of cervical cancer screening within the United States and worldwide.

There are multiple points regarding cervical cancer screening that warrant emphasis. No screening or triage test is perfect. Because all tests have limitations in their sensitivity, it is inevitable that not all cervical precancer or cancer will be detected. Colposcopy also has limitations in its ability to detect significant epithelial lesions and should not be considered a *gold standard* when performing cytologic–histologic correlation, particularly for cytologic glandular abnormalities. Vaccination is further decreasing the positive predictive value of cytology, HPV testing, and colposcopy.

Each cytopathology laboratory will need to make its decisions regarding which testing platform(s) to choose based on multiple factors that are specific to its own environment, striving to obtain an optimal balance between risk stratification versus cost and efficiency for its patient population. All laboratories will not, and cannot, perform all tests. The Enduring Consensus Guidelines for Cervical Cancer Screening and Management (referred to hereinafter as *Enduring Guidelines*, described below) will continue to evolve as new data and FDA-approved technologies become available. It is also important to note that the Enduring Guidelines recommendation may not always perfectly correlate with the respective test label indications and specifications. In response to the recent, continuing, active period of newly FDA-approved technologies and updated screening and management guideline recommendations, this report presents considerations and practical guidance for those looking to incorporate clinician and self-collected primary HPV testing and/or DS/extended HPV genotyping for the triage of primary HPV cervical cancer screen-positive patients. The objectives and topic highlights discussed are summarized in Table 1.

Review of current screening and management guidelines

The 2019 ASCCP Risk-Based Management Consensus Guidelines for the Management of Abnormal Cervical Cancer Screening Tests (referred to hereinafter as the *2019 ASCCP Guidelines*) were

TABLE 1 Overview of the objectives and topic highlights.

Objectives/topic highlights
Practical information for the implementation of primary human papillomavirus testing (clinician-collected and self-collection), extended genotyping, and dual-stain testing into the workflow of the cytopathology laboratory
An overview of the trial evidence supporting extended genotyping and dual-stain immunocytochemistry for the triage of primary human papillomavirus screen-positive women

predicated on the paradigm of equal management for equal risk.[9] Large, longitudinal studies have demonstrated the superior sensitivity of high-risk HPV (hrHPV)–based testing (which includes both HPV screening and co-testing) compared with cytology alone. Therefore, HPV-based screening through the inclusion of hrHPV testing (referred to hereinafter as *HPV testing*) in the initial screening process, is considered the most effective testing strategy, particularly because HPV testing has become more readily available throughout the United States and the world. The 2019 ASCCP Guidelines recommended partial HPV genotyping and additional reflex triage testing (e.g., cytology) for primary HPV screen-positive women and underscored that HPV16/18–positive women, irrespective of the reflex triage (cytology) result, at a minimum, should undergo colposcopy, with the option of expedited treatment available for those with high-risk cytology results (e.g., high-grade squamous intraepithelial lesion [HSIL]). As illustrated in Figure 1, non-HPV16/18 HPV–positive women (or *other* high-risk HPV genotypes) can be triaged by cytology, with follow-up in 12 months for individuals who have normal cytology and colposcopy performed for those who have abnormal cytology.[9,10]

With HPV-vaccinated populations now in the screening cohort, the decreasing prevalence of precancer/cancer caused by vaccine genotypes supports the implementation of primary HPV screening.[11,12] In 2020, the ACS recommended primary HPV testing every 5 years as the preferred screening strategy for average risk patients with a cervix, with start of screening at age 25 years using HPV testing platforms FDA-approved for that indication.[13] In this transition period, until the next guideline release, co-testing every 5 years and cytology testing alone every 3 years were deemed acceptable for situations in which access to FDA-approved primary HPV screening is not available.

After the release of its guidelines recommending primary HPV testing as the preferred screening method, the ACS, in response to concerns by stakeholders that the United States was not ready for this transition, launched the Primary HPV Screening Initiative (PHSI) in the fall of 2021.[14] Nearly 100 volunteers representing numerous stakeholders with expertise in cervical cancer prevention participated, addressing concerns both by identifying barriers and by creating resources and educational offerings to support the transition (these resources can be found at https://cervicalroundtable.org/). Members of the Laboratory Infrastructure PHSI Workgroup have been working together since 2021 to develop resources for laboratories, collaborating with the College of American Pathologists on ensuring the quality of primary HPV testing through accreditation checklist changes (reflected in the December 2024 College of American Pathologists checklists), and presenting educational offerings on primary HPV screening at several national and local meetings.[15]

The USPSTF is an independent volunteer panel of nationally recognized experts supported by the Agency for Healthcare Research and Quality.[16] The focus of the USPSTF is the review of evidence-based medicine to make recommendations for clinical prevention services, such as cervical cancer screening. During its process of guideline determination, the USPSTF collaborates with partner organizations, engages expert reviewers, and encourages public comment submission. The last release of its cervical cancer screening recommendations was published in 2018, with the final recommendations including three *A-level, high-certainty* options: every 3 years with cervical cytology alone in patients aged 21–29 years and every 5 years with primary HPV testing, every 5 years with co-testing, or every 3 years with cervical cytology for those aged 30–65 years.[17] Because the USPSTF reviews and updates its

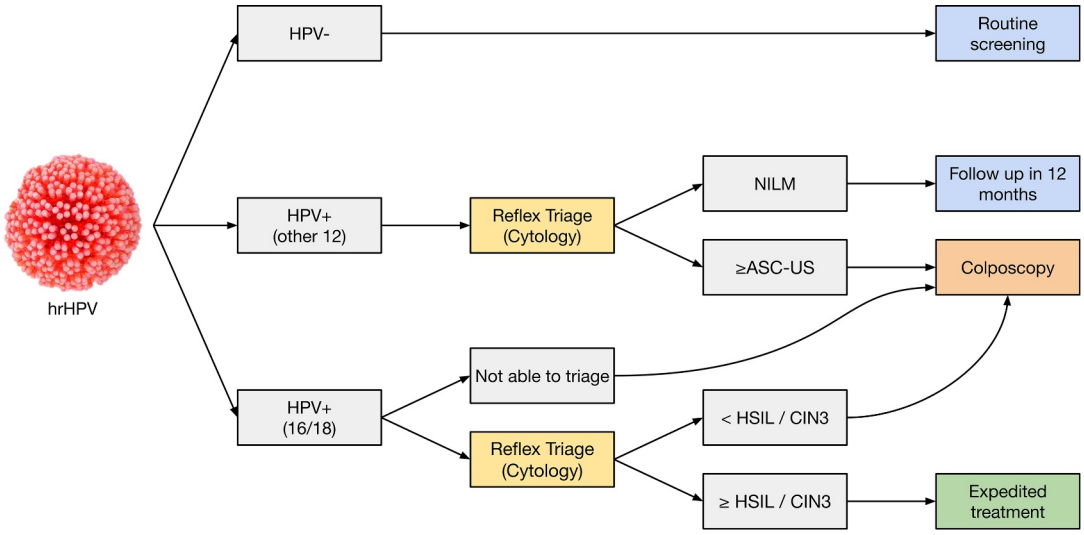


FIGURE 1 2019 ASCCP risk-based management consensus guidelines. Refer below to enduring guideline recommendations for triage of HPV+ cases by dual stain and/or extended genotyping. − indicates negative; +, positive; ASC-US, atypical squamous cells of undetermined significance; CIN 3, cervical intraepithelial neoplasia 3; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy.

screening recommendations at approximately 5-year to 7-year intervals, the review process for cervical cancer screening was recently completed, and the draft guidelines were released for public comment on December 10, 2024.[18] These USPSTF draft recommendations propose screening for cervical cancer every 3 years with cervical cytology alone in women aged 21–29 years and then every 5 years with clinician-collected or patient-collected hrHPV primary screening in women aged 30–65 years. As an alternative to HPV primary screening for women aged 30–65 years, the USPSTF recommends continued screening every 3 years with cervical cytology alone or screening every 5 years with hrHPV testing in combination with cytology (co-testing). Final recommendations are pending at the time of this publication.

ONGOING UPDATES TO CERVICAL CANCER SCREENING AND MANAGEMENT GUIDELINES

The Enduring Guidelines effort is a standing committee whose task is to continuously evaluate new technologies and approaches to cervical cancer screening, management, and surveillance. The committee members include experts in cervical cancer prevention who represent 20 national organizations and patient advocacy groups, the majority of whom also served on the 2019 ASCCP Guidelines consensus committee.[19] Further details regarding the Enduring Guidelines are available at: https://dceg.cancer.gov/enduring-guidelines and at: https://www.asccp.org/clinical-practice/guidelines/enduring-guidelines. The focus of the committee is examination of the data investigating the utility of new technologies and approaches that became available after the 2019 ASCCP Guidelines process to increase targeted cancer prevention in high-risk individuals while also decreasing unnecessary invasive and costly procedures for individuals at low risk.

