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Recommendations for Use of p16/Ki67 Dual Stain for Management of Individuals Testing Positive for Human Papillomavirus

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Objectives: The Enduring Consensus Cervical Cancer Screening and Management Guidelines Committee developed recommendations for dual stain (DS) testing with CINtec *PLUS* Cytology for use of DS to triage high-risk human papillomavirus (HPV)-positive results.

Methods: Risks of cervical intraepithelial neoplasia grade 3 or worse were calculated according to DS results among individuals testing HPV-positive using data from the Kaiser Permanente Northern California cohort and the STudying Risk to Improve DisparitiES study in Mississippi. Management recommendations were based on clinical action thresholds developed for the 2019 American Society for Colposcopy and Cervical Pathology Risk-Based Management Consensus Guidelines. Resource usage metrics were calculated to support decision-making. Risk estimates in relation to clinical action thresholds were reviewed and used as the basis for draft recommendations. After an open comment period, recommendations were finalized and ratified through a vote by the Consensus Stakeholder Group.

Results: 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 testing DS-positive. One-year follow-up with HPV-based testing is recommended for individuals testing DS-negative, except for HPV16- and HPV18-positive results, or high-grade cytology in cotesting, where immediate colposcopy referral is recommended. Risk estimates were similar between the Kaiser Permanente Northern California and STudying Risk to Improve DisparitiES populations. In general, resource usage metrics suggest that compared with cytology, DS requires fewer colposcopies and detects cervical intraepithelial neoplasia grade 3 or worse earlier.

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Conclusions: Dual stain testing with CINtec *PLUS* Cytology is acceptable for triage of HPV-positive test results. Risk estimates are portable across different populations.

Key Words: cervical cancer, screening, recommendations, dual stain, human papillomavirus, triage

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Secondary cervical cancer prevention efforts in adults in the United States include population-based screening, colposcopy triage, and treatment of precursor lesions. Using risks of precancer and cancer to determine management optimizes cancer prevention by reducing unnecessary procedures for low-risk patients while focusing diagnostic testing and treatment on high-risk patients who are most likely to benefit. In 2019, a national consensus conference developed the American Society for Colposcopy and Cervical Pathology (ASCCP) Risk-Based Management Consensus Guidelines for management of individuals with high-risk human papillomavirus (HPV), abnormal cytology, and cervical biopsy results (hereafter referred to as 2019 Guidelines).[1] Recommendations are based on immediate and 5-year risks for cervical precancer (including cervical intraepithelial neoplasia grade 3 [CIN3] and adenocarcinoma in situ) and cancer (collectively defined as CIN3+)[1] and linked to clinical action thresholds. Subsequently, the approach was expanded to use of 3-year risks of CIN3+ when 5-year risks are not available.[2]

The Enduring Consensus Cervical Cancer Screening and Management Guidelines (Enduring Guidelines) process was established to incorporate new data and integrate new technologies and approaches into the existing guidelines framework. In March 2020, the US Food and Drugs Administration approved the use of $p16^{ink4a}$/Ki-67 dual stain (DS) for cytology (commercially available in the United States as CINtec *PLUS* Cytology [Roche Diagnostics, Indianapolis, IN])[3] for triage to inform management of individuals with positive HPV results from screening with primary HPV testing or with cotesting. Dual staining of cytology specimens detects a marker of HPV-related oncogene activity (p16, a tumor suppressor protein) and a marker of cell proliferation (Ki-67) that, when detected together in the same cell, is indicative of cell cycle dysregulation associated with transforming HPV infections and strongly associated with precancerous cellular changes (CIN3+).[4–6] The Enduring Guidelines Committee convened a series of virtual meetings to review the available evidence regarding risk of CIN3+ after DS testing in individuals with HPV-positive results and to make recommendations based on the principle of equal management of equal risks.

