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Original Article

Comparative performance of standalone E6/E7 mRNA testing versus a combined DNA genotyping and viral load assay for detecting cervical high-grade lesions: a retrospective paired study

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Abstract: Objective: To compare the diagnostic performance of standalone E6/E7 mRNA testing versus a combined HPV DNA genotyping and viral load assay for detecting cervical high-grade lesions (CIN2+) and to validate performance, biological rationale, and clinical utility. Methods: This single-center, retrospective, paired diagnostic accuracy study included 150 women in the primary cohort and 100 in an independent validation cohort, all referred for colposcopy. Participants underwent both the E6/E7 mRNA test (Aptima) and the combined DNA test (genotyping + Hybrid Capture 2 viral load) within one month prior to biopsy. Histopathology was the reference standard. Analyses included diagnostic performance metrics, correlation of viral load with histopathology, subgroup assessments, and follow-up of 65 DNA+/mRNA- women. Results: In the primary cohort, the sensitivity of the mRNA assay (87.7%) was not significantly different from that of the DNA test (94.7%, P=0.221), but the specificity was significantly higher (P<0.001). The mRNA signal strongly correlated with histopathological severity (ρ=0.72, P<0.001) and was stronger than the DNA load (ρ=0.38, P<0.001). Validation confirmed these results. Subgroup analyses showed superior mRNA specificity in women <30 years (96.7% vs. 60.0%) and for ASCUS triage (92.7% vs. 63.4%). The mRNA assay effectively refined risk among DNA-positive patients. Conclusion: The E6/E7 mRNA assay provides significantly higher specificity and positive predictive value for CIN2+ detection than a comprehensive DNA-based strategy, without significant sensitivity loss. Its reproducible performance, biological rationale, and proven safety support its role in enhancing precision screening, particularly for ASCUS, young women, and HPV DNA-positive individuals.

Keywords: Cervical cancer screening, E6/E7 mRNA testing, HPV DNA testing, CIN2+, diagnostic accuracy

Introduction

Cervical cancer remains a significant global health challenge, ranking as the fourth most common cancer among women worldwide. In 2022 alone, it accounted for an estimated 660,000 new cases and 350,000 deaths, with a disproportionate burden falling on low- and middle-income countries where access to preventive services is limited [1]. The primary and necessary etiological agent for nearly all cases of cervical cancer is persistent infection with high-risk human papillomavirus (hr-HPV) [2]. While most HPV infections are transient and resolve spontaneously, the small fraction that persists can initiate a multistep process of carcinogenesis, making the detection of clinically significant infections a cornerstone of secondary prevention efforts.

Current international screening guidelines have largely adopted hr-HPV DNA testing as the primary screening modality, owing to its high sensitivity for detecting high-grade cervical intraepithelial neoplasia (CIN2+) [3]. However, the principal limitation of this approach is its suboptimal specificity [4]. HPV DNA tests cannot distinguish between transient infections, which

pose little to no risk of progression, and transforming infections that are actively driving oncogenesis. This lack of specificity leads to the over-detection of clinically insignificant infections, resulting in a substantial number of unnecessary colposcopy referrals, which in turn elevates healthcare costs, strains resources and can cause significant anxiety and psychological distress for patients [5].

The molecular basis of HPV-driven carcinogenesis lies in the activity of two viral oncoproteins, E6 and E7. The persistent expression of these proteins is essential for both the initiation and maintenance of the malignant phenotype. The E6 protein primarily functions by promoting the degradation of the p53 tumor suppressor protein, thereby abrogating cell cycle arrest and apoptosis [6]. Concurrently, the E7 oncoprotein binds to and inactivates the retinoblastoma (pRb) tumor suppressor protein, releasing E2F transcription factors and driving uncontrolled cell proliferation [7]. Because the transcription of E6 and E7 genes is a direct prerequisite for these transforming events, the detection of their corresponding mRNA transcripts offers a more direct biological indicator of active oncogenic processes, as opposed to the mere presence of viral DNA.

Reflecting this biological rationale, numerous clinical studies and meta-analyses have compared the diagnostic performance of HPV E6/E7 mRNA testing against HPV DNA testing for the detection of CIN2+ lesions. The general consensus from these investigations is that E6/E7 mRNA assays demonstrate a specificity that is consistently and significantly higher than that of DNA tests, while maintaining a comparable, albeit sometimes slightly lower, sensitivity [8, 9]. The improved specificity directly translates to a lower rate of false-positive results and, consequently, a reduction in unnecessary colposcopy referrals, addressing a key limitation of current screening paradigms.

The clinical utility of a more specific biomarker is particularly acute in the management of women with equivocal cytological findings, such as atypical squamous cells of undetermined significance (ASCUS). ASCUS is the most common abnormal cytological result and represents a diagnostic “gray area”, encompassing a wide spectrum of underlying pathologies from benign reactive changes to high-grade precancerous lesions [10]. While hr-HPV DNA testing is the standard triage method for ASCUS, its low positive predictive value in this population exacerbates the problem of over-referral. Therefore, the development and validation of improved triage strategies with higher specificity, such as E6/E7 mRNA testing, is a critical and unmet need to refine risk stratification for this large group of women.

Furthermore, E6/E7 mRNA testing demonstrates advantages over standalone DNA tests, and its performance relative to comprehensive DNA-based strategies combining genotyping with viral load assessment remains unestablished [11, 12]. Therefore, this study was designed not only to directly compare standalone E6/E7 mRNA testing against a combined DNA strategy integrating genotyping with viral load assessment for CIN2+ detection but also to address these broader evidence gaps.

Methods

Study design

This single-center, retrospective, paired, and blinded comparative diagnostic accuracy study was conducted to evaluate the performance of standalone E6/E7 mRNA detection against a combined strategy of HPV genotyping and HC2-HPV DNA viral load testing in cervical screening. Data for this study were sourced from the deidentified clinical database of The First Affiliated Hospital of Soochow University, encompassing eligible patients screened between February 1, 2021, and December 31, 2023. The research protocol received full approval from the Institutional Review Board (IRB) of The First Affiliated Hospital of Soochow University (Approval No. 20251234). In accordance with the ethical principles of the Declaration of Helsinki, the IRB granted a waiver of the requirement for individual patient consent due to the retrospective nature of the study, which relied exclusively on the analysis of anonymized data and presented no intervention or risk to the patients. All patient data were handled with strict confidentiality. This study was designed and is reported in adherence to the Standards for Reporting of Diagnostic Accuracy Studies (STARD 2015) guidelines.