Based on current demographics, it is expected that approximately 90% of individuals undergoing primary HPV screening will have a negative screen and will require no further testing, whereas the approximately 10% who test positive will require a follow-up colposcopy or risk-based surveillance determined by the genotype, any other available triage result, and prior history according to 2019 ASCCP Guidelines and subsequent Enduring Guidelines recommendations.[9,19,20] Enduring Guideline recommendations for the incorporation of DS immunocytochemistry and extended genotyping into the 2019 ASCCP Guidelines have been published and have been incorporated into clinical decision-support tools.[21,22] Recommendations for self-collection have been finalized and are scheduled for publication in early 2025.

VAGINAL SELF-COLLECTION: FDA APPROVAL, COLLECTION, WORKFLOW, ADVANTAGES, AND CURRENT LIMITATIONS

FDA approval

HPV self-collection is generally defined as vaginal sampling without the use of a speculum by the individual who is undergoing screening. To reduce barriers to screening, particularly in inaccessible settings and with unscreened or underscreened patient populations, self-collection of vaginal specimens was approved by the FDA in May 2024 for collection in a health care setting after the patient is given medical guidance and instruction.[23] Because previously unscreened or underscreened patient populations gain the benefit of screening through self-collection, it is likely that patient demographics of the screening population will change with its adoption in the future. Because the prevalence of hrHPV types varies between differing demographic patient populations, further studies need to be conducted in historically underserved populations to identify which HPV types are relevant to those underserved populations. Similarly, vaccinated individuals will be protected against genotypes included in the vaccine they have received, and the future of screening in vaccinees will likely look different from that in unvaccinated individuals. Additional studies and data will inform future risk stratification of HPV-positive patients by extended HPV genotyping.

Self-collected vaginal specimens can be tested for HPV as an alternative specimen type either when cervical sampling is contraindicated or if a cervical specimen cannot be obtained. Although follow-up in the United States is limited, meta-analysis indicate that the performance of self-collected specimens is similar to that of provider-collected specimens, with both methodologies having similar pooled sensitivities, which are higher than cytology alone.[24] In addition, in patients who cannot tolerate a speculum examination and are also unable to collect their own vaginal sample, a vaginal specimen can also be obtained by a provider. To qualify for self-collection, patients should be asymptomatic and eligible for primary HPV testing.

Collection and workflow

As of January 2025, FDA approval applies to the Roche cobas (Roche Diagnostics) and BD Onclarity (Becton, Dickinson and Company) HPV testing platforms. After collecting the vaginal specimen in a health care setting, the specimen is sent to the laboratory according to Roche and BD instructions for use, which contain details on collection devices and transport.[25,26] Patients who are positive for HPV16 and/or HPV18 are recommended to proceed directly to colposcopy with concurrent cytology collection. The remaining patients who test positive for *other* HPV genotypes on self-collected specimens will require an additional office-based visit with a clinician-collected cervical specimen for further triage testing to determine the need for colposcopy. This is a critical caveat for laboratories to be aware of and monitor their testing protocols: *unlike clinician-obtained cervical samples, reflex cytology and DS cannot be performed on self-collected vaginal specimens.* In addition, follow-up of HPV-negative, self-collected specimens is currently recommended at 3 years by the Enduring Guidelines Workgroup (vs. 5 years) until further data are available. Thus it is advisable for laboratories to consider setting up separate electronic medical record orders for primary HPV testing on cervical (clinician-collected) versus vaginal

(self-collected) specimens so that workflow in the laboratory and clinical management for the patient are clearly distinguished.

Advantages and limitations

Self-collected vaginal HPV testing has several advantages in overcoming limitations, which are health system infrastructure-based and provider-based (such as limited provider access and availability) as well as patient-based (such as physical, social, or emotional barriers or discomfort with speculum-based examinations). Self-collection does have operational limitations, including the inability to perform reflex cytology or DS testing for non-16/18 (*other types*) HPV-positive samples. Because this sampling method is primarily directed at underscreened patients who may not have ready access to medical care, the subsequent coordination of care for HPV-positive patients may have inherent challenges, either when immediate colposcopy is needed or in the scheduling of an office visit for a clinician-obtained cervical specimen for triage testing. Further guidance and recommendations on self-collection for primary HPV results from the Enduring Guidelines effort are in press at the time of this writing (R. Nayar, personal communication).

Because self-collected vaginal samples currently require immediate transfer to cytologic fixative solutions, they are not approved at this time for home self-collection. Work has been done in the United States on the evaluation of preanalytical variables for primary HPV screening from self-collected vaginal swabs in both health care and home settings.[27] The NCI's *Last Mile* initiative includes SHIP (Self-Sampling for HPV Testing to Improve Cervical Cancer Prevention)—a nationwide multicenter trial and associated studies with independent evaluation of multiple self-collection devices and HPV assays for usability, acceptability, accuracy, and effectiveness. The trial network includes 25 clinical sites across the United States, and participant enrollment began in the fall of 2024.[28]

Overview of FDA-approved HPV test platforms testing and triage reporting

As HPV testing became widely used for ASC-US triage and co-testing over the past 2 decades, the FDA approved multiple testing platforms for the detection of hrHPV in cervical cytology specimens. These platforms have undergone both analytic and clinical validation with high intralaboratory and interlaboratory reproducibility. The number of FDA-approved testing platforms continues to grow. On November 2, 2023, the Abbott Alinity m HPV DNA assay (Abbott Laboratories) was approved by the FDA for primary HPV screening and co-testing[29]. With this additional option, there are currently six FDA-approved HPV testing platforms. Five platforms use DNA-based testing, and the sixth incorporates an RNA-based amplification technique.[30] It is important to note that only three of the six platforms are FDA approved for the indication of primary HPV testing. These include the Roche cobas HPV test with partial genotyping, the Abbott Alinity m HPV assay with extended genotyping, and the BD Onclarity HPV assay with extended genotyping. The multipathology society–sponsored Cytopathology Education and Technology Consortium has strongly advised that only HPV testing platforms approved by the FDA for primary screening are used for that specific indication.[31] This position is supported by the ACS, the 2019 ASCCP guidelines, and the clinical provider professional societies focused on cervical cancer screening and management. The reason for this restriction is the recognition that the clinical validity of primary HPV screening requires large-scale clinical trial data, which are not achievable for home-brew HPV tests.[32,33] It is imperative that cytology managers, supervisors, and laboratory director cytopathologists clearly communicate to ordering clinicians (and patients) that primary HPV testing can only be performed using platforms FDA approved for that specific indication. Thus laboratories should only offer primary HPV testing as an order option if they have an approved platform available. Low risk HPV testing should not be offered for cervical cancer screening and management. There should be no exceptions to this testing policy.

THE THREE FDA-APPROVED HPV TESTING PLATFORMS FOR PRIMARY HPV SCREENING

Roche cobas HPV test: Partial genotyping

The Roche cobas HPV test can be run on both the 4800 and the 6800/8800 testing systems (Roche Diagnostics). It uses amplification of target DNA by polymerase chain reaction (PCR) and nucleic acid hybridization for the detection of 14 hrHPV types in a single analysis. It provides individual results for HPV16 and 18, along with a simultaneous, pooled result for the other 12 high-risk genotypes (HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), all in one run from one patient sample. The assay is FDA approved with multiple collection media, to include Roche cell-collection media, BD SurePath collection vial, and ThinPrep Papanicolaou (Pap) test PreservCyt solution. The test includes an internal control to verify the presence of human cells. The cobas HPV test is also FDA approved for patient triage with CINtec *PLUS* Cytology (DS) immunocytochemistry (Roche Diagnostics). Additionally, it is FDA approved for vaginal self-collection using the Evalyn brush (Rovers Medical Devices) and Copan swab (COPAN Diagnostics Inc.) collection devices. Of note, reflex cytology or DS testing cannot be performed on vaginal self-collected specimens.

BD Onclarity HPV assay: Extended genotyping

The BD Onclarity HPV assay is a qualitative in vitro test for the detection of HPV in clinician-collected cervical specimens using an endocervical brush/spatula combination or broom and placed in a BD SurePath vial or placed in ThinPrep Pap test PreservCyt solution. The test uses amplification of target DNA by PCR and nucleic acid

hybridization for the detection of 14 hrHPV types in a single analysis and includes an internal control to ensure cellular material is present. The test specifically identifies six individual HPV types - 16, 18, 31, 45, 51, and 52 and reports the other eight hrHPV types in groups (HPV types 33/58, 35/39/68, and 56/59/66). It was initially approved in the United States to detect HPV types 16, 18, and 45, with the 11 other types as a group, and subsequently received additional approval for extended genotyping in 2020 to report the nine-valent vaccine types (HPV types 31, 52, or 33/58 [pooled]), and the remaining seven high-risk types, which are less likely to cause cervical disease (HPV types 51, 35/39/68 [pooled], and 56/59/66 [pooled]. The assay is FDA approved for use with both BD SurePath and Hologic ThinPrep liquid-based cytology (LBC) media. When using extended genotyping on a clinician-collected (cervical) sample, the risk stratification may or may not benefit from the addition of cytology or DS testing (see the Enduring Guidelines recommendations).[21,22] Reflex cytology may be performed for HPV16/18-positive samples (to allow for the option of expedited treatment). Reflex cytology or DS is recommended for HPV types 31, 45, 52, 33/58, 51, and 35/39/68 to determine whether colposcopy is indicated. Reflex testing is not needed on HPV types 56/59/66 based on lower risk, and 1-year repeat testing is recommended. However, if cytology is performed for HPV types 56/59/66 (e.g., as part of a co-test) and cytology results are ASC-cannot rule out HSIL or higher, colposcopy is recommended. The BD Onclarity HPV assay is also FDA approved for vaginal self-collection using the Copan swab collection device. Of note, reflex cytology or DS testing cannot be performed on a vaginal self-collected specimens.

