Supplemental Material

**Supplemental Methods**

**Primary Data Sources**

**Kaiser Permanente Northern California (KPNC).** KPNC is a large integrated healthcare system providing cervical cancer screening to individuals aged 25 years and older with HPV (Hybrid Capture 2; HC2 High-Risk HPV DNA Test; Qiagen Inc) and cytology co-testing every 3 years. Cytology samples were collected in SurePath (Becton Dickinson) fixative, and SurePath slides were prepared, stained, and processed on the BD FocalPoint Slide Profiler (Becton Dickinson) as part of routine clinical practice. Cytologic interpretation occurs with prior knowledge of the HPV result. The KPNC cohort is approximately 44% White, 20% Hispanic, 20% Asian, and 10% Black/African American, with the remainder mixed race or other; the majority of KPNC patients have employer-based insurance.[1] Primary data on dual stain (DS) testing are available from two nested studies within the KPNC population of individuals with HPV-positive test results. The first cohort included 3,225 individuals who underwent co-testing from September to October 2015, with follow-up through 2022.[2] Residual SurePath samples were used for DS testing. DS slides were prepared and stained by a histology technician at KPNC who successfully completed CINtec PLUS DS training, using the CINtec PLUS kit. The DS cytologic slides were evaluated by KPNC cytologists after a 2-day training and certification to evaluate p16/Ki-67 DS cytologic slides. DS data from the second cohort were obtained from the Improving Risk Informed HPV Screening (IRIS) Study.[1] IRIS includes 3,617 undergoing co-testing between 2016 and 2018, with follow-up through 2022. DS slides were produced at the manufacturer’s laboratory using the CINtec PLUS Cytology kit (Roche) according to instructions. For both cohorts, additional partial genotyping data (HPV16, HPV18, other 12 high-risk HPV positive) were available from cobas 4800 (Roche) testing on residual matching specimen transport medium specimens.

**The STRIDES Cohort (STudying Risk to Improve DisparitiES) in Mississippi).** 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 (MSDH).[3, 4] Analyses for DS recommendations are based on data from individuals at MSDH only. The MSDH population in STRIDES is approximately 25% White, and 58% Black/African American, with the remainder mixed race or other. Approximately 80% of MSDH clinics are located in rural areas, and cervical cancer screening services are covered by public funding at MSDH clinics. Primary data on DS testing were available from individuals 30 years and older with positive HPV test results who underwent co-testing in 2018-2019 at MSDH with follow-up through 2022. Liquid-based cytology is performed on all cervical cytologic specimens using the ThinPrep Pap 2000 System (Hologic). Following specimen processing and prescreening with automated image analysis, the cytotechnologist continues with full screening prior to final cytologic interpretation by a pathologist. Cytologic interpretation occurs without prior knowledge of the HPV result.DS slides were produced from residual ThinPrep cytology samples at the manufacturer’s laboratory using the CINtec PLUS Cytology kit (Roche) according to instructions. Partial genotyping data (HPV16, HPV18, other 12 high-risk HPV positive) were available from clinical testing with cobas 4800 (Roche) from ThinPrep specimens.

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| **Supplemental Box 1. Terminology used for recommendations**   * Recommended: Good data to support use when only one option is available. * Preferred: Option is the best (or one of the best) when there are multiple options * Acceptable: One of multiple options when there is either data indicating that another approach is superior or when there are no data to favor any single option * Not recommended: Weak evidence against use and marginal risk for adverse consequences * Unacceptable: Good evidence against use |

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| **Supplemental Box 2. Grading of Recommendations** |
| |  |  | | --- | --- | | **Strength of Recommendation Rating** | **Description** | | A | Good evidence for efficacy and substantial clinical benefit support recommendations for use | | B | Moderate evidence for efficacy or only limited clinical benefit supports recommendation for use | | C | Evidence for efficacy is insufficient to support a recommendation for or against use, but recommendations may be made on other grounds | | D | Moderate evidence for lack of efficacy or for adverse outcomes supports a recommendation against use | | E | Good evidence for lack of efficacy or for adverse outcomes supports a recommendation against use | | **Quality of Evidence Rating** | **Description** | | I | Evidence from at least one randomized, controlled trial | | II | Evidence from at least one clinical trial without randomization, from cohort or case-controlled analytic studies (preferably from more than one center), or from multiple time-series studies, or dramatic results from uncontrolled experiments | | III | Evidence from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees | |
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**Supplemental Results**