Study population

The cohort for this study was retrospectively identified from the electronic medical records (EMR) database of the Department of Gynecology at The First Affiliated Hospital of Soochow University. We screened the records of all female patients who underwent electronic colposcopy between February 1, 2021, and December 31, 2023. Initial eligibility was based on a referral for colposcopy due to an abnormal cervical cytology result of ASCUS or higher or a positive high-risk HPV DNA test, in line with established clinical guidelines [13]. All patient data were fully deidentified prior to analysis to ensure confidentiality.

Primary analysis cohort: Inclusion criteria for the primary paired diagnostic accuracy analysis stipulated that patients must have (1) been aged ≥21 years at the time of screening, consistent with screening guidelines applicable during the study period [14]; (2) received both the standalone E6/E7 mRNA detection test and the combined HPV genotyping with HC2-HPV DNA viral load test within one month prior to the scheduled colposcopy and cervical biopsy; (3) a definitive histopathological diagnosis obtained from a multipoint cervical biopsy or a cervical conization procedure, serving as the gold standard; and (4) complete and traceable records, including clinical history, laboratory results, and final pathology reports. This resulted in a cohort expected to comprise women with varying risks, from those with minor cytological abnormalities to those with higher-grade referrals, allowing for a broad assessment of test performance across a clinically relevant spectrum.

Exclusion criteria: Patients were excluded from the primary and validation cohorts for any of the following reasons: (1) a prior documented history of treatment for cervical cancer or high-grade cervical intraepithelial neoplasia (CIN2+); (2) being in a state of pregnancy or lactation at the time of testing; (3) a known immunocompromised status, including but not limited to HIV infection, organ transplant recipients, or current use of systemic immunosuppressive medications, due to the established impact on HPV persistence and progression risk [15]; (4) a history of a total hysterectomy; or (5) significant missing data for key variables, including test results or final pathological diagnosis, which would preclude a complete analysis.

Independent validation cohort: To externally validate our findings, an independent validation cohort was assembled by applying identical inclusion and exclusion criteria to a consecutive series of patients who underwent colposcopy at the same institution between January 1, 2024, and December 30, 2024.

Follow-up cohort for longitudinal safety assessment: Additionally, to evaluate the long-term safety of triage based on E6/E7 mRNA status, a separate follow-up cohort was identified from the initially excluded patient records. This cohort comprised women who were (1) positive by the combined DNA test (Test Method B), (2) negative by the E6/E7 mRNA assay (Test Method A), and (3) had a baseline histopathological diagnosis of <CIN2. These patients were required to have at least one follow-up clinical assessment (repeat cytology, HPV test, or histology) recorded in the EMR 12 to 24 months after their initial evaluation.

Index tests

To systematically evaluate the clinical performance of different technological approaches in cervical cancer screening, this study employed two mainstream testing strategies. Test Method A focused on detecting viral oncogenic activity, whereas Test Method B adopted a comprehensive strategy integrating precise viral genotyping with a semiquantitative assessment of the infection level. This design allows for a direct comparison of the two strategies in terms of sensitivity, specificity, and their value in guiding clinical risk stratification.

Test Method A: E6/E7 mRNA testing: Detection of E6/E7 mRNA from exfoliated cervical cell specimens was performed using the Aptima HPV Assay (Hologic, Inc., San Diego, CA, USA). This assay utilizes transcription-mediated amplification (TMA) to qualitatively detect E6/E7 messenger RNA (mRNA) from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). By targeting the expression of the viral E6/E7 oncogenes, this method provides a direct marker of potentially transforming infections rather than the mere presence of viral DNA [16]. All testing and result interpretation were conducted in strict compli-

ance with the manufacturer's standard operating procedure. This procedure relies on prevalidated internal analytical thresholds to definitively differentiate positive from negative signals, with the final qualitative result ('Positive' or 'Negative') being automatically generated by the instrument software. For the purpose of correlating viral oncogenic activity with disease severity, the semiquantitative relative light unit (RLU) values generated by the Aptima assay were also recorded and analyzed.

Test Method B: combined HPV genotyping and viral load assessment: Test Method B was a multidimensional and tiered comprehensive testing strategy designed to assess the risk of cervical lesions by providing more complete virological information. This approach consisted of two parallel and interrelated steps: precise genotyping of high-risk HPV and semiquantitative viral load assessment.

HPV genotyping was conducted using the HPV GenoArray Test Kit (Guangdong Hybribio Biotech Co., Ltd., Chaozhou, China). This test employs a polymerase chain reaction (PCR) and flow-through hybridization gene chip-based method to specifically identify the same 14 high-risk HPV genotypes [17]. Concurrently, viral load was assessed semiquantitatively using the Hybrid Capture 2 (HC2) High-Risk HPV DNA Test (Qiagen, Gaithersburg, MD, USA). A positive viral load result was defined by a relative light units to calibrator cutoff (RLU/CO) ratio of ≥1.0, a clinically validated threshold associated with a higher risk of high-grade cervical lesions [18, 19]. The continuous RLU/CO values from the HC2 test were recorded for subsequent correlation analyses.

A specimen was considered positive for the combined Test Method B and subsequently stratified into one of two risk categories: Type 1 (Highest-Risk) Positive: Defined by a genotyping result positive for HPV 16 or HPV 18, irrespective of the viral load measurement; Type 2 (High-Risk) Positive: Defined by a genotyping result positive for one of the other 12 high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68) and a concurrent positive viral load, defined as a Hybrid Capture 2 (HC2) result of ≥1.0 RLU/CO.