Abbott Alinity m HPV assay: Extended genotyping

This PCR-based, multiplex testing platform is intended for use with the Alinity m system. It identifies 14 hrHPV genotypes, reporting HPV types 16, 18, and 45 with concurrent reporting of the other high-risk genotypes in two groups (HPV types 31/33/52/58 and 35/39/51/56/59/66/68). All individual HPV genotypes and the two groups are identified in a single reaction and are reported together. Like the Onclarity assay, an internal control ensures specimen adequacy, and it is approved for use with both BD SurePath and Hologic ThinPrep LBC media. Abbott Alinity m is not currently approved for vaginal self-collection.

All three assays described above are indicated for use in routine cervical cancer screening according to professional medical guidelines, including triage of ASC-US cytology, co-testing (or adjunctive screen) with cytology, and primary HPV screening of women to assess the risk for cervical precancer and cancer. Patients should be followed up in accordance with professional medical guidelines, results from prior screening, medical history, and other risk factors.

Corporate representatives and customer service agents from each manufacturer can offer additional information on quality-control HPV panels and verification procedures. For billing, Current Procedural Terminology codes 87624 (hrHPV detection) and 87625 (separately reported genotype detection) or 87626 (partial/extended genotype detection in the same test) should be submitted, as appropriate. Reimbursement amounts depend on the third-party payor and geographic region for federal payments (Centers for Medicare and Medicaid Services). The characteristics of the three testing platforms approved for primary HPV testing are listed in Table 2, with emphasis on the two platforms that offer extended genotyping for triage of positive primary screening results and approval for use with CINtec *PLUS* cytology (DS).

LABORATORY WORKFLOW CONSIDERATIONS IF OFFERING PRIMARY HPV TESTING WITH TRIAGE

Currently, the FDA-approved methods for the triage of primary HPV screen-positive patients include cervical cytology, HPV genotyping, and CINtec *PLUS* cytology (DS). The Roche cobas HPV test, the BD Onclarity HPV assay, and the Abbott Alinity m HPV assay all provide specific genotyping for HPV16/18, with the BD and Abbott platforms providing additional individual and/or group genotype information. If your cytopathology laboratory is considering offering primary HPV testing as an in-house screening option, there are several workflow considerations to keep in mind:

1. Is your current HPV testing platform approved for a primary screening indication?
2. Is your Pap test vial approved for testing on your primary HPV testing platform?
   - The LBC preservative approved for use varies with the testing platform and, within certain manufacturers, varies between the size (moderate-throughput vs. high-throughput) of the specific platform.
3. What will be your chosen testing method for the triage of your primary HPV screen-positive patients based on current professional society guidelines/recommendations versus your laboratory's capabilities?
4. If using a testing platform that offers extended genotyping, what are your specific laboratory information system requirements to set up extended genotyping reporting?

NEED FOR TRIAGE TOOLS WITH THE OPTIMAL BALANCE OF SENSITIVITY AND SPECIFICITY

Because most HPV infections are transient, particularly in younger women with a low risk of cervical intraepithelial neoplasia (CIN) grade 3 or greater (CIN3+), triage of HPV-positive patients is needed to avoid unnecessary downstream *harms*. Triage strategies should identify which HPV-positive patients require colposcopy/biopsy, while also reducing overdiagnosis, unnecessary referral to colposcopy, and overtreatment. The triage test should have high negative predictive value to maximally reassure patients who test negative.[34] As a triage test, cervical cytology has several significant advantages,

TABLE 2 Summary of human papillomavirus (HPV) testing platforms approved for primary HPV testing.

Assay	Roche cobas 48/68/8800	BD Onclarity	Abbott Alinity m
Detection of:	HPV DNA	HPV DNA	HPV DNA
No. of HPV genotypes	14	14	14
Assay type	L1 PCR	E6, E7 PCR	L1 PCR
Internal control for specimen adequacy	Yes	Yes	Yes
HPV16/18 (with or without HPV45) genotyping results provided	Yes	Yes	Yes
Extended genotyping reported	No	Yes	Yes
		16, 18, 45, 31, 51, 52, (33, 58)	16, 18, 45, (31, 33, 52, 58)
		(56, 69, 66), (35, 39, 68)	(35, 39, 51, 56, 59, 66, 68)
Cytologic media	Roche cell-collection media BD SurePath collection vial ThinPrep Pap test PreservCyt solution	BD SurePath collection vial ThinPrep Pap test PreservCyt solution	BD SurePath collection vial ThinPrep Pap test PreservCyt solution
CPT codes	87624	87624 and 87626	87624 and 87626
Use with CINtec *PLUS* cytology	Yes	No[a]	No[a]

Abbreviations: CPT, Current Procedural Terminology; PCR, polymerase chain reaction.
[a]Triage of extended genotyping by dual stain or other methods currently under review.

which include high specificity for CIN when abnormal (LSIL or worse) and being readily available in the United States because it is a component of each of the currently used screening strategies. Based on these factors, cervical cytology was the recommended triage test in the 2019 ASCCP guidelines for positive primary HPV test results. However, it also has disadvantages. Because of its relatively low sensitivity for high-grade precursor lesions, patients who have negative cytology results require retesting at a relatively short interval.[35] This limited sensitivity places women who have non-HPV16/18 and negative cervical cytology at risk for precancer. The need for optimal risk stratification led to research and development followed by studies evaluating alternative triage tests.

Genotyping: Partial/limited versus extended

The International Agency for Research in Cancer (IARC) defines four major HPV genotype risk groups—HPV types 16 and 18/45, HPV16-related types, and the remaining lower risk types. Currently, 12 HPV genotypes are considered carcinogens (IARC class 1), and one is considered a probable carcinogen (IARC class 2A). However, the risk for an individual patient depends on the specific genotype(s) present and the patient's history. In a general screening population in which HPV positivity is approximately 10%, the expected genotype frequency is estimated at 1.5% for HPV16, 0.5% for HPV18, and 8% for other HPV types.

Tests with *no genotyping* are those in which all genotypes included within the assay are reported as a single result, without separating individual genotypes or groups of genotypes. Partial (or limited) genotyping refers to specifically reporting the presence of HPV16 and HPV18 (with or without HPV45), and all remaining genotypes are detected as a pool and reported as *other*. Partial genotyping has proven beneficial in directing risk-based clinical management because HPV16 and HPV18 together account for the majority (approximately 70%) of all invasive cervical cancers.[36] Within partial genotyping, HPV16 is particularly important because it is associated with approximately 50% of CIN3+.

The detection and specific identification of HPV16 and HPV18 (and/or HPV45) are also crucial because they account for approximately 90% of endocervical adenocarcinomas (EAs), and cervical cytology has a relatively low sensitivity for glandular lesions. Because of the relative sensitivity of cytology for squamous versus glandular lesions, EAs now comprise approximately one fourth of all cervical cancers in the United States.[37] When cervical cytology raises concern for a glandular abnormality, its correlation with the HPV test status is critical. The concurrent detection of HPV types 16/18/45 and atypical glandular cell (AGC) cytology confers a highly elevated risk of EA/EA in-situ because the finding of AGC cytology (compared with normal cytology) increases the risk of EA/EA in-situ 20-fold. In recognition of the powerful prediction of this combination of findings, the significant risk AGC cytology offers in the setting of an HPV16/18/45–positive patient must be considered when weighing the benefit of other, non-cytology triage strategies.[38] It should also be noted that cervical cytology was never intended to screen for endometrial abnormalities, which are HPV-negative and are more likely to present with symptoms such as bleeding.

Extended genotyping identifies individual oncogenic genotypes beyond HPV16 and HPV18/45, providing more specific information

on the combined 12 *other* hrHPV genotypes typically reported as a pooled result by FDA-approved HPV testing platforms with partial genotyping. Whereas Arbyn et al. defined extended HPV genotyping as reporting at least six individual genotypes,[32] the World Health Organization extended genotyping guidelines and efforts occurring internationally refer to any genotyping beyond HPV16/18(45) as extended genotyping, with no *minimum* number of channels or genotypes required for this qualification. The Enduring Guidelines Committee considers extended genotyping to be the reporting of individual HPV genotypes or groups *beyond partial genotyping*.

