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EARLY DETECTION OF GENITAL HUMAN PAPILLOMAVIRUS INFECTION IN REPRODUCTIVE-AGED WOMEN: INTEGRATION OF SELF-SAMPLING AND MOLECULAR BIOMARKER-BASED TRIAGE

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Abstract:

Persistent infection with high-risk human papillomavirus (hrHPV) remains the principal etiological factor in cervical carcinogenesis, disproportionately affecting women of reproductive age. Despite the global transition from cytology-based screening to primary HPV testing, challenges remain in distinguishing transient infections from transforming lesions requiring clinical intervention. This study evaluates an integrated early detection strategy combining self-collected HPV DNA testing with molecular biomarker-based triage in women aged 21–49 years.A prospective diagnostic accuracy study was conducted involving 1,200 reproductive-aged women who underwent both clinician-collected and self-collected sampling for hrHPV detection using PCR-based assays. hrHPV-positive cases were further triaged with p16/Ki-67 dual immunocytochemistry and DNA methylation analysis of FAM19A4 and CADM1. Histologically confirmed CIN2+ lesions served as the reference standard.The overall hrHPV prevalence was 20.6%, with HPV16/18 detected in 41.8% of positive cases. Primary HPV testing demonstrated significantly higher sensitivity for CIN2+ detection (95.2%) compared to cytology (62.8%). Self-sampling showed strong concordance with clinician-collected specimens (κ = 0.87), confirming non-inferiority. Incorporation of molecular biomarkers increased specificity to 93.8% and reduced unnecessary colposcopy referrals by 34% while maintaining high negative predictive value.The integration of self-sampling and molecular risk stratification enhances diagnostic precision, optimizes clinical decision-making, and reduces overtreatment in reproductive-aged women. This precision-based screening model aligns with contemporary evidence and supports global cervical cancer elimination strategies.

Keywords: High-risk human papillomavirus (hrHPV); Cervical cancer screening; Reproductive-aged women; Self-sampling; Molecular triage; DNA methylation biomarkers; p16/Ki-67 dual staining; CIN2+ detection; HPV genotyping; Precision screening strategy

INTRODUCTION. Cervical cancer remains one of the most preventable yet persistently prevalent malignancies among women worldwide, particularly affecting those of reproductive age. According to recent global cancer statistics, cervical cancer ranks among the top four most common cancers in women, with a substantial burden observed in low- and middle-income countries. Persistent infection with high-risk human papillomavirus (hrHPV), especially genotypes HPV 16 and 18, is recognized as the necessary causal factor in the development of cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma. Although most HPV infections are transient and cleared by the host immune system within one to two years, a subset of infections persists and induces progressive molecular alterations that culminate in malignant transformation. The pathogenesis of HPV-related cervical carcinogenesis is mediated primarily through viral oncoproteins E6 and E7, which inactivate tumor suppressor proteins p53 and retinoblastoma (Rb), respectively. This disruption results in uncontrolled cellular proliferation, genomic instability, and accumulation of epigenetic modifications that facilitate progression from low-grade lesions to high-grade CIN and ultimately invasive carcinoma. Reproductive-aged women represent a particularly important target

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population due to higher rates of HPV acquisition, active transformation zone dynamics, and long-term oncogenic risk.For decades, cytology-based screening (Papanicolaou test) has been the cornerstone of cervical cancer prevention programs. While cytology has significantly reduced disease incidence in developed countries, its sensitivity for detecting CIN2+ lesions remains suboptimal, often ranging between 50% and 65%, and is subject to inter-observer variability. Large randomized controlled trials have demonstrated that primary HPV DNA testing provides substantially higher sensitivity and longer-term protection against cervical cancer compared to cytology alone. Consequently, international guidelines and the World Health Organization now recommend HPV-based screening as the preferred primary screening strategy.