Gold standard: reference standard

The definitive diagnosis for all study participants was established through histopathological examination, which served as the reference standard. All subjects underwent colposcopy, during which any suspicious lesions were subjected to multipoint biopsy or a diagnostic cervical conization procedure. The resulting tissue specimens were independently evaluated by two senior gynecological pathologists who were blinded to the results of both the E6/E7 mRNA and the combined DNA index tests. The classification of lesions was based on the microscopic assessment of key morphological features on hematoxylin and eosin (H&E) stained sections, including nuclear atypia, mitotic activity, and loss of epithelial maturation, strictly adhering to the criteria outlined in the 5th edition of the World Health Organization (WHO) Classification of Tumors for Female Genital Tumors [20]. For cases of diagnostic uncertainty, particularly in distinguishing between CIN1 and CIN2, p16 immunohistochemistry was employed as an ancillary stain to confirm the presence of a transforming HPV infection, in line with the lower anogenital squamous terminology (LAST) recommendations [21]. Any discordant diagnoses between the two primary pathologists were resolved by a third, more senior pathologist to reach a final consensus. For the purpose of diagnostic accuracy analysis, the final histopathological results were dichotomized into two groups: low-grade or no lesion (<CIN2), which included normal histology, inflammation, and CIN1; and high-grade lesions (≥CIN2+), which included CIN2, CIN3, adenocarcinoma in situ (AIS), and invasive carcinoma. The endpoint of ≥CIN2+ was selected as the gold standard for clinically significant disease, as it represents the threshold for clinical intervention according to major consensus guidelines [22].

Sample size calculation

This study was designed as a retrospective comparative analysis. Therefore, the sample size was not determined by a formal a priori power calculation but was instead based on the consecutive series of all eligible patients who underwent both diagnostic tests within the defined study period (February 1, 2021, to December 31, 2023) and met all inclusion and

exclusion criteria. The final cohort consisted of 150 paired samples.

To validate the adequacy of this sample size for the study's objectives, a post hoc power analysis was conducted. The analysis focused on comparing the specificity of the standalone E6/E7 mRNA assay versus the combined HPV DNA test, as improving specificity to reduce unnecessary colposcopy referrals represents a key clinical advantage. Based on the observed specificities in our cohort for detecting ≥CIN2 (90.3% for the E6/E7 mRNA assay vs. 71.0% for the combined test), the absolute difference was 19.3%. A difference of this magnitude is considered clinically significant for altering patient management strategies.

The power calculation was performed for McNemar's test for paired proportions using data from the 93 patients without ≥CIN2. With a two-sided significance level (α) of 0.05, the sample of 93 individuals provided over 95% power to detect the observed 19.3% difference in specificity, assuming a conservative total proportion of discordant pairs of 25%. This confirms that the final sample size was sufficient to draw robust conclusions regarding the comparative diagnostic performance of the two tests.

Statistical analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize baseline characteristics; continuous variables were presented as the mean ± standard deviation (SD) or median with interquartile range (IQR), while categorical variables were presented as frequencies and percentages (n, %). To evaluate diagnostic performance, 2×2 contingency tables were constructed to calculate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), along with their 95% confidence intervals (CIs), for the detection of CIN2+ by each testing method. McNemar's test, a paired chi-square test, was employed to compare the differences in sensitivity and specificity between the two paired testing methods within the same patient cohort. Furthermore, receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUC) was calculated to assess the overall discriminative ability of each test. The Z test was used to determine the statistical significance of the difference between the AUCs. The nonparametric Kruskal-Wallis test, followed by post hoc Dunn's test with Bonferroni correction, was used to compare semiquantitative viral load (RLU for mRNA, RLU/CO for DNA) across ordered histopathological grades (normal/CIN1/CIN2/CIN3+). The strength of the association between viral load and disease severity was quantified using Spearman's rank correlation coefficient (ρ). Stratified analyses were performed for prespecified subgroups, including age groups (<30 vs. ≥30 years) and patients with ASCUS cytology. For the longitudinal follow-up analysis, the cumulative incidence of progression to CIN2+ was estimated using the Kaplan-Meier method, and the difference between groups defined by baseline mRNA status was compared using the log-rank test. All statistical tests were two-sided, and a *P* value of <0.05 was considered statistically significant.

Results

Study population flow and baseline characteristics

A total of 323 patient records were initially screened for eligibility. After applying the inclusion and exclusion criteria, 150 patients formed the final paired analysis cohort for this study. The detailed participant flow, including reasons for exclusion, is depicted in **Figure 1**. The baseline clinical and pathological characteristics of the 150 included patients are summarized in **Table 1**. The mean age of the cohort was 39.1 ± 8.9 years. Patients were stratified by the reference standard histopathological diagnosis into two groups: 93 patients (62.0%) were classified as <CIN2 (including normal histology, inflammation, or CIN1), and 57 patients (38.0%) were classified as ≥CIN2+ (comprising CIN2, CIN3, adenocarcinoma in situ, or invasive carcinoma). The distribution of referral cervical cytology results differed significantly between the two groups (P<0.001), with a higher proportion of HSIL+ findings in the ≥CIN2+ group (38.6%) than in the <CIN2 group (6.5%). The results of the combined Test B (HPV genotyping with viral load) also showed a significantly different distribution (P<0.001). Notably, a Type 1 (Highest-Risk) positive result (HPV 16/18) was found in 63.2% of the ≥CIN2+ group versus 25.8% of the <CIN2 group.