Need for extended genotyping

Although partial genotyping detects HPV types 16 and 18/45, which are the most critical HPV genotypes in cervical carcinogenesis, sensitivity of risk stratification for the larger proportion of *other* HPV types is limited.[5] Integrating extended genotyping into recommended testing schemes is currently a challenge in the United States because the five FDA-approved HPV testing platforms that offer genotyping do not report their respective partial and/or extended type results in a similar fashion, and only three are approved for primary HPV testing. Some results are integrated, whereas others require an additional reflex order and separate test performed from the original specimen. For laboratorians and cytopathologists with medical oversight of cytopathology laboratories, the ordering and reporting characteristics of HPV testing platforms are major factors to consider when examining which platform(s) to implement. The risk conferred by the *other* individual 12 HPV genotypes is not the same. In particular, the 3-year cumulative incidence rates of CIN3+ for genotypes HPV types 31, 33, 35, and 52 have been identified as comparable with the rate HPV18, with each significantly higher than the rate for the remaining 12 *other* genotypes.[39] The efficacy of extended genotyping and cervical cytology for the risk stratification of HPV-positive patients was examined using data from the baseline phase of the Onclarity trial.[40] Extended genotyping in combination with cervical cytology triage was compared with previously published data for CIN3+ detection using HPV16/18 primary screening in combination with p16/Ki-67 DS immunocytochemistry. The results illustrated sensitivity, specificity, and colposcopies for each case of CIN3+ detected that were similar to those of primary HPV screening stratified by HPV16/18 genotyping and DS. In addition, extended genotyping allows the ability to detect genotype-specific persistence, which can help optimize disease detection and reduce the need for unnecessary colposcopic procedures.[41,42] The relative advantages and disadvantages of partial/extended genotyping are summarized in Table 3.

Management of extended genotyping results

The Enduring Guidelines recommendations for extended HPV genotyping have been published and are pending incorporation into clinical decision support tools for risk-based management.[22]

BIOMARKER TRIAGE

Although HPV genotyping is a nonmorphologic biomarker of patient risk for precancer or cancer, other molecular or immunohistochemical targets have also been studied for triage. HPV-based screening (primary HPV testing and co-testing) has been proven to improve sensitivity for the detection of high-grade lesions (precancer) compared with cervical cytology alone based screening.[43–47] Biomarkers offer an additional tool for triaging patients who are primary HPV screen-positive to the appropriate clinical management.

TABLE 3 Partial/extended genotyping: Advantages/disadvantages.

Advantages	Disadvantages
High sensitivity	Lack of cytomorphologic subtyping as squamous or glandular, which may guide management (obtained if reflex cytology is performed)
Fast throughput (integrated into the HPV testing platform)	Multiple risk subgroups (or individual risk genotypes) to assess in determining patient management (should improve with further incorporation into risk-based management applications)
Potentially avoids the cost and preparation time of an additional triage test (see Enduring Guidelines recommendations)	Challenging to determine the most effective follow-up for patients who are positive for a lower risk genotype (or genotype group), although extended genotyping can provide cost-effective triage when combined with concurrent cytology results
Does not require microscopic expertise in cytopathology	If used alone for triage determination, would require more colposcopies to identify CIN3+
Can be used with self-collected patient samples	Need for additional triage tests to further stratify relatively lower risk patients (non-HPV16/18)
Potential for future incorporation of automation	

Abbreviations: CIN3+, ≥ cervical intraepithelial neoplasia grade 3; Enduring Guidelines, Enduring Consensus Guidelines for Cervical Cancer Screening and Management; HPV, human papillomavirus.

Dual stain

$P16^{INK4a}$ (p16; inhibitor of cyclin-dependent kinase 4) is a tumor suppressor protein that promotes cell-cycle arrest. Focal p16 staining may be seen in normal physiologic conditions in cervical squamous metaplastic cells and endocervical cells. In cervical dysplasia, overexpression of p16 is regarded as a surrogate biomarker for transforming HPV infections. Ki-67, a commonly used proliferation marker, is a nuclear and nucleolar protein that is expressed in all phases of the cell cycle except for resting cells (G0 phase). Under physiologic conditions, the expression of Ki-67 is mutually exclusive of the antiproliferation protein p16. In epithelial cells expressing the hrHPV E6/E7 oncoproteins, cell-cycle dysregulation allows for the co-expression of Ki-67 and the functional protein p16. This simultaneous co-expression serves as a genotype-independent and morphology-independent indicator of the presence of transforming HPV infections and underlying high-grade disease.[48–50] The incorporation of this test allows for further risk stratification and the identification of patients for whom colposcopy (or other risk-based intervention) is indicated.

In 2020, the FDA approved CINtec *PLUS* Cytology as the first biomarker test for triage of HPV-positive patients. It is FDA approved for the triage of HPV-positive women in either primary HPV screening or co-testing scenarios.[51] Its specific FDA-approved indications are to determine the need for referral to colposcopy within either of the strategies of primary HPV screening or co-testing, specifically:

- Women aged 25–65 years who are primary screen HPV test-positive for the *other 12* HPV genotypes; and
- Women aged 30–65 who have Pap cytology that is negative for intraepithelial lesion or malignancy and are HPV-positive for the *other 12* HPV genotypes.

The recommended use of DS has recently been expanded beyond the original FDA approval by the Enduring Guidelines process based on additional data.[21] For triage of positive HPV results from screening with primary HPV testing (with or without genotyping) or with cytology cotesting, colposcopy is recommended for individuals who test DS-positive. One-year follow-up with HPV-based testing is recommended for those who test DS-negative, except for those who test HPV16-positive and HPV18-positive or have high-grade cytology identified in co-testing, for whom immediate colposcopy referral is recommended.

DS is intended for use with the cobas HPV test on the 4800 or 6800/8800 test systems. A positive test result (immunocytochemical co-expression of the two biomarkers) should be followed by colposcopy.[21,52] The CINtec *PLUS* Cytology test is a qualitative immunocytochemical assay approved by the FDA for use with cervical cytology specimens collected by a clinical provider in the ThinPrep Pap test PreservCyt solution. The assay is intended for the simultaneous detection of p16 and Ki-67 proteins using a multiplex assay with primary antibodies to each of these proteins, and the test kit includes a ready-to-use primary antibody cocktail for use with the Ventana BenchMark ULTRA immunocytochemical instrument with diaminobenzidine and Fast Red detection (Roche Diagnostics). The dual immunocytochemical-stained cytology preparation allows for the simultaneous detection of p16 and Ki-67 immunoreactivity in the same cell as a biomarker combination, which is indicative of cell-cycle dysregulation and transforming HPV infection.

The performance of DS for triaging hrHPV-positive patients for the detection of CIN3+ has been evaluated in multiple countries, demonstrating comparable or better overall performance to that of cytology.[53] Compared with Pap cytology triage, DS led to fewer colposcopies and earlier detection of CIN3, with greater negative predictive value. However, in a co-testing setting, DS should *not* be used for the triage of high-risk cervical cytology results, which include the Bethesda System categories of ASC-cannot rule out HSIL, AGC, and HSIL or higher[54]. Patients with these cervical cytology results should proceed directly to colposcopy according to the Enduring Guidelines.

Dual-stain interpretation

Educational information regarding CINtec *PLUS* Cytology is available on the Roche Diagnostics website, with detailed guidance regarding interpretation included in the Interpretation Guide for CINtec *PLUS* Cytology (Roche Diagnostics, 04-30-2020, Revision C).[55]

DS is less subjective than a morphologic review, but it is not strictly objective because it requires cytologic (albeit not morphologic) interpretation by a trained, qualified pathologist. Prescreening by a cytologist is advised, if possible, but it is not required. If screened by a cytologist, dual-stained cells or those with a suspicion of being dual-stained should be marked (dotted) on the slide for pathologist review. According to the authors who have implemented DS testing (N.J. and T.L.), the time required for a cytologist to screen a DS slide is similar to or slightly longer than that needed for a morphologic review of a Pap-stained slide.

There are three potential final results for each case: *unsatisfactory*, *negative*, or *positive*. The criteria for a satisfactory specimen are identical to those for a morphologic cervical cytology/Pap test. An unsatisfactory slide is one that does not contain any dual-stained cells and also does not meet criteria for squamous cellularity or has >75% of squamous cells obscured, as determined by the Bethesda System for Reporting Cervical Cytology.[56] A negative result is a slide with sufficient well-visualized squamous cellularity to be satisfactory that does not contain any dual-stained cells.

During the final evaluation of a slide screened by a cytologist, the pathologist should review the slide to:

- Examine for the presence of dual-stained cell(s), which determine a positive result;
- If no unequivocal dual-stained cells(s) are present, then determine whether cells marked as suspicious are in fact DS-positive or DS-negative;

- Determine whether slides initially screened as negative for dual-stained cells do in fact contain dual-stained cells that were missed during the initial screening; and
- For screened slides with no dual-stained cells, determine whether the slide is satisfactory for interpretation and is a negative case or whether it is unsatisfactory.

DS offers a simple criterion for test positivity. A positive result is the detection of one or more epithelial cells showing double (dual-stained) immunoreactivity (Figure 2). Dual-stained cells may be isolated or within cell clusters. If an isolated dual-stained cell is identified, the stained nucleus and cytoplasm must be clearly allocated to the same cell in the same plane of focus. After controlling for plane of focus and over/underlapping, the presence of at least one dual-stained cell is considered a positive test result irrespective of the morphologic features (such as the cell size or nuclear/cytoplasmic ratio) of that cell. This is a change in practice for both cytologists and pathologists. Like with a cervical cytology slide, the finding of a single abnormal (dual-stained) cell indicates a satisfactory specimen irrespective of the background squamous cellularity.