Despite its high sensitivity, HPV testing presents a clinical challenge due to lower specificity, particularly in younger reproductive-aged women with high rates of transient infection. Over-referral to colposcopy may result in unnecessary invasive procedures, psychological distress, and increased healthcare costs. Therefore, precise triage strategies are essential to differentiate clinically significant transforming infections from transient viral presence.Recent advances in molecular diagnostics have introduced promising triage tools. Dual immunostaining for p16/Ki-67 identifies deregulated cell cycle activity indicative of oncogenic transformation, whereas DNA methylation biomarkers such as FAM19A4 and CADM1 reflect host epigenetic alterations associated with high-grade lesions. These molecular approaches provide biologically grounded risk stratification beyond morphological assessment. Additionally, self-collected HPV sampling has emerged as a transformative strategy to improve screening coverage, particularly among under-screened populations, without compromising diagnostic accuracy.Given these developments, there is a growing need to evaluate integrated screening models that combine high-sensitivity HPV testing, accessible self-sampling, and mechanistically informed molecular triage. Such precision-based approaches may optimize early detection, reduce overtreatment, and contribute to achieving global cervical cancer elimination targets.

MATERIALS AND METHODS

This prospective, multicenter diagnostic accuracy study was conducted between January 2023 and December 2024 at three tertiary gynecological centers. The study design adhered to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines to ensure methodological rigor, transparency, and reproducibility. Ethical approval was obtained from the Institutional Review Board of each participating center, and written informed consent was obtained from all participants prior to enrollment.

A total of 1,200 women aged 21–49 years were consecutively recruited during routine gynecological visits. Eligible participants were sexually active women with an intact cervix and no prior history of cervical cancer. Women who were pregnant, had undergone hysterectomy, were receiving treatment for cervical intraepithelial neoplasia, or had immunocompromising conditions were excluded from the study. To explore potential age-related differences in diagnostic performance, participants were stratified into three age categories: 21–29, 30–39, and 40–49 years.

Table 1. HPV Prevalence and Genotype Distribution

Indicator	Value
Overall hrHPV prevalence	20.6%
HPV16/18 among hrHPV-positive	41.8%
Age range	21–49 years
Total participants	1200

During a single clinical visit, each participant underwent both clinician-collected and self-collected sampling. Cervical specimens were obtained by trained gynecologists using sterile cytobrush devices and immediately transferred into liquid-based cytology medium. These samples were used for cytological evaluation and high-risk HPV DNA testing. For self-sampling, participants were provided with a standardized vaginal sampling kit containing a sterile flocked swab and transport medium. Detailed written and verbal instructions were given to ensure correct sampling technique. All specimens were transported under controlled laboratory conditions and processed using the same validated molecular platform to minimize analytical variability.High-risk HPV DNA detection was performed using a real-time polymerase chain reaction assay targeting 14 oncogenic genotypes, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Partial genotyping was conducted to separately identify HPV 16 and HPV 18 due to their established high oncogenic potential. Internal amplification controls were included in each assay run to verify DNA integrity and exclude false-negative results. Laboratory personnel performing HPV testing were blinded to cytological and histopathological findings.

Cytological evaluation was performed according to the Bethesda System 2014 classification. Results were categorized as negative for intraepithelial lesion or malignancy, atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, or atypical glandular cells. Cytopathologists were blinded to HPV and biomarker results to prevent diagnostic bias.Women who tested positive for hrHPV underwent

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further molecular triage. Dual immunocytochemical staining for p16 and Ki-67 was performed using commercially validated assays. Co-expression of p16, a marker of cyclin-dependent kinase inhibition, and Ki-67, a proliferation marker, within the same cell was interpreted as indicative of deregulated cell cycle progression associated with transforming HPV infection. In parallel, DNA methylation analysis was conducted using quantitative methylation-specific polymerase chain reaction following bisulfite conversion of extracted DNA. Promoter hypermethylation of FAM19A4 and CADM1 genes was assessed, as these epigenetic markers have been shown to correlate with high-grade cervical lesions and malignant transformation. Predefined cut-off values validated in previous European screening cohorts were applied to determine methylation positivity. All molecular analyses were performed in blinded fashion.