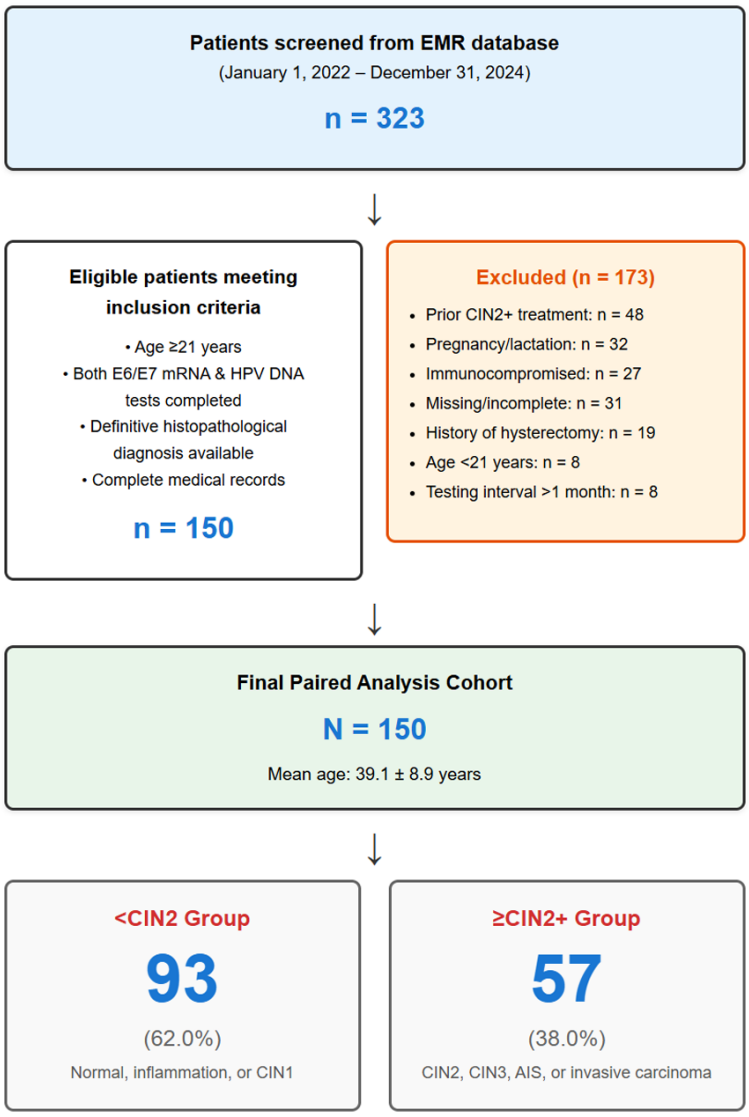

Figure 1. Flow diagram showing patient selection and stratification.

Overall diagnostic performance for detecting CIN2+

The diagnostic performance of the standalone E6/E7 mRNA assay (Test Method A) and the combined HPV genotyping with viral load assay (Test Method B) for detecting CIN2+ lesions was evaluated and is summarized in **Table 2**. Test Method B demonstrated a slightly higher sensitivity of 94.7% (95% CI, 85.4%-98.9%), correctly identifying 54 out of 57 cases, compared to Test Method A, which had a sensitivity of 87.7% (95% CI, 76.3%-94.9%), identifying 50 cases. However, this difference in sensitivity between the two paired tests was not statistically significant (P=0.221). In contrast, a substantial and significant difference was observed in specificity. Test Method A showed a significantly higher specificity of 90.3% (95% CI, 82.1%-95.7%) compared to the 71.0% (95% CI, 60.6%-80.0%) specificity of Test Method B (P< 0.001). This was due to Test Method A generating only 9 false-positive results among the 93 patients without CIN2+, whereas Test Method B produced 27 false-positive results. Consequently, the PPV for Test Method A was considerably higher at 84.7% (95% CI, 72.6%-93.0%) versus 66.7% (95% CI, 55.5%-76.6%) for Test Method B. Both assays maintained high and comparable NPV, at 92.3% (95% CI, 84.4%-96.9%) for Test Method A and 95.7% (95% CI, 87.8%-99.1%) for Test Method B.

E6/E7 mRNA viral load demonstrates a strong correlation with the severity of cervical histopathology

To investigate the biological rationale underlying the superior specificity of the E6/E7 mRNA assay, we analyzed the relationship between the quantitative signal of both tests and the severity of the underlying histopathology (**Table 3**). The semiquantitative values (relative light units, RLU for Aptima mRNA; RLU/CO for HC2 DNA) were compared across four histologically confirmed groups: normal/cervicitis (n=28), CIN1 (n=35), CIN2 (n=32), and CIN3+ (n=25, including 22 CIN3 and 3 carcinomas). As anticipated, the E6/E7 mRNA viral load exhibited a strong and statistically significant positive correlation with disease severity (**Figure 2A**). The median mRNA levels demonstrated a stepwise increase from the normal/cervicitis group through CIN1 and CIN2 to the highest levels in the CIN3+ group (Kruskal-Wallis test, P<0.001). Post hoc analysis revealed significant differences between all adjacent pathological grades (all P<0.05). In

Table 1. Baseline Clinical and Pathological Characteristics of the Included Study Population (N=150)

Characteristic	Overall (N=150)	<CIN2 (n=93)	≥CIN2+ (n=57)	Statistics	*P* value
Age, years, Mean ± SD	39.1 ± 8.9	37.8 ± 8.5	41.3 ± 9.1	t=-2.46	0.015
Referral Cytology, n (%)				χ^2=35.82	<0.001
NILM	28 (18.7)	25 (26.9)	3 (5.3)		
ASCUS	56 (37.3)	41 (44.1)	15 (26.3)		
LSIL	44 (29.3)	25 (26.9)	19 (33.3)		
HSIL+	22 (14.7)	6 (6.5)	16 (28.1)		
Final Histopathology, n (%)			-	-	
<CIN2	93 (62.0)	93 (100)	0 (0)		
≥CIN2+	57 (38.0)	0 (0)	57 (100)		
Of which CIN2	-	-	32 (56.1)*		
Of which CIN3	-	-	22 (38.6)*		
Of which AIS/Invasive Ca.	-	-	3 (5.3)*		
Combined Test B Result, n (%)				χ^2=31.65	<0.001
Negative	40 (26.7)	35 (37.6)	5 (8.8)		
Type 1 Positive (HPV 16/18)	55 (36.7)	24 (25.8)	31 (54.4)		
Type 2 Positive (Other hr-HPV & VL+)	55 (36.7)	34 (36.6)	21 (36.8)		

Notes: *Percentages for lesion grades (CIN2, CIN3, AIS/invasive Ca.) are calculated based on the total number of patients in the ≥CIN2+ group (n=57). SD, Standard Deviation; NILM, Negative for Intraepithelial Lesion or Malignancy; ASCUS, Atypical Squamous Cells of Undetermined Significance; LSIL, Low-grade Squamous Intraepithelial Lesion; HSIL+, High-grade Squamous Intraepithelial Lesion or worse; CIN, Cervical Intraepithelial Neoplasia; AIS, Adenocarcinoma in situ; Ca., Carcinoma; hr-HPV, high-risk Human Papillomavirus; VL+, positive viral load (HC2 RLU/CO ≥1.0). Statistical tests: Independent samples t test for age; Pearson's chi-square test for categorical variables. A *P* value <0.05 was considered statistically significant.