Although there are no specific guidelines for reporting/classifying the dual-stained cell as glandular versus squamous, and dual stained-positive cells are identified irrespective of morphology, it is recommended to provide as much detail as possible regarding the dual-stained cell type (i.e., squamous or glandular) observed in the pathology report. Providing this cell-type context could affect the management, such as with EA in-situ, and allow the most appropriate clinical follow-up and/or further sampling to be performed. An example of such reporting is as follows:

"Positive for dual stained cells (see note).

Note: The positive cells have a glandular morphology; clinical correlation with appropriate sampling is suggested."

Increasing patient age, particularly ages older than 55 years, has been associated with unsatisfactory reporting because of inadequate squamous cellularity in addition to other cytologic features typically seen in postmenopausal patients.[57] Because of their studies, Benevolo and colleagues stress that the variables routinely encountered in each step of the procedure (sampling, immunostaining, and interpretation) and the characteristics of the screened population (such as age) are all factors that influence DS interpretation. Those authors have demonstrated, with results similar to other reports, that the interpretation reproducibility for DS positivity is comparable to that of cervical cytology.[58,59]

Examples of interpretation issues: Cell clusters and background staining

The evaluation of cell clusters and background staining must follow interpretation algorithms described by the manufacturer. Figure 3 illustrates examples of DS-positive cells in clusters for which the more clearly dual stained-positive cells can be appreciated at the edges of the tight cell groups.

Background staining

To be interpreted as a positive cell for CINtec *PLUS* Cytology, the brown p16 stain within the cytoplasm of a cell with a red Ki-67–stained nucleus must be more intense than the nonspecific

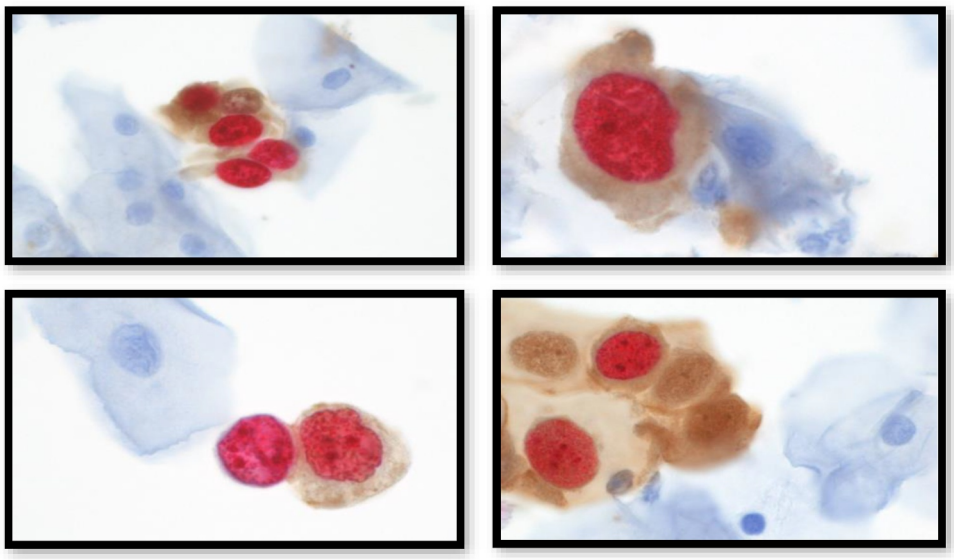


FIGURE 2 P16/Ki-67 dual-stained immunocytochemistry (CINtec *PLUS* Cytology). Cytoplasmic brown p16 staining and nuclear red Ki-67 staining are shown: one or more dual-stained cell(s) indicate a *positive* test result (https://diagnostics.roche.com/us/en/products/tests/cintec-plus.html; accessed October 10, 2024).

brown background staining of terminally differentiated squamous cells. Figure 4 illustrates Ki-67–positive cells in which the interpretation of the cytoplasmic brown staining (true p16 staining vs. nonspecific background) is problematic. In each of these examples, other foci of unequivocal DS-positive cells were identified. Care is required to not overinterpret cell groups like those illustrated if the findings are limited to weak cytoplasmic staining, which is similar in intensity to adjacent superficial squamous cells.

Training is imperative

Although more objective than cytology, interpretation of DS, in part, is a subjective test and requires proper training to ensure safe implementation in routine practice. A Danish study performed by Hammer et al. studied reviewer concordance in an older patient population (aged 45 years and older). Those authors determined that novice evaluators required a significant amount of experience to attain accurate DS interpretation in this age group.[60]

Validation/training

Roche provides a detailed analytical validation procedure with specific points pertaining to each preparation step, to include reagents/materials, sample size, specimen types, equipment, prestaining and staining procedures, postprocessing mounting/coverslipping, and acceptance criteria. The recommended number of gynecologic cytology cases required for validation is 30–50 residual HPV-positive and/or HSIL and LSIL vial samples. These cases may be derived from the laboratory's clinical practice or as commercial samples.

Potential for the incorporation of AI

To further reduce the subjective analytic steps (cytology) involved in cervical cancer screening, the incorporation of artificial intelligence (AI) into DS result determination has been examined by Wentzensen and colleagues.[61] By using a cloud-based, whole-slide imaging platform with a deep-learning classifier trained on biopsy-based gold standards, those authors compared AI-based DS with routine cervical cytology and manual DS. They observed that AI-based DS had a lower positivity

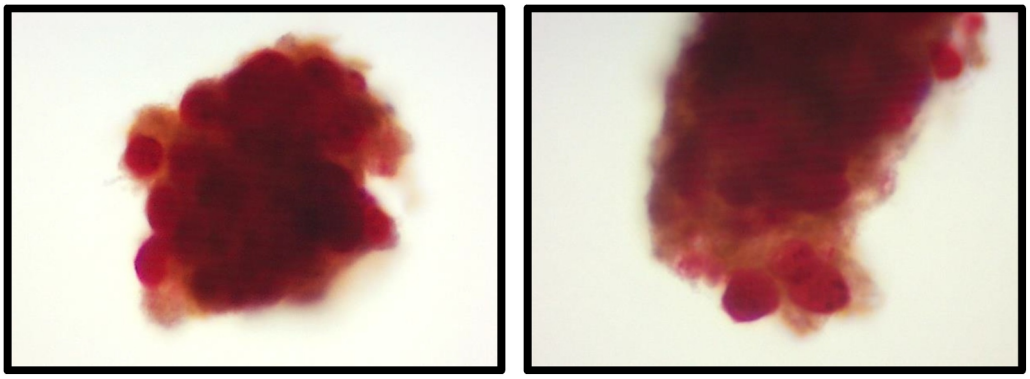


FIGURE 3 Interpretation of cell clusters using p16/Ki-67 dual-stained immunocytochemistry (CINtec *PLUS* Cytology). Dual-stained cells are located both at the edge of the cell clusters and centrally, with diffuse p16 staining (brown cytoplasmic) and Ki-67 staining (nuclear red; original magnification ×400). Slides courtesy of Quest Diagnostics.

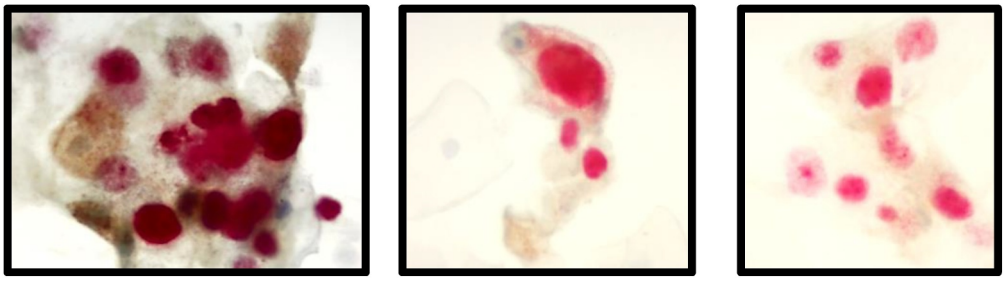


FIGURE 4 Interpretation of weak cytoplasmic staining when evaluating p16/Ki-67 dual-stained immunocytochemistry (CINtec *PLUS* Cytology). Cells with strong Ki-67 (nuclear red) staining and weak brown cytoplasmic staining are shown. Cytoplasmic staining must be more intense than the nonspecific brown background staining of terminally differentiated squamous cells to be interpreted as a positive dual-stained cell (original magnification ×600). Slides courtesy of Quest Diagnostics.

rate than both cytology and manual DS and reduced referral to colposcopy by one third. They concluded that AI not only provided benefits of automation and objectivity but also delivered equal sensitivity while increasing specificity and reducing unnecessary colposcopies.

Dual-stain reimbursement

The recent Current Procedural Terminology code for a qualitative multiplex assay is 88344 (immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody procedure). The Medicare Physician Fee Schedule rates differ by geographic locality, and all decisions regarding the specific code(s) to report are the responsibility of the cytopathology laboratory. There is no additional reimbursement for the cytologist screening, if performed.