METHODS

The overall principles and approach of the Enduring Guidelines evidence review, risk assessment, and development of recommendations, are summarized separately (Wentzensen et al.). The Enduring Guidelines effort is conducted by 20 organizations under clinical leadership from ASCCP and American Cancer Society, scientific and analytic leadership from the National Cancer Institute, and with consultation from the Centers for Disease Control and Prevention (CDC). The CDC is not a member organization and did not participate in the final voting process. The purpose of the DS evidence review was to evaluate risks of CIN3+ among individuals with HPV-based (primary HPV or cotesting) screen-positive test results according to DS triage testing results (positive or negative) and to define management based on clinical action thresholds for systems that choose to adopt DS. These recommendations do not include a comparative effectiveness evaluation of DS and other technologies and cannot address whether DS should be adopted in a specific setting. Cost-effectiveness was also not evaluated because it is beyond the scope of Enduring Guidelines.

The Risk Assessment group, led by researchers at the National Cancer Institute, conducted an extensive data analysis effort to produce CIN3+ and cancer risk estimates for the following clinical scenarios, informed by current FDA indications: 1) triage of HPV-positive test results when limited genotyping is not available from the screening test; 2) triage of HPV-positive test results when limited HPV genotyping is provided by the screening test; 3) triage of HPV-positive test results used in conjunction with cytology (cotesting); and 4) triage of HPV-positive test results in follow-up after abnormal screening results, colposcopy, and treatment. For these recommendations, the term "limited genotyping" refers to FDA-approved assays for primary screening that report HPV16 and HPV18 separately, including assays that provide HPV16, HPV18, and other 12 high-risk types (HR12) and assays that report additional genotypes beyond HPV16 and HPV18. Currently, DS has not been proposed as a stand-alone primary screening test or for triage of cytology results; nor is DS proposed for refining the management of individuals testing HPV-negative. Thus, risk assessments and recommendations were not addressed for these scenarios.

The Evidence Assessment Working Group, including clinicians, pathologists, content experts, and representatives of national organizations, reviewed the CIN3+ risk estimates in relation to clinical action thresholds and drafted recommendations, considering the precision of risk estimates and additional appropriate information such as cancer risk and resource usage metrics. Draft recommendations were affirmed by the Consensus Stakeholder Group, whose members were instructed to share the draft recommendations with their respective organizations for further input. The draft recommendations were subsequently presented at the 2023 ASCCP annual meeting followed by a 6-week open public comment period, publicized by stakeholder organizations. Further revisions were made before recommendations were presented at a final Consensus Stakeholder meeting in June 2023, where public comments were reviewed and additional revisions were proposed and considered. The final recommendations were confirmed by a virtual vote of organization representatives, passing the required two-thirds majority vote for all recommendations. The terminology for these recommendations and grading of recommendation strength (A–E), and quality of evidence (I–III), followed that in the 2019 Guidelines (Supplemental Boxes 1 and 2, http://links.lww.com/LGT/A335).[1]

Primary Data Sources

The 2019 Guidelines used risk estimate data from Kaiser Permanente Northern California (KPNC) to develop risk thresholds and management recommendations[7] for the US population. Primary data on DS testing were available from the KPNC population of individuals aged 25 years and older with HPV-positive results.[5,8] To assess the performance of these approaches in a racially, geographically, and economically distinct population not served by a comprehensive, integrated health care system like KPNC, we also evaluated data from the STudying Risk to Improve DisparitiES (STRIDES) study. The STRIDES is a diverse, statewide cohort study of individuals undergoing cervical cancer screening and management in the state of Mississippi at the University of Mississippi Medical Center or the Mississippi State Department of Health.[9,10] Both primary data sources are described in more detail in the Supplemental Material, http://links.lww.com/LGT/A335.