Table 2. Diagnostic performance of E6/E7 mRNA (Test Method A) vs. Combined HPV DNA testing (Test Method B) for detecting CIN2+ (N=150)

Parameter	E6/E7 mRNA	Combined Test	*P* value[1]
Test Results			
True Positive	50	54	
False Positive	9	27	
True Negative	84	66	
False Negative	7	3	
Performance Metrics, % (95% CI)			
Sensitivity	87.7 (76.3-94.9)	94.7 (85.4-98.9)	0.221
Specificity	90.3 (82.1-95.7)	71.0 (60.6-80.0)	<0.001
Positive Predictive Value	84.7 (72.6-93.0)	66.7 (55.5-76.6)	-
Negative Predictive Value	92.3 (84.4-96.9)	95.7 (87.8-99.1)	-

Notes: Performance metrics are presented as percentages with 95% confidence intervals. Abbreviations: CI, confidence interval; TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; NPV, negative predictive value. [1]*P* values were calculated using McNemar's test for paired comparisons of sensitivity and specificity between the two tests.

contrast, the DNA viral load, while also showing a significant overall trend (Kruskal-Wallis test, P=0.002), displayed considerable overlap and variability, particularly within the lower-grade lesions (normal/CIN1/CIN2), as visualized in **Figure 2B**. This was quantitatively confirmed by Spearman's rank correlation analysis, which showed a significantly stronger correlation coefficient for mRNA load (ρ=0.72, P<0.001) with the ordinal histopathology scale than for DNA load (ρ=0.38, P<0.001).

Comparison of overall diagnostic accuracy and analysis of discordant results

The overall diagnostic accuracy, as measured by the area under the ROC curve, was excellent and comparable between the standalone E6/

Table 3. Semiquantitative Viral Load of E6/E7 mRNA and HPV DNA Across Histopathological Grades

Histopathological Group	n	E6/E7 mRNA (RLU), Median (IQR)	HPV DNA (RLU/CO), Median (IQR)
Normal/Cervicitis	28	0.15 (0.08-0.41)	1.25 (0.45-3.80)
CIN1	35	0.95 (0.30-2.85)	2.90 (1.10-12.50)
CIN2	32	4.20 (1.50-15.75)	5.65 (1.80-25.30)
CIN3+	25	28.50 (12.40-98.20)	18.70 (8.15-105.50)
Kruskal-Wallis *P* value		<0.001	0.002
Spearman's ρ (*P* value)		0.72 (<0.001)	0.38 (<0.001)

Notes: IQR, Interquartile Range; RLU, Relative Light Units; RLU/CO, Relative Light Units/Cutoff. Statistical comparisons were made using the Kruskal-Wallis test. The strength of the monotonic relationship was assessed using Spearman's rank correlation coefficient (ρ).

E7 mRNA assay (AUC=0.890; 95% CI, 0.828-0.952) and the combined DNA test (AUC= 0.885; 95% CI, 0.821-0.949), with no statistically significant difference (P=0.789). The crossing of the ROC curves (**Figure 3**) visually underscores the fundamental trade-off between the two methods: the DNA test achieved marginally higher sensitivity, while the mRNA assay provided superior specificity.

To delve into the clinical implications of this trade-off, we analyzed the 26 cases (17.3% of the cohort) with discordant results. The predominant pattern, "E6/E7 mRNA Negative/ Combined Test Positive" (n=24), was highly informative. Within this group, 18 out of 24 patients (75.0%) had a final histopathological diagnosis of <CIN2 (**Table 4**). This finding provides direct evidence that the mRNA assay's higher specificity effectively corrects a substantial number of false-positive results generated by the DNA-based strategy, thereby substantiating its potential to reduce unnecessary colposcopy referrals. The remaining 6 cases in this category represented CIN2+ lesions missed by the mRNA assay. Conversely, the rarer "E6/E7 mRNA Positive/Combined Test Negative" pattern (n=2) was critically significant, as both patients (100%) were confirmed to have CIN2+. This demonstrates the mRNA assay's unique capacity to identify a subset of clinically significant, transforming infections that were overlooked by the combined DNA genotyping and viral load algorithm, likely owing to its direct detection of active oncogenic transcription even in instances of low viral DNA load or integration.

Validation of diagnostic performance in an independent cohort

To externally validate the diagnostic performance observed in our primary cohort, we applied the same inclusion and exclusion criteria to an independent, consecutive series of patients who underwent screening and colposcopy at our institution between January 1, 2024, and December 30, 2024. This validation cohort comprised 100 women. The baseline characteristics of the validation cohort were similar to those of the primary cohort, with a mean age of 38.5 ± 9.2 years and a CIN2+ prevalence of 36.0% (36/100). As summarized in **Table 6**, the diagnostic performance of both tests in the validation cohort closely mirrored the findings from the primary analysis. The sensitivity of the E6/E7 mRNA assay (88.9%, 95% CI: 74.7-95.6) was, again, not significantly different from that of the combined DNA test (97.2%, 95% CI: 85.8-99.9; P=0.375). Crucially, the E6/E7 mRNA assay maintained its superi or specificity, which was 89.1% (95% CI: 78.4-94.9) compared to 70.3% (95% CI: 58.2-80.1) for the combined DNA test (P=0.004). This significant difference in specificity directly translated to a higher PPV for the mRNA assay (80.0% vs. 61.4%). The nearly identical performance profile was visually confirmed by the ROC curves, where the AUC for the E6/E7 mRNA assay in the validation cohort was 0.895 (95% CI: 0.830-0.960), demonstrating excellent agreement with the AUC of 0.890 (95% CI: 0.828-0.952) observed in the primary cohort (**Figure 4**).