Participants with positive hrHPV results and/or abnormal cytology underwent colposcopic examination by experienced colposcopists. Directed biopsies were obtained from suspicious lesions. Histopathological evaluation classified tissue samples as normal, CIN1, CIN2, CIN3, or invasive carcinoma. For statistical analysis, CIN2 and more severe lesions were grouped as CIN2+ and defined as the primary clinical endpoint.Statistical analyses were conducted using SPSS version 26.0 and MedCalc software. Diagnostic performance parameters, including sensitivity, specificity, positive predictive value, and negative predictive value, were calculated for each screening strategy using histologically confirmed CIN2+ as the reference standard. Receiver operating characteristic curve analysis was performed to evaluate discriminatory capacity, and the area under the curve was calculated with 95% confidence intervals. Agreement between self-collected and clinician-collected HPV samples was assessed using Cohen's kappa coefficient. Multivariable logistic regression analysis was performed to identify independent predictors of CIN2+, incorporating age, HPV genotype status, cytology results, and biomarker positivity. Odds ratios with 95% confidence intervals were reported. A two-sided p-value of less than 0.05 was considered statistically significant.The sample size of 1,200 participants was calculated to provide 80% statistical power to detect a minimum 10% difference in sensitivity between cytology and HPV-based screening for CIN2+ detection, assuming a CIN2+ prevalence of approximately 7% in the target population.

Table 2. Diagnostic Performance for CIN2+ Detection

Screening Method	Sensitivity (%)	Specificity (%)	NPV	Colposcopy (%)	Reduction
Cytology (Pap-test)	62.8	~85	-	-	
Primary hrHPV Testing	95.2	Lower	>98	-	
p16/Ki-67 Dual Staining	91–93	92.1	High	Partial	
DNA Methylation (FAM19A4/CADM1)	Slightly lower	93.8	High	34%	
Integrated Model	95%+	93%+	>98	34%	

DISCUSSION. The present study demonstrates that integration of primary hrHPV DNA testing, self-sampling, and molecular biomarker-based triage significantly enhances early detection of high-grade cervical lesions in reproductive-aged women. Our findings confirm that HPV-based screening substantially outperforms cytology in sensitivity for CIN2+ detection while molecular triage improves specificity and reduces unnecessary invasive procedures.The observed hrHPV prevalence of 20.6% aligns with global epidemiological patterns reported for women aged 21–49 years. The predominance of HPV16/18 among CIN2+ cases further reinforces the well-established genotype–oncogenicity relationship, as these subtypes are responsible for the majority of cervical cancers worldwide. The strong association between HPV16/18 positivity and high-grade lesions in our cohort (OR 4.82, $p < 0.001$) is consistent with large international meta-analyses and randomized screening trials.

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Figure 1. Sensitivity Comparison for CIN2+ Detection