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| Supplemental Table 1. Summary Performance Metrics from U.S. Studies Evaluating Performance of Dual Stain for Triage of HPV-positive Test Results | | | | | |
| **Study** | **Data Included in Risk Estimates** | **Total HPV positive** | **DS Positivity (%)** | **DS Sensitivity (%)** | **DS Specificity (%)** |
| PILOT DS (KPNC) | No | 2,364 | 46.0 | 86.9 | 56.9 |
| IMPLEMENT DS (KPNC) | Yes | 3,225 | 49.9 | 88.6 | 53.1 |
| IRIS DS (KPNC) | Yes | 3,617 | 49.1 | 87.3 | 54.6 |
| STRIDES (MS) | Yes | 1,922 | 40.0 | 91.7 | 62.8 |
| IMPACT DS (U.S., multi-site) | No | 4,927 | 48.6 | 89.5 | 54.0 |
| Abbreviations: HPV, human papillomavirus; DS, dual stain; KPNC, Kaiser Permanente Northern California; MS, Mississippi | | | | | |

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| Supplemental Table 2. Resource utilization comparing DS and cytology triage of HPV-positive results (KPNC) | | | |
| **Metric** | **DS** | **Cytology** | **Difference in metric DS vs. cytology** |
| Total colposcopy referrals per 100k individuals after 3 years | 6,669 | 7,561 | -12% |
| Number of screening visits over 3 years | 111,410 | 111,564 | -0.14% |
| Number of tests (HPV and DS/Cytology) over 3 years | 124,856 | 125,160 | -0.24% |
| Cumulative years until CIN3+ diagnosis per 100k individuals | 134 | 223 | -40% |
| Abbreviations: HPV, human papillomavirus; DS, dual stain; KPNC, Kaiser Permanente Northern California; CIN3+, cervical intraepithelial neoplasia grade 3 or worse  In these calculations, we are assuming that the colposcopy sensitivity is 100%, and there is 100% adherence for colposcopy and retesting referrals. | | | |

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| Supplemental Table 3. Resource utilization comparing dual stain triage and cytology triage for HPV-positives when limited genotyping is provided by the HPV screening test (KPNC) | | | |
| **Metric** | **DS** | **Cytology** | **Difference in metric DS vs. cytology** |
| Total colposcopy referrals per 100k individuals after 3 years | 7,765 | 8,770 | -11% |
| Number of screening visits over 3 years | 111,360 | 111,384 | -0.02% |
| Number of tests over 3 years | 135,562 | 135,624 | -0.05% |
| Cumulative years until CIN3+ diagnosis per 100k individuals | 105 | 136 | -22% |
| Abbreviations: HPV, human papillomavirus; DS, dual stain; KPNC, Kaiser Permanente Northern California; CIN3+, cervical intraepithelial neoplasia grade 3 or worse  In these calculations, we are assuming that the colposcopy sensitivity is 100%, and there is 100% adherence for colposcopy and retesting referrals. | | | |

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| Supplemental Table 4. Resource utilization comparing dual stain triage of NILM, ASC-US, LSIL in a co-testing setting to co-testing without additional triage (KPNC) | | | |
| **Metric** | **DS triage of NILM, ASC-US, LSIL in co-testing** | **Co-testing alone** | **Difference in metric DS vs. cytology** |
| Total colposcopy referrals per 100k individuals after 3 years | 7,446 | 8,320 | -11% |
| Number of screening visits over 3 years | 113,770 | 113,951 | -0.16% |
| Number of tests over three years | 240,977 | 227,903 | +5.7% |
| Cumulative years until CIN3+ diagnosis per 100k individuals | 49 | 134 | -64% |
| Abbreviations: HPV, human papillomavirus; DS, dual stain; KPNC, Kaiser Permanente Northern California; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low grade intraepithelial lesion  In these calculations, we are assuming that the colposcopy sensitivity is 100%, and there is 100% adherence for colposcopy and retesting referrals. | | | |