Performance of the E6/E7 mRNA assay within DNA test risk strata

To evaluate the added clinical value of the E6/E7 mRNA assay (Test Method A) for risk stratification, its performance was analyzed within the subgroups of patients who had tested positive with the combined DNA test (Test Method B). This analysis focused on the 110 patients stratified into the highest-risk (Type 1 Positive,

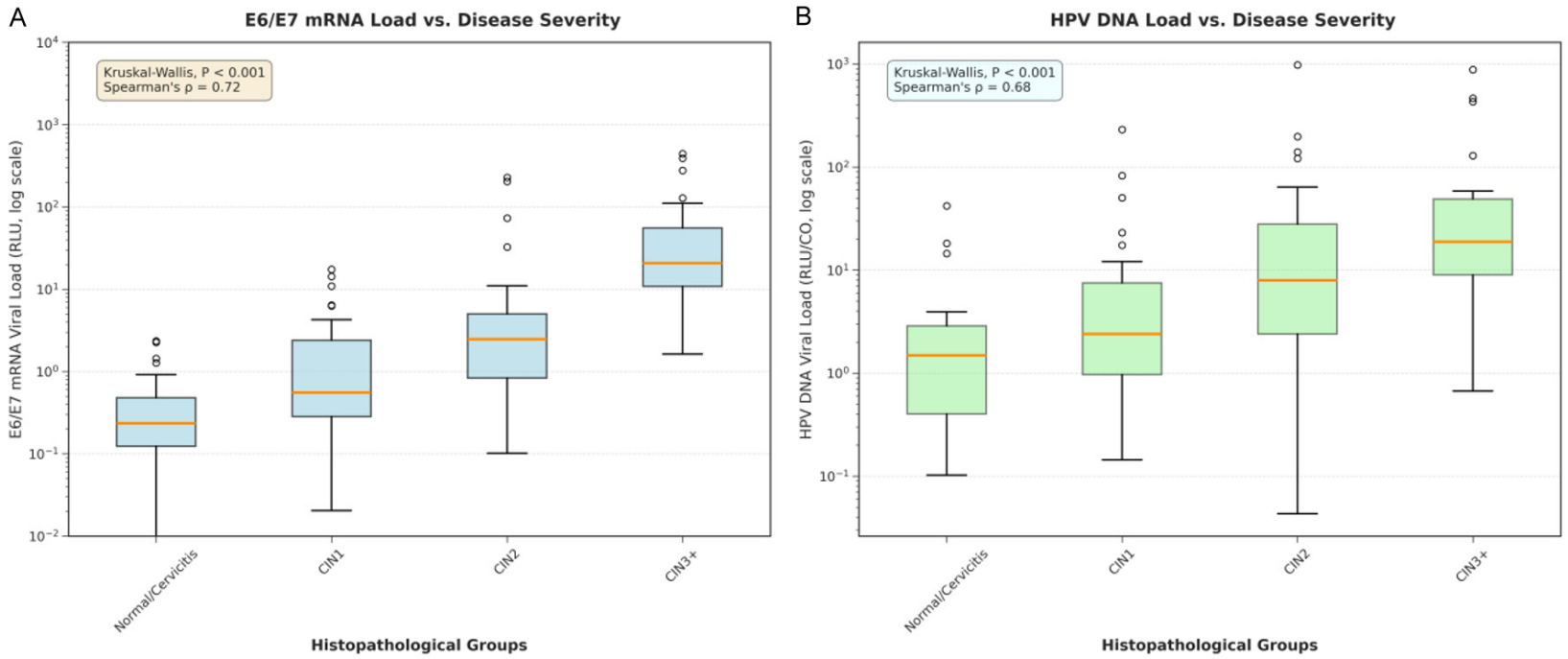

Figure 2. Correlation between viral load and cervical histopathological severity. Box plots illustrating the distribution of semiquantitative viral load measurements across four histopathologically confirmed groups (normal/cervix, CIN1, CIN2, and CIN3+). A. E6/E7 mRNA viral load (RLU, relative light units) measured by the Aptima HPV assay. B. HPV DNA viral load (RLU/CO, relative light units/cutoff) detected by the Hybrid Capture 2 assay. Box plots display the median (orange line), interquartile range (box), 1.5× interquartile range (whiskers), and outliers (circles). Y-axes are plotted on a logarithmic scale. Statistical comparisons were performed using the Kruskal-Wallis test, and the strength of monotonic relationships was assessed using Spearman's rank correlation coefficient (ρ).

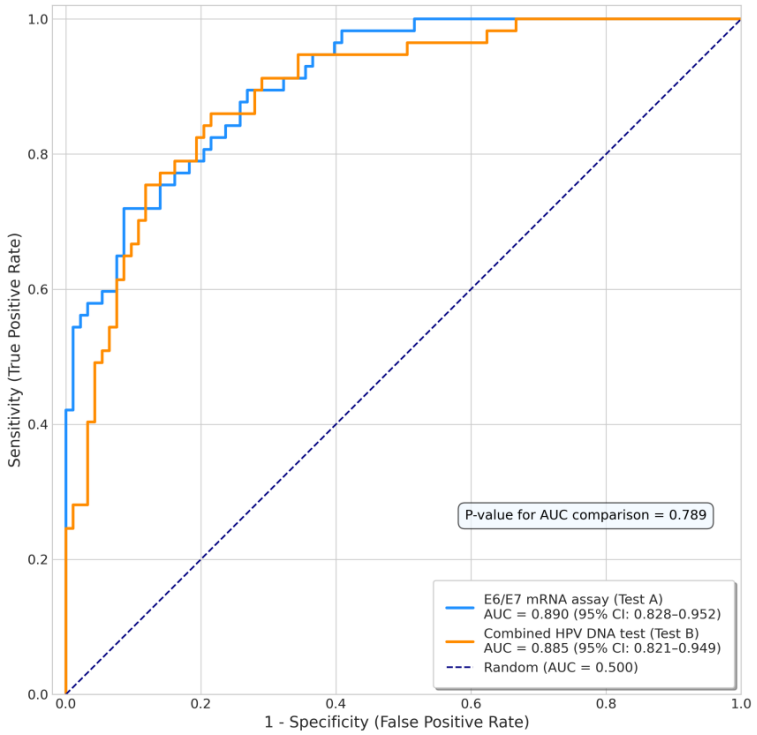

Figure 3. Comparison of ROC curves for the detection of CIN2+. The figure displays the receiver operating characteristic (ROC) curves for the standalone E6/E7 mRNA assay (Test Method A) and the combined HPV DNA test (Test Method B) in detecting CIN2+. The y-axis represents sensitivity, while the x-axis represents 1 - specificity. The diagonal dashed line indicates the performance of a random classifier (AUC=0.5). The area under the curve (AUC), its 95% confidence interval (95% CI), and the *P* value for the comparison between the two AUCs are provided for each respective test.

n=55) and high-risk (Type 2 Positive, n=55) groups (**Table 6**).