CONCLUSIONS OF MAJOR TRIALS EXAMINING THE EFFICACY OF DUAL-STAIN TRIAGE COMPARED WITH CERVICAL CYTOLOGY

The Improved Primary Screening and Colposcopy Triage (IMPACT) trial examined the clinical performance of DS for the triage of HPV-positive women in a large primary screening population in the United States.[52,62] This prospective observational screening study enrolled over 35,000 women aged 25–65 years in 32 US sites. The results supported DS as a safe and effective option for the triage of primary HPV-screened women because it demonstrated improved risk stratification (higher sensitivity) than cytology-based strategies both at baseline and at 1-year follow-up, irrespective of the HPV genotype.

Through retrospective analysis of the ATHENA (Addressing The Need for Advanced HPV Diagnostics) trial, DS testing exhibited better performance compared with Pap cytology for the triage of HPV-positive women, with improved sensitivity and at least equal specificity for the detection of CIN3+.[63] Like cytology, DS also allows for inclusion of the additional information provided by partial genotyping.[64]

EEMAPS (European Equivocal or Mildly Abnormal Papanicolaou Study) was a retrospective analysis of the performance of DS in the triage of ASC-US and LSIL Pap cytology for the detection of CIN2+.[65] Differences in the sensitivity of cytology versus DS are observed based on the extent of screening and the prevalence of disease. Pap cytology (cytomophologic) interpretation is a more complex process with greater subjectivity than DS.

PALMS (Primary ASC-US and LSIL Marker Study) was a large, pan-European study performed to determine the sensitivity and specificity of DS compared with Pap cytology and HPV testing in screening for CIN2+.[66] The results demonstrated a positive DS rate that was comparable to the positive rate of abnormal Pap cytology, with DS significantly more sensitive in detecting CIN2+ than Pap cytology and with comparable specificity. As observed in EEMAPS, DS was also more specific than HPV testing (in women aged 30 years and older), although it was slightly less sensitive.

Olivas and colleagues have published a comprehensive review of ancillary techniques in cervical cytology.[67] Advantages and disadvantages of genotyping (partial/extended) and DS for primary HPV screen-positive patients are listed in Tables 3 and 4, respectively (expanded from Olivas, et al.).

Management of dual-stain results

The Enduring Guidelines Consensus Committee has published its recommendations for DS testing,[21] and these have been incorporated into clinical decision support tools for risk-based management.

Although CINtec *PLUS* Cytology is approved by the FDA on ThinPrep specimens, its performance on SurePath samples has been extensively evaluated and incorporated into routine clinical practice at Kaiser Permanente Northern California, whose data have been extensively used by the Enduring Guidelines Workgroup.[54,68]

FLOW-CHART ALGORITHMS FOR PRIMARY HPV TEST IMPLEMENTATION

If your cytopathology laboratory wishes to implement primary HPV testing, there are multiple important decision points for cytopathology laboratories to consider. Examples of major considerations include, but are not limited to, the following: (1) the need for a testing platform FDA approved for a primary HPV screening indication, (2) which LBC platform(s) is currently used, and (3) how the triage of primary HPV test-positive cases will be performed. Each of these decisions involves multiple factors, including finances, the depth of laboratory staffing at multiple levels (such as technical/preparatory, information technology, and laboratory information system support),

TABLE 4 Dual stain: Advantages/disadvantages.

Advantages	Disadvantages
Provides actual cervical cell-based information (compared with solely risk-based information)	Low throughput
Greater specificity than Pap cytology for triage of primary screen HPV-positive patients	Requires a specific immunocytochemistry/immunohistochemistry staining platform
Relatively low technical complexity	Not integrated into the HPV testing platform
Potential for digital imaging and application of AI	Requires additional microscopic training to accurately interpret
	Requires cytopathology expertise, which is in increasingly short supply
	May not be able to separate squamous and glandular abnormalities in each positive case

Abbreviations: AI, artificial intelligence; HPV, human papillomavirus; Pap, Papanicolaou test.

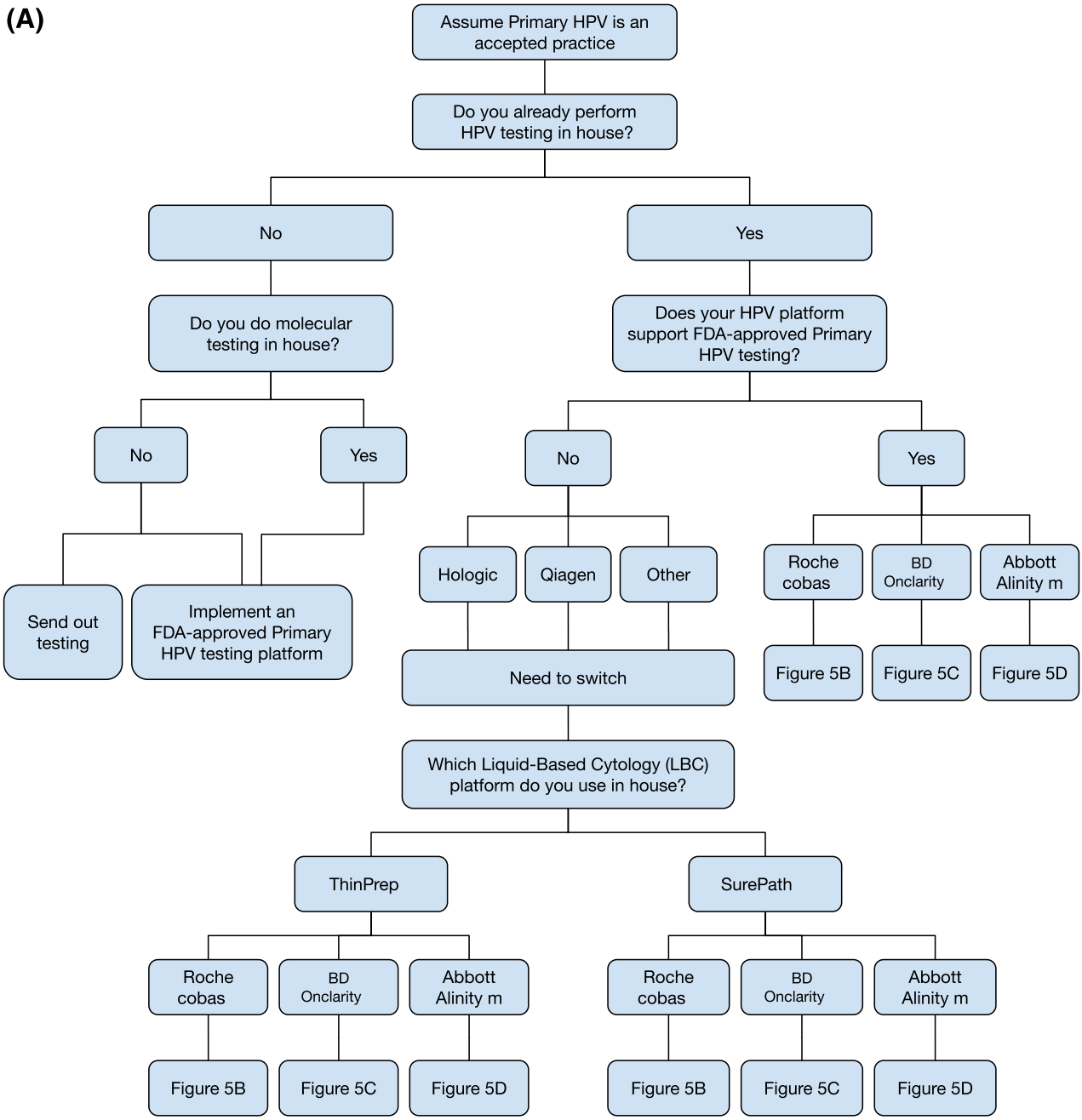


FIGURE 5 (A) Laboratory implementation considerations for primary HPV screening for cervical cancer with triage test options. (B) Triage of Roche primary HPV screening (cobas HPV; *follow Enduring Guidelines dual-stain recommendations; Clarke et al., 2024[21]). (C) Triage of BD primary HPV screening (Onclarity; Becton, Dickinson and Company; *follow Enduring Guidelines extended genotype recommendations; Massad et al., 2025[22]). (D) Triage of Abbott primary HPV screening (Alinity m; *follow Enduring Guidelines extended genotype recommendations; Massad et al., 2025[22]). (E) Primary HPV screening (other test platforms). − indicates negative; +, positive; +/−, with or without; ASC-US, atypical squamous cells of undetermined significance; Enduring Guidelines, Enduring Consensus Guidelines for Cervical Cancer Screening and Management; FDA, US Food and Drug Administration; GT, genotyping; HPV, human papillomavirus; IHC, immunocyto-histochemistry; Pap, Papanicolaou test; XGT, extended genotyping.