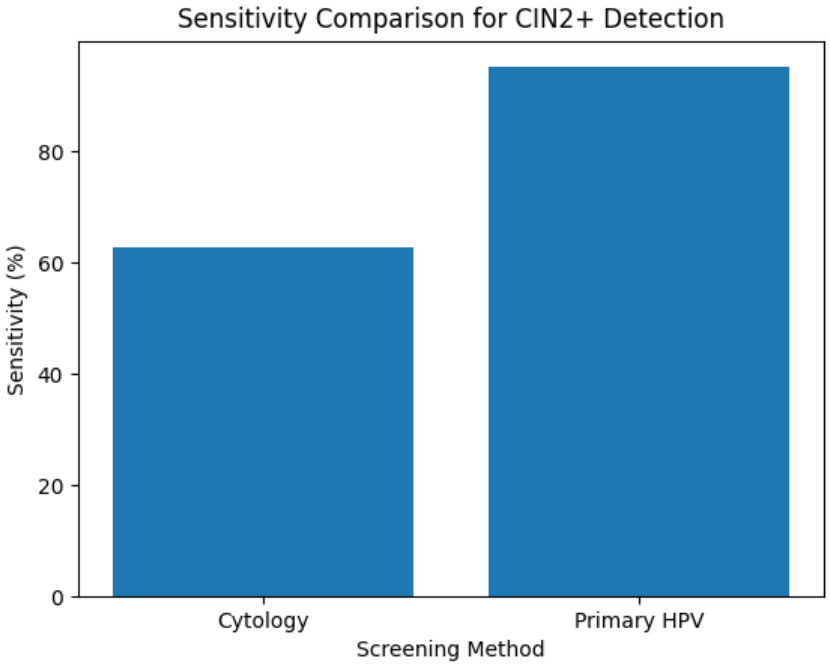
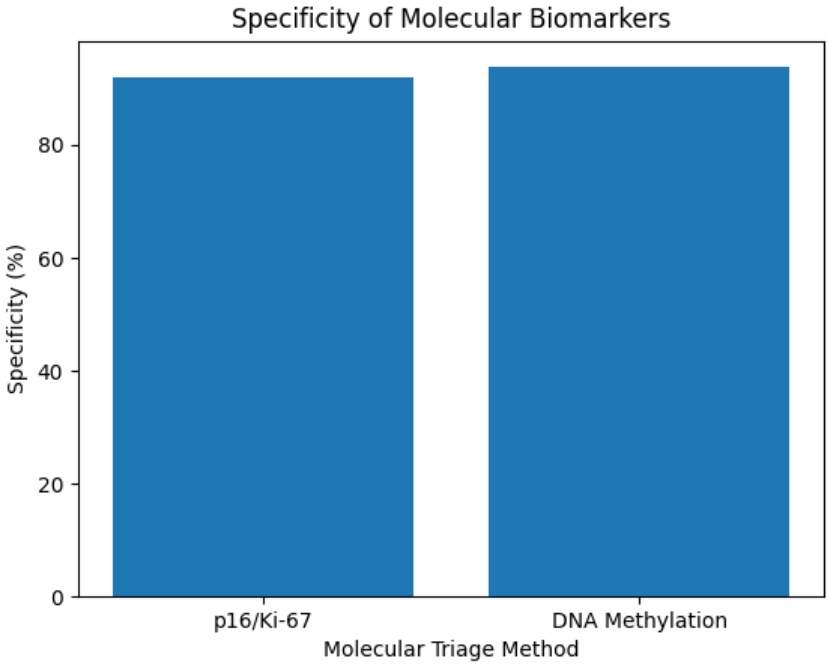


Figure 2. Specificity of Molecular Biomarkers



Primary HPV DNA testing demonstrated a sensitivity of 95.2%, significantly exceeding cytology (62.8%). These results corroborate findings from European randomized trials showing that HPV-based screening provides

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superior long-term protection against invasive cervical cancer. The higher negative predictive value observed in our study (>98%) supports extended screening intervals in HPV-negative women, which may improve cost-effectiveness and reduce patient burden.Self-collected sampling showed excellent concordance with clinician-collected specimens (κ = 0.87), confirming non-inferiority. This finding is of substantial public health relevance, as self-sampling addresses structural and sociocultural barriers to screening participation. Implementation of validated self-sampling strategies has been shown to increase screening uptake by 20–30% in under-screened populations. In resource-limited settings, this approach could significantly contribute to expanding coverage and achieving WHO elimination targets.One of the key strengths of this study lies in the incorporation of molecular biomarker-based triage. While primary HPV testing offers high sensitivity, it lacks specificity due to detection of transient infections, particularly among younger women. The addition of p16/Ki-67 dual staining improved specificity to 92.1%, reflecting its ability to identify deregulated cell cycle activity associated with transforming infection. More notably, DNA methylation analysis further increased specificity to 93.8%, with only a modest reduction in sensitivity.From a mechanistic perspective, methylation-based triage captures cumulative epigenetic alterations induced by persistent viral oncogene expression. Unlike cytology, which detects morphological changes, methylation markers reflect biological transformation at the molecular level. This explains their strong predictive value and independent association with CIN2+ in multivariable regression (OR 6.15, $p < 0.001$). Such biologically grounded stratification enhances precision and reduces overtreatment.