In the highest-risk subgroup of 55 patients positive for HPV 16/18 (Type 1), the E6/E7 mRNA assay remained highly sensitive for detecting high-grade disease. As shown in **Table 3**, Part A, the assay identified 30 of the 31 CIN2+ cases (sensitivity of 96.8%). Crucially, for the 21 patients in this high-risk stratum who tested negative on the mRNA assay, the NPV was 95.2%, indicating a very high probability of being free from high-grade disease. The PPV for an mRNA-positive result in this group was 88.2%.

The clinical utility of the mRNA assay was particularly evident in the high-risk subgroup of 55 patients who were positive for other high-risk HPV types with a significant viral load (Type 2). Within this cohort, the standalone E6/E7 mRNA test served as an effective secondary triage tool (**Table 5**, Part B). The test was positive in only 24 of these 55 patients, significantly improving the PPV from an initial 38.2% (21 CIN2+ cases out of 55) to 75.0%. More importantly, the test was negative in 31 patients, of whom 28 were correctly identified as not having CIN2+. This resulted in a high NPV of 90.3%, effectively downgrading the risk for a majority (56.4%) of patients in this less specific risk category, thereby identifying a large subset of individuals with transient or non-transforming infections who could avoid immediate colposcopy.

Stratified analysis by age reveals enhanced superiority of E6/E7 mRNA testing in women under 30 years

Given the higher prevalence of transient HPV infections in younger women, which contributes to the low specificity of DNA-based tests in this population, we performed a stratified analysis by age. The cohort was divided into two groups: women under 30 years of age (n=48) and those 30 years or older (n=102). As summarized in **Table 7**, the diagnostic performance of both tests differed markedly between these groups. In the <30 years subgroup, the specificity of the E6/E7 mRNA assay was 96.7% (95% CI: 83.3-99.9%), significantly outperforming the combined DNA test, which had a specificity of only 60.0% (95% CI: 42.2-76.1%; P< 0.001). This 36.7-percentage-point difference in specificity was substantially larger than the 19.3-point difference observed in the overall cohort. Consequently, the PPV of the mRNA assay in young women was exceptionally high (93.3%), more than double that of the DNA test (41.7%). Both tests maintained high sensitivity in this age group, with no significant difference (P=1.000). In the ≥30 years subgroup, the superiority of the mRNA assay in specificity remained significant (87.3% vs. 76.4%, P= 0.021), but the magnitude of the difference was less pronounced.

Table 4. Final histopathological diagnosis in cases with discordant test results (n=26)

Discordance Category	Total Cases (n)	Final Histology <CIN2, n (%)	Final Histology ≥CIN2+, n (%)
1. E6/E7 mRNA Negative/Combined Test Positive	24	18 (75.0%)	6 (25.0%)
2. E6/E7 mRNA Positive/Combined Test Negative	2	0 (0.0%)	2 (100.0%)

Notes: This table includes all patients from the paired cohort (n=150) whose results for the two index tests were discordant, based on calculations derived from **Table 2**. Percentages are calculated row-wise.

Table 5. Diagnostic Performance of E6/E7 mRNA (Test Method A) vs. Combined HPV DNA Testing (Test Method B) for Detecting CIN2+ in the Independent Validation Cohort (N=100)

Parameter	E6/E7 mRNA	Combined Test	*P* value[1]
Test Results			
True Positive	32	35	
False Positive	7	24	
True Negative	57	40	
False Negative	4	1	
Performance Metrics, % (95% CI)			
Sensitivity	88.9 (74.7-95.6)	97.2 (85.8-99.9)	0.375
Specificity	89.1 (78.4-94.9)	70.3 (58.2-80.1)	0.004
Positive Predictive Value	82.1 (67.3-91.4)	61.4 (48.5-72.9)	-
Negative Predictive Value	93.4 (83.5-97.8)	97.6 (87.5-99.6)	-

Notes: Performance metrics are presented as percentages with 95% confidence intervals. Abbreviations: CI, confidence interval; TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; NPV, negative predictive value. [1]*P* values were calculated using McNemar's test for paired comparisons of sensitivity and specificity between the two tests.

Table 6. Performance of the E6/E7 mRNA assay in subgroups stratified by combined DNA test results (n=110)

Stratum/E6/E7 mRNA Result	Final Histology ≥CIN2+ (n)	Final Histology <CIN2 (n)	Total (n)	Performance Metric	Value % (95% CI)
A. Type 1 Positive (HPV 16/18)			55		
mRNA Positive	30	4	34	PPV	88.2 (73.4-95.3)
mRNA Negative	1	20	21	NPV	95.2 (77.3-99.2)
B. Type 2 Positive (Other hr-HPV)			55		
mRNA Positive	18	6	24	PPV	75.0 (55.1-88.0)
mRNA Negative	3	28	31	NPV	90.3 (75.1-96.7)

Notes: This table presents the performance of E6/E7 mRNA testing within predefined risk strata based on combined DNA test results. The positive predictive value (PPV) and negative predictive value (NPV) were calculated for E6/E7 mRNA positivity and negativity, respectively, within each stratum. CI, confidence interval.

Stratified analysis in the atypical squamous cells of undetermined significance (ASCUS) population

Given the clinical challenge of managing individuals with ASCUS cytology, a stratified analysis was performed to evaluate the diagnostic performance of both testing methods within this specific subgroup (n=56). The results of this crucial analysis are presented in **Table 8**.