Other Data Sources

Like the 2019 Guidelines, the current recommendations were based on data from primary sources to calculate CIN3+ and cancer risk estimates. In addition, we reviewed summary performance estimates from studies published in the literature evaluating CINtec *PLUS* Cytology for triage of HPV-positive results, including those

from industry and international settings.[11,12] These data were not included in the risk assessment calculations for the development of recommendations (Wentzensen et al.); however, they were considered as additional supporting evidence in this process (Supplemental Table 1, http://links.lww.com/LGT/A335).

Risk-Based Approach

Consistent with the process for the 2019 Guidelines, a risk-based approach was used to determine clinical actions.[13] For each clinical scenario listed previously, we estimated the immediate and 3-year risks of developing CIN3+ using prevalence-incidence mixture models in KPNC data.[2] Prevalence-incidence mixture models consist of a logistic regression model for CIN3+ prevalent at baseline (i.e., immediate risk) and a Cox proportional hazards model for incident CIN3+ occurring after baseline. In STRIDES, we estimated immediate CIN3+ risks only because the duration of follow-up is currently insufficient to estimate cumulative 3-year risks and management confidence probabilities. The resulting clinical actions are based on risk thresholds determined by the 2019 Guidelines. For tests used to triage HPV-positive results, like DS, the colposcopy risk threshold is central to the recommendations: colposcopy is recommended when the immediate risk of CIN3+ is 4%–24%, whereas a 1-year return is recommended when the 5-year CIN3+ risk is 0.55% or higher. Management confidence probabilities for risk-based management recommendations were calculated as previously described,[13] which incorporate both the precision of the risk estimates and how close they are to a clinical action threshold. The management confidence probability is the probability that the risk estimated using another random sample of individuals with the same test results from the same population would have the same recommendation.

Resource Utilization

Clinical resource usage metrics were computed for different scenarios. These included number of colposcopy referrals, number of tests performed, and time to CIN3+ diagnosis (in years), which was estimated under the simplified assumptions that a) colposcopy referral followed stated recommendations for DS and the 2019 Guidelines for cytology, b) 100% colposcopy attendance among those referred, and c) 100% CIN3+ detection at colposcopy. Fewer colposcopy referrals, tests, and fewer years to CIN3+ diagnosis were considered positive attributes of a testing strategy. Resource usage metrics were estimated using test performance data from KPNC and applied to a hypothetical population of 100,000 individuals undergoing screening with primary HPV testing or cotesting in scenarios where positive HPV test results were triaged with either cytology or DS, followed from the baseline HPV-positive result through 3 years of follow-up. Importantly, each resource usage analysis is a distinct comparison between the respective approaches. Resource usage metrics should not be used for comparison across different recommendations.

- Key Points:

1. These recommendations only apply to FDA-approved DS cytology assays. Currently, CINtec PLUS Cytology (Roche Diagnostics) is the only DS test with FDA approval. The performance of other, non–FDA-approved p16/Ki67 assays may not be similar, and the generalizability of these recommendations cannot be assumed.
2. These recommendations apply only to results obtained in asymptomatic women; symptomatic women should undergo testing according to relevant protocols.[14,15]
3. Because of limited data availability for multiple rounds of testing or for specific clinical scenarios, estimates for downstream risks of CIN3+ are either not available or are insufficient to allow for the development of risk-based recommendations for all possible scenarios related to DS testing. For example, this includes a scenario of postcolposcopy management when the squamocolumnar junction was not fully visualized and no histologic CIN2+ was found on colposcopic biopsy/endocervical curettage. In these situations where recommendations are not available, clinical judgment and shared decision-making should consider the 2019 Guidelines[1] and 2017 Colposcopy Standards,[16] where applicable, and may also consider the increased risk that follows from DS-positive test results and the decreased risk that follows from DS-negative test results. Additional recommendations may be generated as more data become available allowing robust risk estimation.