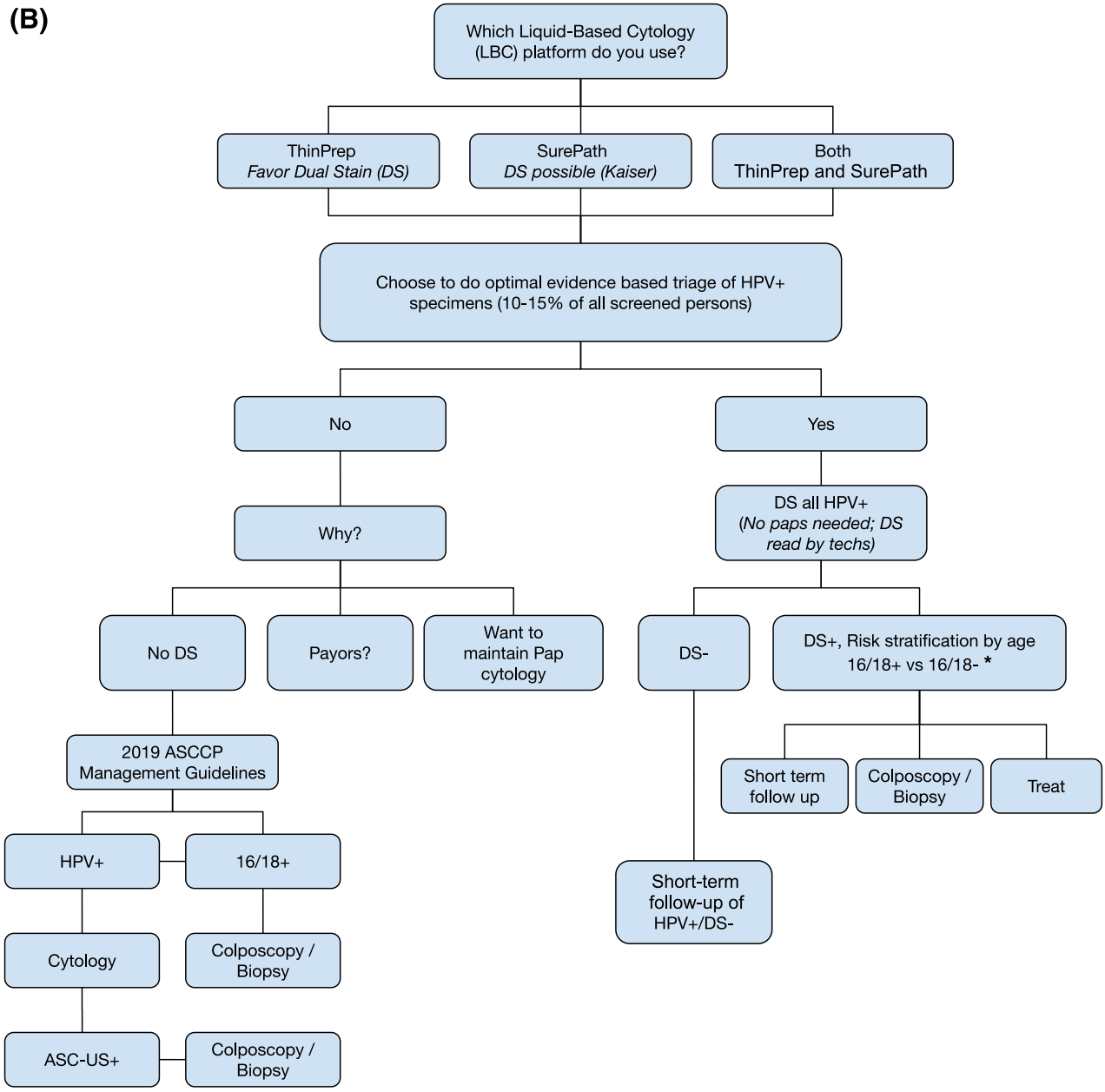


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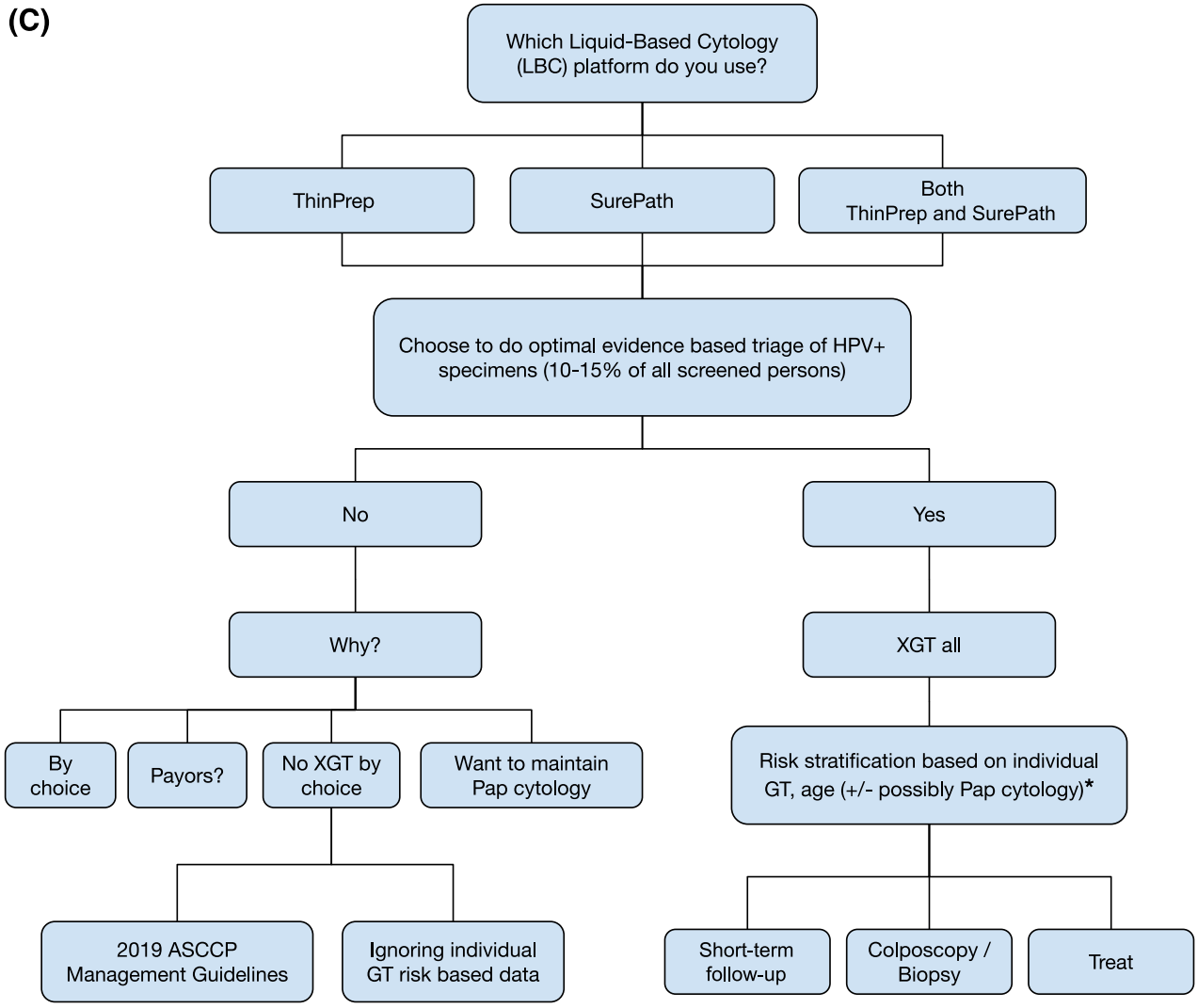