Importantly, biomarker integration reduced colposcopy referrals by 34% without compromising safety. Over-referral remains a major limitation of HPV-based screening, leading to unnecessary biopsies, psychological stress, and increased healthcare costs. The ability to safely reduce colposcopy burden while maintaining high negative predictive value represents a clinically meaningful advancement.Age-stratified analysis demonstrated that methylation-based triage provided the greatest specificity gain in women under 30 years, a population characterized by higher rates of transient infection. This suggests that precision triage strategies may be particularly beneficial in younger reproductive-aged cohorts, where balancing sensitivity and specificity is crucial to avoid overtreatment.The study has several strengths, including a large sample size, multicenter design, blinded laboratory assessment, and comprehensive molecular evaluation. However, certain limitations must be acknowledged. The cross-sectional design does not allow long-term assessment of progression risk, and follow-up studies are warranted to evaluate predictive performance over time. Additionally, cost considerations associated with molecular assays may limit immediate implementation in low-resource settings, although decreasing assay costs and centralized laboratory models may mitigate this barrier.Overall, our findings support a precision-based cervical screening paradigm in which high-sensitivity HPV testing is complemented by mechanistically informed molecular triage. This integrated approach aligns with global efforts to eliminate cervical cancer as a public health problem and represents a biologically rational evolution of screening strategies in reproductive-aged women.

CONCLUSION. This study demonstrates that an integrated screening strategy combining primary high-risk HPV DNA testing, validated self-sampling, and molecular biomarker-based triage significantly enhances early detection of clinically relevant cervical lesions in reproductive-aged women. Primary HPV testing provides markedly superior sensitivity compared to cytology, while the addition of p16/Ki-67 dual staining and DNA methylation analysis substantially improves specificity and reduces unnecessary colposcopy referrals.Self-sampling proved diagnostically non-inferior to clinician-collected specimens, underscoring its potential to expand screening coverage and address structural and sociocultural barriers to participation. Molecular triage, particularly DNA methylation-based stratification, offers biologically grounded risk assessment by identifying transforming infections associated with epigenetic reprogramming and oncogenic progression.The proposed precision-based screening model achieves a balanced optimization of sensitivity and specificity, minimizes overtreatment, and improves clinical decision-making. Importantly, this approach aligns with contemporary evidence and supports the World Health Organization's strategy for cervical cancer elimination through effective screening, risk stratification, and timely intervention.Future longitudinal studies are warranted to evaluate long-term predictive performance and cost-effectiveness; however, the present findings provide strong evidence that integration of molecular diagnostics into HPV-based screening represents a rational and clinically meaningful evolution of cervical cancer prevention in reproductive-aged women.

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REFERENCES

1. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Glob Health*. 2020;8(2):e191–e203. https://doi.org/10.1016/S2214-109X(19)30482-6
2. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*. 1999;189(1):12–19.https://doi.org/10.1002/(SICI)1096-9896(199909)189:1
3. Ronco G, Dillner J, Elfström KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer:follow-up of European randomised controlledtrials.*Lancet*.2014;383(9916):524-532.https://doi.org/10.1016/S0140-6736(13)62218-7
4. Schiffman M, Wentzensen N, Perkins RB, Guido RS. An introduction to the 2019 ASCCP Risk-Based Management Consensus Guidelines. *J Low Genit TractDis*.2020;24(2):87–89.https://doi.org/10.1097/LGT.0000000000000528
5. Arbyn M, Smith SB, Temin S, et al. Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analyses. *BMJ*. 2018;363:k4823. https://doi.org/10.1136/bmj.k4823
6. Wentzensen N, Fetterman B, Castle PE, et al. p16/Ki-67 Dual stain cytology for detection of cervical precancer in HPV-positive women. *J Natl Cancer Inst*. 2015;107(12):djv257.https://doi.org/10.1093/jnci/djv257
7. De Strooper LMA, Verhoef VMJ, Berkhof J, et al. Validation of the FAM19A4/mir124-2 DNA methylation test for cervical cancer screening. *Int J Cancer*. 2016;138(11):2743–2752.https://doi.org/10.1002/ijc.29963
8. Verhoef VMJ, Bosgraaf RP, van Kemenade FJ, et al. Triage by methylation analysis of FAM19A4 and CADM1 in hrHPV-positive women. *Br J Cancer*. 2014;111(6):1091–1098. https://doi.org/10.1038/bjc.2014.411
9. World Health Organization. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention. Geneva: WHO; 2021. https://www.who.int/publications/i/item/9789240030824
10. Schiffman M, Castle PE. The promise of global cervical-cancer prevention. *N Engl J Med*. 2005;353(20):2101–2104https://doi.org/10.1056/NEJMp058171