Recommendations and Supporting Evidence:

I. Dual stain for triage of HPV-positive results when limited genotyping is not available from the screening test

Recommendation: ***DS is acceptable for triage of individuals testing HPV-positive when limited genotyping is not available. If using DS to triage HPV-positive test results without genotyping, colposcopy is recommended for individuals testing HPV-positive and DS-positive, and 1-year return is recommended for individuals testing HPV-positive and DS-negative (A-II).***

Rationale and Risk Estimates Supporting Recommendation: Risk estimates from KPNC supporting the recommendation for DS triage of individuals testing HPV-positive are shown in Table 1. The risks for HPV-positive/DS-positive exceeded the colposcopy threshold in all scenarios, even for those with previous HPV-negative screening results, indicating that history does not change patient management when DS is used. The HPV-positive, DS-positive results had an immediate CIN3+ risk of 9.5%, which met the colposcopy referral threshold of 4%–24%. In contrast, HPV-positive, DS-negative results met criteria for a 1-year return (immediate risk 0.75%, 3-year risk 1.5%). At the 1-year return, repeat testing with HPV-based testing is recommended, consistent with the 2019 Guidelines. A previous HPV-negative test result reduced the estimated immediate CIN3+ risks but did not change management: DS-positive met the colposcopy referral threshold (4.9%) and DS-negative met the 1-year return threshold (immediate risk 0.16%, 3-year risk 1.2%). The immediate CIN3+ risks for DS triage of individuals testing HPV-positive were similar in the STRIDES cohort (11.5% for DS-positive, 0.7% for DS-negative), indicating similar test performance in different populations (Table 2). Data on 3-year follow-up and history were not available in STRIDES. This is a recommendation for HPV tests without genotyping. Tests approved for primary screening in the United States currently include limited genotyping (i.e., testing separately for HPV16 and HPV18) information.

Resource Utilization Metrics: Models comparing DS triage of HPV-positive results to cytology triage of HPV-positive results used the preceding guideline for DS management and 2019 Guidelines for cytology management. Under these assumptions using test performance data from KPNC, DS triage of HPV-positive results yielded 12% fewer total colposcopies and 40% fewer years to CIN3+ diagnoses compared with triage with cytology (Supplemental Table 2, http://links.lww.com/LGT/A335).

II. Dual stain for triage of HPV-positive results when limited genotyping is provided by the screening test

Recommendation: ***A combination of DS and limited genotyping (provided by the screening HPV test) is acceptable for triage of***

TABLE 1. Dual Stain for Triage of HPV-Positive Results in KPNC[a]

History	Current test result	N	% of Total	CIN3+ cases	CIN3+ immediate risk (%)	CIN3+ 3-y cumulative risk (%)	Clinical management recommendation	Management confidence probability (%)
Not considered	HPV+/DS+	3,384	4.0	362	9.5	12.3	**Colposcopy**	100
Not considered	HPV+/DS−	3,458	4.1	44	0.75	1.5	**1-y return**	100
HPV-negative	HPV+/DS+	710	1.6	48	4.9	7.9	**Colposcopy**	86
HPV-negative	HPV+/DS−	991	2.2	9	0.16	1.2	**1-y return**	97

[a]These results apply to HPV test results when limited genotyping is not available from the screening test.

CIN3+ indicates cervical intraepithelial neoplasia grade 3 or worse; DS, dual stain; HPV, human papillomavirus; KPNC, Kaiser Permanente Northern California.

individuals testing HPV-positive. If using DS to triage HPV-positive test results with limited genotyping, colposcopy is recommended for individuals testing positive for HPV16 or HPV18. For individuals testing positive for the pool of HR12, colposcopy is recommended when DS is positive and 1-year return is recommended when DS is negative (A-II).