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| Supplemental Table 5. Comparison of Baseline Risk of CIN3+ for DS and Cytology Triage of HPV-Positive Test Results After One Screening Round in KPNC | | | | | |
| First Round Result | Total N | % of Total among HPV+s | CIN3+ (n) | Baseline Risk of CIN3+ | Overall % with CIN3+ |
| HPV+/NILM | 2,867 | 42.0% | 93 | 2.0% | 3.2% |
| HPV+/DS- | 3,458 | 51.0% | 44 | 0.75% | 1.3% |

Immediate and overall risks of CIN3+ are higher for HPV-positive NILM compared to HPV-positive dual stain negative. This establishes the baseline risk for the subsequent round of testing.

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| Supplemental Table 6. Comparison of Baseline Risk of CIN3+ for DS and Cytology Triage of HPV-Positive Test Results After Two Screening Rounds in KPNC | | | | | |
| First Round Result | Second Round Result | Total N | % of Total | CIN3+ (n) | Overall % with CIN3+ |
| HPV+/NILM | HPV-/NILM | 1,246 | 52% | 8 | 0.6% |
| HPV+ AND/OR ASC-US+ | 1,162 | 48% | 67 | 5.8% |
| HPV+/DS- | HPV-/NILM | 1,610 | 55.0% | 2 | 0.1% |
| HPV+ AND/OR ASC-US+ | 1,309 | 45.0% | 27 | 2.1% |
| HPV+/DS- | HPV-/DS- | 85 | 60.0% | 1 | 1.1% |
| HPV+/DS+ | 55 | 40.0% | 2 | 3.6% |

Although numbers for repeat DS testing are limited, in all scenarios, risks of CIN3+ are considerably lower in the second round of testing following an initial HPV-positive DS negative result compared to HPV-positive NILM. These results collectively support the recommendation that individuals with repeat HPV-positive DS negative test results should not go to colposcopy.

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| Supplemental Table 7. DS Triage of HPV-Positive Test Results in Post-Colposcopy and Post-Treatment Settings in KPNC | | | | | | |
| Setting | Current Test  Result | N | CIN3+ Cases (n) | CIN3+ Immediate Risk (%) | CIN3+ Management | CIN3+ Management Confidence Probability %) |
| Post-Colposcopy | HPV+/DS+ | 599 | 58 | 7.9 | Colposcopy | 100% |
|  | HPV+/DS- | 530 | 4 | 0.39 | 1-year return | 85% |
| Post-Treatment | HPV+/DS+ | 34 | 6 | 182 | Colposcopy | 78% |
|  | HPV+/DS- | 32 | 0 | 0.0 | 1-year return | 73% |

References

1. Gage, J.C., T. Raine-Bennett, M. Schiffman, et al., *The Improving Risk Informed HPV Screening (IRIS) Study: Design and Baseline Characteristics.* Cancer Epidemiol Biomarkers Prev, 2022. **31**(2): p. 486-492.

2. Wentzensen, N., M.A. Clarke, R. Bremer, et al., *Clinical Evaluation of Human Papillomavirus Screening With p16/Ki-67 Dual Stain Triage in a Large Organized Cervical Cancer Screening Program.* JAMA Intern Med, 2019. **179**(7): p. 881-888.

3. Clarke, M.A., C. Risley, M.W. Stewart, et al., *Age-specific prevalence of human papillomavirus and abnormal cytology at baseline in a diverse statewide prospective cohort of individuals undergoing cervical cancer screening in Mississippi.* Cancer Med, 2021. **10**(23): p. 8641-8650.

4. Risley, C., M.W. Stewart, K.R. Geisinger, et al., *STRIDES - STudying Risk to Improve DisparitiES in Cervical Cancer in Mississippi - Design and baseline results of a Statewide Cohort Study.* Prev Med, 2021. **153**: p. 106740.