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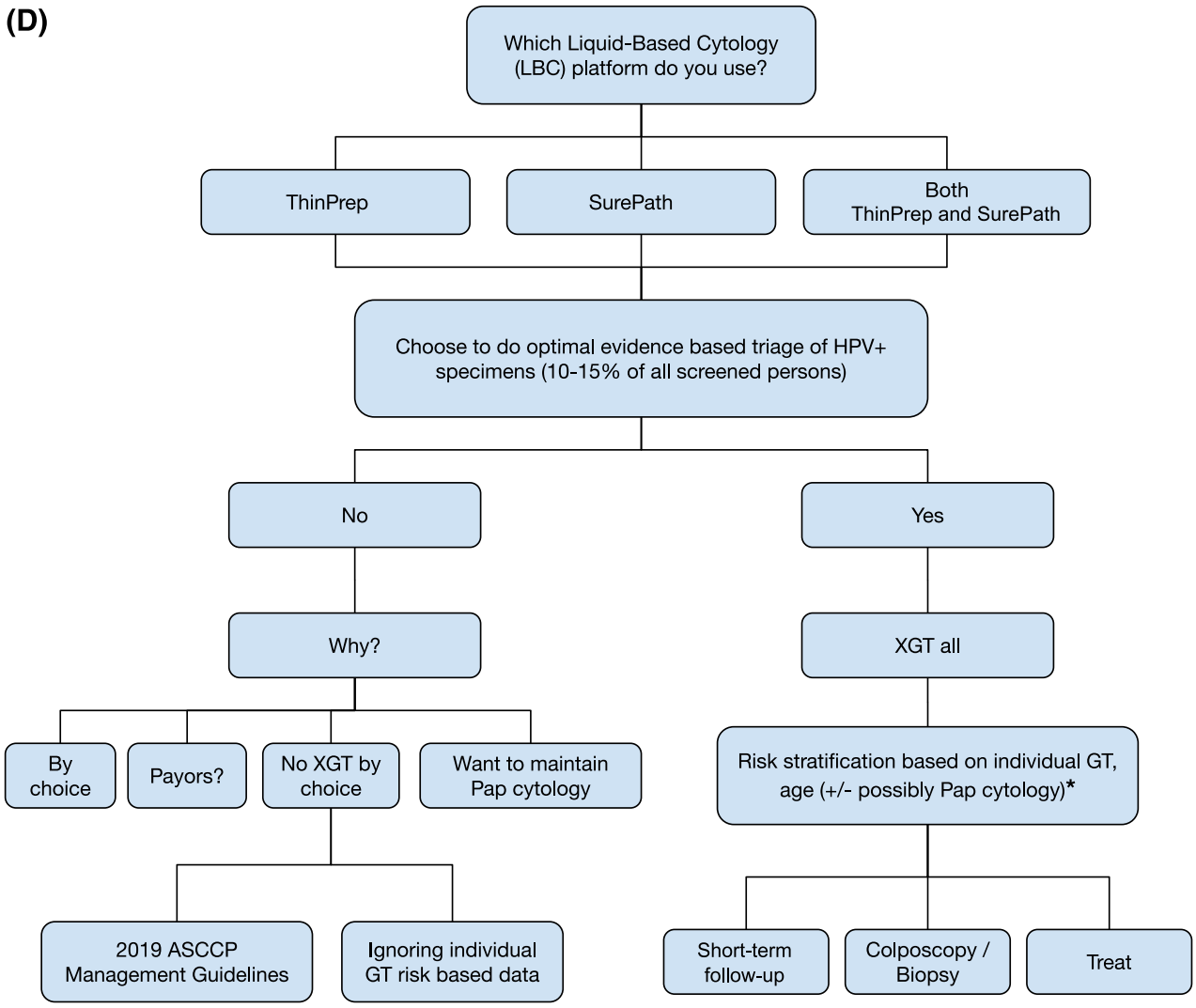


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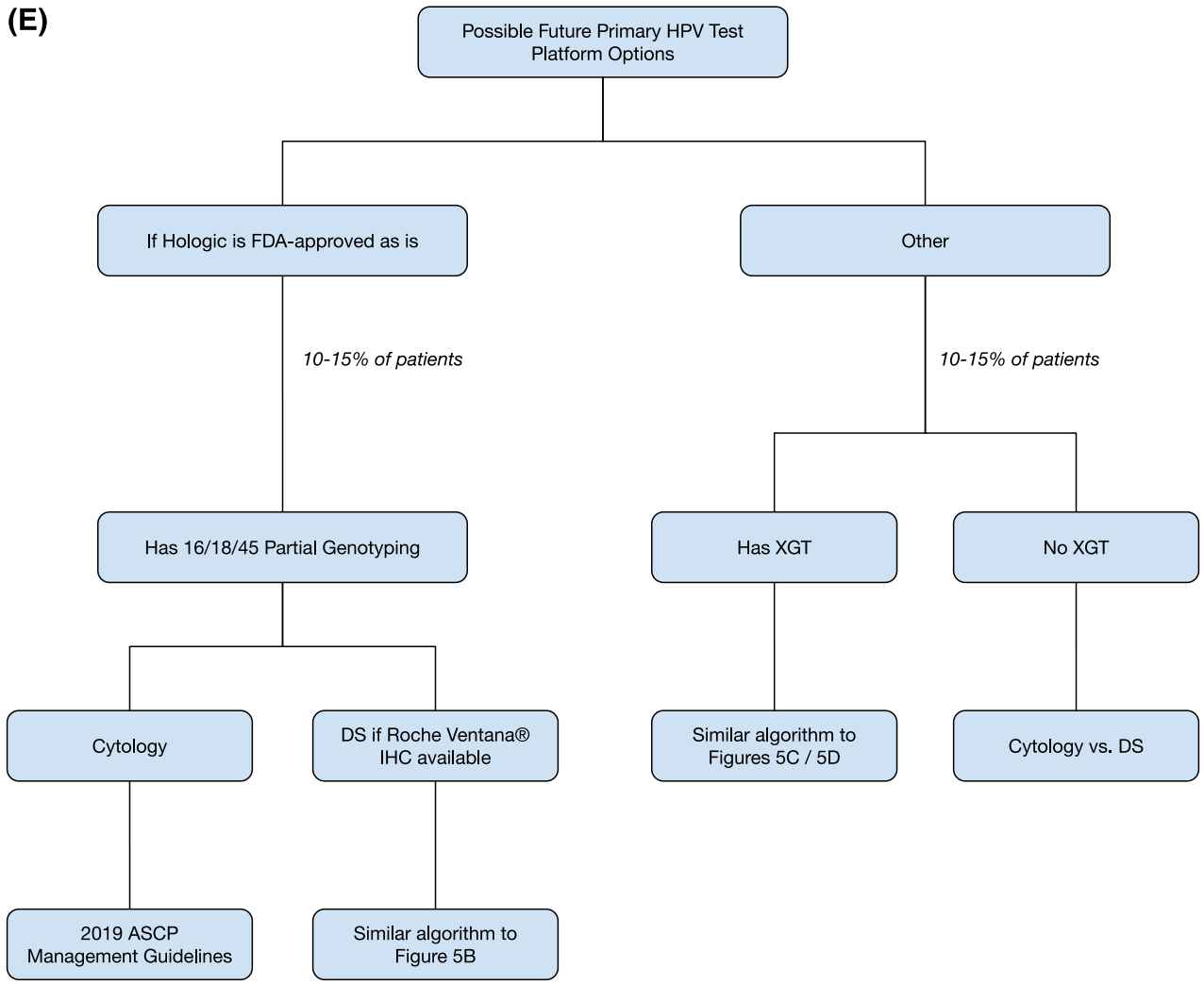


FIGURE 5 (Continued)

and expertise in cytomorphologic interpretation (cytologist and cytopathologist staff). Clinical provider test-ordering practices, acceptance of new screening guideline recommendations, and the adoption of primary HPV testing vary significantly throughout the country, and these factors must also be considered. For use as a tool to guide cytopathology laboratories and health system leaders in their decision making, flowcharts illustrating decision points and options have been developed through the ACS PHSI[69] and are provided in Figure 5A–E.[21,22]

ONGOING RESEARCH AND NEXT STEPS

Additional triage strategies currently under evaluation include markers of host methylation, which have been identified as increased in cervical precancer/cancer, in addition to HPV genome methylation testing.[70] Further study of the long-term effect of HPV vaccination on the prevalence of hrHPV in the United States is also needed in addition to examining HPV genotypes in underscreened or unscreened patient populations that hopefully will receive the benefit of screening through vaginal self-collection.

The updated USPSTF draft recommendations endorsed primary HPV screening by clinician and self-collection methods, with cytology and cotesting as alternate strategies. The open comment period for the draft concluded in January 2025, and final recommendations are pending.[18] It remains to be determined whether the ACS and USPSTF final guidelines will align and how professional organizations will endorse screening practices in the United States.

The time for laboratories to offer primary HPV cervical cancer screening is now. For cytopathology laboratories yet to offer primary HPV testing, there are several practical issues to consider before verification and implementation, as detailed above. These practical issues include, but are not limited to, the availability of an FDA-approved testing platform for primary HPV screening, the type(s) of LBC testing performed, the effect on workflow processes, the triage of hrHPV-positive screen results, the ordering of testing and reporting of results through the electronic medical record and integration with the laboratory information system, billing, client

education, and implications for the laboratory's quality-assurance program. The College of American Pathologists Laboratory Accreditation Program's 2024 checklists include modified and new requirements that pertain to primary HPV screening and dual stain. The approval of self-collection by the FDA in May 2024 affects laboratory and clinical provider workflows for ordering, screening, and triage of HPV-positive results from these vaginal specimens and management of test-positive patients.

Although cervical cytology has high specificity and has historically served as the triage mechanism for primary HPV-positive women, extended genotyping and biomarker assays such as DS offer additional triage test options. DS offers at least equivalent specificity to cytology; and, if chosen as a triage option, it requires additional cytologist and pathologist training and is not a fully objective assessment.

A comprehensive process exists for updating the 2019 ASCCP Cervical Cancer Screening and Management Guidelines through the Enduring Guidelines Committee, with information regarding the incorporation of DS and extended genotyping into the risk-based guidelines recently published.[21,22] The Enduring Guidelines Committee recommendations for the management of vaginal self-collection results are in press, and it is anticipated that they will be available in early 2025.

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CONFLICT OF INTEREST STATEMENT

Mark H. Stoler reports personal/consulting fees for clinical trial design and expert pathology services in HPV-related vaccine, diagnostic, and therapeutic trials from Merck, Roche/Ventana, BD Life Sciences, Abbott Molecular, Inovio Pharmaceuticals, and Frantz Viral Therapeutics outside the submitted work. The remaining authors disclosed no conflicts of interest.

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