Rationale and Risk Estimates Supporting Recommendation: Risk estimates from KPNC supporting the recommendation for DS triage of individuals testing HPV-positive when limited genotyping is provided by the screening test are shown in Table 3. Risk estimates are shown for DS-positive and DS-negative test results in strata of limited genotyping. Strata are ordered hierarchically, from the most to least carcinogenic HPV types: HPV16, followed by HPV18, followed by HR12. All strata with DS-positive results met the colposcopy threshold of 4%–24% immediate CIN3+ risk. Immediate CIN3+ risks are similar in the KPNC cohort (23.0% for HPV16-positive, 11.3% for HPV18-positive, and 5.6% for HR12-positive; Table 3) and STRIDES cohort (24.2% for HPV16 +, 5.6% for HPV18+, and 7.9% for HR12+; Table 4). The risks for HPV HR12-positive/DS-negative met the 1-year return threshold in both the KPNC cohort and STRIDES cohort (immediate CIN3+ risk in both cohorts of 0.5%; 3-year risk in KPNC cohort of 1.1%). At the 1-year return, repeat testing with HPV-based testing (HPV alone or HPV/cytology cotesting) is recommended, consistent with the 2019 Guidelines.

Special considerations for HPV16 and HPV18: The CIN3+ risks of HPV-positive/DS-negative results were below the colposcopy threshold for all genotype categories and met criteria for a 1-year return. However, HPV16 and HPV18 are most strongly associated with cervical cancer. Although the CIN3+ risks of individuals testing HPV16-positive, DS-negative or HPV18-positive, DS-negative are below the colposcopy referral threshold, additional follow-up data to ensure a very low risk of cancer are required to support risk-based management for this group. In the interim, it is recommended that all HPV16- and HPV18-positive results are referred to colposcopy, independent of DS result.

***Resource Usage Metrics*:** Models assumed management of DS results according to recommendations as described previously and cytology results per 2019 Guidelines. Under these assumptions using test performance data from KPNC, triaging HPV HR12-positive results with DS yields 11% fewer total colposcopies and 22% fewer years to CIN3+ diagnoses compared with triage with cytology (Supplemental Table 3, http://links.lww.com/LGT/A335).

III. DS for triage of HPV-positive cytology results in cotesting

Recommendation: ***In a cotesting setting, DS is acceptable for triage of individuals with HPV-positive test results and negative for intraepithelial lesion or malignancy (NILM), atypical squamous cell of undetermined significance (ASC-US), or low-grade squamous intraepithelial lesion (LSIL) cytology. If using DS to triage HPV-positive cotesting results without genotyping, colposcopy is recommended for individuals testing HPV-positive with NILM, ASC-US, or LSIL cytology, and positive for DS, and a 1-year return is recommended for individuals testing HPV-positive with NILM, ASC-US, or LSIL cytology, and negative for DS (A-II).***

If using DS to triage HPV-positive cotesting results of NILM, ASC-US or LSIL with limited genotyping, colposcopy is recommended for individuals testing positive for HPV16 or HPV18. For individuals testing positive for the pool of HR12 with NILM, ASC-US, or LSIL cytology, colposcopy is recommended when DS is positive and 1-year return is recommended when DS is negative (A-II).

Use of DS in individuals with cytology results of atypical squamous cells, cannot rule out high-grade (ASC-H), atypical squamous cells, cannot rule out high-grade (AGC), or high-grade squamous intraepithelial lesion (HSIL) is not recommended, and if obtained, should not guide management (D-III).

Rationale and Risk Estimates Supporting Recommendation: Risk estimates from KPNC supporting the recommendation for DS triage of HPV-positive NILM, ASC-US, and LSIL cotesting results

TABLE 2. Dual Stain Triage of HPV-Positive Results in STRIDES[a,b]

History	Current test result	N	% of Total	CIN3+ cases	CIN3+ immediate risk (%)	Clinical management recommendation
Not considered	HPV+/DS+	768	7.6	88	11.5	**Colposcopy**
Not considered	HPV+/DS−	1,113	11.0	8	0.7	**1-y return**

[a]These results apply to HPV test results when limited genotyping is not available from the screening test.

[b]Duration of follow-up in the STRIDES cohort is currently not sufficient to estimate cumulative 3-year risks and management confidence probabilities; follow-up is ongoing.

STRIDES indicates STudying Risk to Improve DisparitiES in Mississippi

TABLE 3. Dual Stain Triage of HPV-Positive Results When Limited Genotyping Is Provided by the Screening Test in KPNC

Current test result	N	% of Total	CIN3+ cases	CIN3+ immediate risk (%)	CIN3+ 3-y cumulative risk (%)	Clinical management recommendation	Management confidence probability%
DS+/HPV16+	681	0.8	172	23.0	29.4	**Colposcopy**	88
DS−/HPV16+	325	0.4	15	2.6	5.0	**Special situation**	N/A
DS+/HPV18+	200	0.2	26	11.3	15.2	**Colposcopy**	100
DS−/HPV18+	137	0.2	2	1.1	2.4	**Special situation**	N/A
DS+/HR12+	2,503	3.0	164	5.6	7.6	**Colposcopy**	100
DS−/HR12+	2,996	3.5	27	0.53	1.1	**1-y return**	100

HR12 indicates positive for pooled 12 high-risk HPV types.

are shown in Table 5. Among individuals with HPV-positive NILM, ASC-US, or LSIL results, all DS-positive results met the colposcopy threshold of 4%–24%, and DS-negative results met the threshold for repeat testing in 1 year. At the 1-year return, repeat testing with HPV-based testing (HPV alone or HPV/cytology cotesting) is recommended, consistent with the 2019 Guidelines. The immediate CIN3+ risks of HPV-positive NILM, ASC-US, or LSIL cytology with DS-positive results were 4.1%–6.6%. The CIN3+ risks of HPV-positive NILM, ASC-US, or LSIL cytology with DS-negative results were 0.53%–0.87% (immediate) and 0.92%–1.6% (3 years). Immediate CIN3+ risks for DS-positive and DS-negative ASC-US and LSIL were similar in the STRIDES cohort (7.5% and 8.0% vs 0% and 1.1%); follow-up is still underway for HPV-positive NILM in STRIDES (Table 6).

The 2019 Guidelines recommend colposcopy for HPV16-positive and HPV18-positive in the setting of NILM, ASCUS, and LSIL cytology results, and they recommend colposcopy or, in some cases, expedited treatment for ASC-H, AGC, and HSIL cytology results; currently, DS results should not alter management in these situations.

Resource Utilization Metrics. As in the previous section, the models managed DS results per the aforementioned recommendations and cotesting results per 2019 Guidelines. Under these assumptions using test performance data from KPNC, cotesting with DS triage results in 11% fewer total colposcopies and 64% fewer years to CIN3+ diagnoses compared with cotesting alone. Dual stain triage results in a 5.7% increase of number of tests through 3 years (Supplemental Table 4, http://links.lww.com/LGT/A335).

IV. Use of DS in follow-up after abnormal screening results, colposcopy, or treatment (surveillance settings)

Recommendation: ***When patients are being followed after (a) abnormal screening test results that did not require colposcopy, (b) colposcopy, or (c) treatment, it is acceptable to use DS according to the guidelines for management of an initial abnormal screening test result (B-II). For example, when using HPV-based testing (primary HPV or cotesting) colposcopy is recommended after an HPV-positive/DS-positive result, and 1-year follow-up is recommended after an HPV-positive, DS-negative result, except in the case of HPV16-positive, HPV18-positive, AGC, ASC-H, or HSIL, for which management according to the 2019 Guidelines is recommended (colposcopy or expedited treatment).***

For patients with 3 or more consecutive HPV-positive, DS-negative results, follow-up in 1 year or colposcopy is acceptable (C-III).

Rationale and Risk Estimates Supporting Recommendation: Data in the screening and triage setting indicate that DS provides greater risk discrimination than cytology and that history has less impact on the risk estimate of a DS result than a cytology result (see Table 1 and Supplemental Tables 5 and 6, http://links.lww.com/LGT/A335). Limited DS data were available to assess CIN3+ risk estimates in different follow-up settings in KPNC. Among those with 2 consecutive 1-year repeat HPV-positive, DS-negative test results, the risk of CIN3+ was below the colposcopy referral threshold (1.1%; Supplemental Table 6, http://links.lww.com/LGT/A335). In the postcolposcopy and posttreatment settings, the CIN3+ risks among individuals with HPV-positive, DS-positive test results were above the colposcopy referral threshold (7.9% and 182%, respectively) and among those with HPV-positive, DS-negative results, the risks were below the colposcopy referral threshold (0.39% and 0.0%, respectively; Supplemental Table 7, http://links.lww.com/LGT/A335). These data suggest that DS provides similar risk stratification in follow-up after abnormal screening results, colposcopy, or treatment as in the screening/triage settings. It follows that DS can be used in the settings of follow-up after abnormal screening tests, colposcopy, or treatment

TABLE 4. Dual Stain Triage of HPV-Positive Results When Limited Genotyping Is Provided by the Screening Test in STRIDES[a]

Current test result	N	% of Total	CIN3+ cases	CIN3+ immediate risk (%)	Clinical management recommendation
DS+/HPV16+	178	1.8	43	24.2	**Colposcopy**
DS−/HPV16+	110	1.1	2	1.8	**Special situation**
DS+/HPV18+	72	0.7	4	5.6	**Colposcopy**
DS−/HPV18+	84	0.8	0	0.0	**Special situation**
DS+/HR12+	518	5.1	41	7.9	**Colposcopy**
DS−/HR12+	919	9.1	4	0.5	**1-y return**

[a]Duration of follow-up in the STRIDES cohort is currently not sufficient to estimate cumulative 3-year risks and management confidence probabilities; follow-up is ongoing.

STRIDES indicates STudying Risk to Improve DisparitiES in Mississippi.

TABLE 5. Dual Stain Triage of HPV-Positive NILM, ASC-US, or LSIL Cytology Results in a Cotesting Setting in KPNC

Current test result	N	% of Total	CIN3+ cases	CIN3+ immediate risk (%)	CIN3+ 3-y cumulative risk (%)	Clinical management recommendation	Management confidence probability%
HPV+/NILM/DS+	1,003	1.4	73	4.6	8.6	**Colposcopy**	79
HPV+/NILM/DS−	1,864	2.7	20	0.60	1.5	**1-y return**	100
HPV+/ASC-US/DS+	978	1.0	82	6.6	9.9	**Colposcopy**	100
HPV+/ASC-US/DS−	954	1.0	15	0.87	1.6	**1-y return**	100
HPV+/LSIL/DS+	942	0.9	46	4.1	5.9	**Colposcopy**	58
HPV+/LSIL/DS−	595	0.6	7	0.53	0.92	**1-y return**	100

ASC-US indicates atypical squamous cells of undetermined significance; LSIL, low-grade intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy.

using the same recommendations outlined for the screening/triage settings. Specifically, DS can be used for triage of HPV-positive test results when primary HPV testing is used, or for triage of HPV-positive NILM, ASCUS, or LSIL results when cotesting is used.

Data are not yet available for more than 2 repeat results of HPV-positive, DS-negative. If HPV-positive, DS-negative results persist through 3 consecutive, annual repeat tests (i.e., ≥2 years), clinical judgment should be used for management, and either continued repeat testing or referral to colposcopy may be considered.

V. Unsatisfactory DS results

Recommendation: ***When a DS result is unsatisfactory due to sampling issues, repeating the sample as soon as convenient and no later than 4 months is acceptable (C-III). If other satisfactory results are available at the time of DS testing that can be used for management according to risk (e.g., HPV16- or HPV18-positive or cytology results), management based on those results is also acceptable*** **(C-III).**

Rationale: DS involves examination of cervical cells and therefore may be unsatisfactory due to insufficient cellularity of the specimen. In these cases, precancer cannot be excluded, and the sample should be recollected within 4 months.[17] When other test results are available at the time of DS testing based on which the patient can be managed according to the 2019 Guidelines, these results may be used for management without repeating DS testing.

SUMMARY AND FUTURE DIRECTIONS

The Enduring Consensus Cervical Cancer Screening and Management Guidelines provide recommendations for the application of DS testing for clinical practices that choose to use DS to triage HPV-positive results. These recommendations were developed based on a thorough review of the available evidence regarding DS testing and risk estimates generated from large studies of individuals testing HPV-positive in 2 distinct and diverse clinical populations.[5,8–10] Although data underlying these recommendations are robust, with high-confidence scores for most risk estimates, more data will be needed to address cancer risk among individuals with HPV16- and HPV18-positive, DS-negative results. In the interim, the 2019 Guidelines for colposcopy for HPV16- and HPV18-positive results were carried forward to maximize safety.[1] In the future, these recommendations may require revision as follow-up data become available, particularly in surveillance settings.

To ensure applicability of DS recommendations to different populations, we assessed risk estimates in 2 very distinct cohorts: KPNC (the main dataset used for the 2019 Guidelines) and STRIDES in Mississippi. The KPNC cohort is approximately 44% White, 20% Hispanic, 20% Asian, and 10% Black/African American, with the remainder of mixed race or other race; most KPNC patients have employer-based insurance. The STRIDES dataset provides additional support for the generalizability of DS performance because it represents a diverse statewide sample, including patients with publicly funded screening, 80% of whom live in rural areas, and 58% of whom identify as Black or African American. Data used to estimate resource usage metrics required at least 3 years of follow-up and came exclusively from KPNC. Although resource usage metrics may differ somewhat in settings with different patient characteristics or test performance, the high concordance of risk estimates between KPNC and STRIDES suggest that the conclusions from the metrics presented here are applicable across populations.

Several options are currently available for risk stratification of individuals who test positive for HPV, including DS, cytology, and limited genotyping. The current recommendations are intended to guide clinical management among those choosing to use CINtec

TABLE 6. Dual Stain Triage of HPV-Positive NILM, ASC-US, or LSIL Cytology Results in a Cotesting Setting in STRIDES[a]

Current test result	N	% of Total	CIN3+ cases	CIN3+ immediate risk (%)	Clinical management recommendation
HPV+/NILM/DS+	332	3.3	7		*Follow-up still underway*
HPV+/NILM/DS−	929	9.2	5		*Follow-up still underway*
HPV+/ASC-US/ DS+	145	1.4	11	7.5	**Colposcopy**
HPV+/ASC-US/ DS−	95	0.9	1	1.1	**1-y return**
HPV+/LSIL/DS+	150	1.5	12	8.0	**Colposcopy**
HPV+/LSIL/DS−	68	0.7	0	0.0	**1-y return**

[a]Duration of follow-up in the STRIDES cohort is currently not sufficient to estimate risks associated with NILM/DS+, cumulative 3-year risks, and management confidence probabilities; follow-up is ongoing.

PLUS (DS); they do not constitute a preference or recommendation for one test or combinations of tests over others. We did not directly compare test accuracy, efficiency, or cost-effectiveness of various strategies when developing recommendations for DS; comparative trials would be needed to assess the accuracy and efficiency of various risk assessment strategies for individuals who test positive for HPV. Further, resource usage metrics are only intended for comparisons of different approaches within each scenario and should not be used for comparisons across different recommendations. Costs may vary, and laboratories and clinical practices can use DS risk estimates and resource usage metrics to inform considerations about whether and how to incorporate DS into clinical practice.

Dual stain is a robust marker of CIN3+ risk and can be incorporated into clinical management strategies. Existing clinical decision support tools (e.g., the ASCCP app) plan to incorporate these recommendations for use of DS. Future opportunities exist to explore the accuracy of DS in primary screening settings, and of DS in combination with novel strategies such as extended genotyping and automated approaches.[18]

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