In this cohort, the standalone E6/E7 mRNA assay (Test Method A) demonstrated a markedly superior specificity of 92.7% (95% CI, 80.6%-97.5%), generating only 3 false-positive results among the 41 patients without high-grade disease. In sharp contrast, the specificity of the combined HPV DNA test (Test Method B) was significantly lower at 63.4% (95% CI, 47.9%-76.8%), producing 15 false positives in the same group. This pronounced difference in

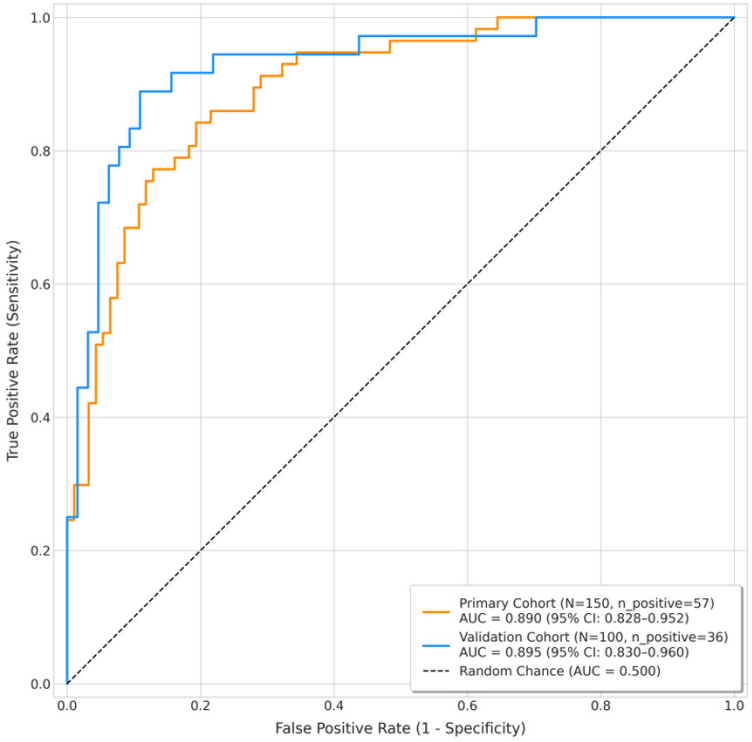

Figure 4. Consistent diagnostic performance of the E6/E7 mRNA assay in the primary and validation cohorts. Receiver operating characteristic (ROC) curves illustrate the diagnostic accuracy of the standalone E6/E7 mRNA assay for the detection of high-grade cervical intraepithelial neoplasia (CIN2+) in the primary cohort (N=150) and the independent validation cohort (N=100). The area under the curve (AUC) for the primary cohort was 0.890 (95% confidence interval [CI], 0.828-0.952), and the AUC for the validation cohort was 0.895 (95% CI, 0.830-0.960).

specificity directly translated to a substantial improvement in PPV. The PPV for the mRNA assay was 81.3% (95% CI, 57.0%-93.4%), while the PPV for the combined test was only 48.3% (95% CI, 30.8%-66.2%).

Both methods maintained high and comparable sensitivity for detecting CIN2+ within the ASCUS group (86.7% for Test A vs. 93.3% for Test B). The NPVs were also high and similar for both assays (95.0% for Test A and 96.3% for Test B), reinforcing their utility in safely ruling out high-grade lesions.

Short-term follow-up validates the safety of triage based on E6/E7 mRNA negativity

To prospectively validate the clinical safety of using E6/E7 mRNA negativity to defer colposcopy in HPV DNA-positive women, we identified a follow-up cohort from the initially excluded patients. This cohort consisted of 65 women who were HPV DNA-positive but E6/E7 mRNA-negative and had a baseline histopathological diagnosis of <CIN2. These women underwent routine clinical surveillance for a median of 18 months (IQR: 14-24 months). As detailed in **Table 9**, the vast majority of these women (96.9%; 63/65) remained free of high-grade disease during the follow-up period, with outcomes including normal cytology, persistent low-grade changes, or clearance of the HPV infection. Only 2 out of 65 patients (3.1%) showed cytological or histological progression to ≥CIN2, yielding an 18-month cumulative risk of progression of 3.1% and affirming an exceptionally high negative predictive value for the mRNA assay in this context. The safety of this triage strategy is further visualized in the Kaplan-Meier curve (**Figure 5**), which plots the cumulative incidence of progression to CIN2+ over time for the mRNA-negative group compared to a matched group of DNA-positive/mRNA-positive women. The cumulative progression rate in the mRNA-negative group remained consistently and significantly lower (log-rank test, P<0.001), providing robust longitudinal evidence that deferring immediate intervention for DNA-positive, mRNA-negative women with an initial <CIN2 diagnosis is a safe and effective management strategy, potentially averting a significant number of unnecessary procedures.

Discussion

This study provides a comprehensive head-to-head comparison of an E6/E7 mRNA-based assay and a combined HPV DNA genotyping and viral load test for the detection of high-grade cervical intraepithelial neoplasia (CIN2+). The central finding is that while both methods demonstrate comparable overall diagnostic accuracy, the E6/E7 mRNA test exhibits significantly superior specificity, a distinction that holds profound implications for clinical prac-

Table 7. Diagnostic performance of E6/E7 mRNA and combined DNA testing stratified by age

Parameter	<30 Years (n=48)		≥30 Years (n=102)	
CIN2+ Prevalence	31.3% (15/48)		41.2% (42/102)	
Test Method	E6/E7 mRNA	Combined Test	E6/E7 mRNA	Combined Test
Sensitivity, % (95% CI)	86.7 (62.1-96.3)	86.7 (62.1-96.3)	88.1 (75.0-95.0)	97.6 (87.4-99.9)
P value (McNemar)	1.000		0.125	
Specificity, % (95% CI)	96.7 (83.3-99.9)	60.0 (42.2-76.1)	87.3 (76.5-93.9)	76.4 (64.4-85.3)
P value (McNemar)	<0.001		0.021	
PPV, % (95% CI)	92.9 (68.5-98.7)	41.7 (25.6-59.8)	81.5 (67.0-90.6)	75.0 (62.6-84.5)
NPV, % (95% CI)	93.5 (79.3-98.2)	93.1 (78.0-98.1)	91.8 (82.2-96.5)	97.9 (89.1-99.6)

Notes: PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval. *P* values for sensitivity and specificity were calculated using McNemar's test for paired comparisons within each age group.

Table 8. Diagnostic performance of the two test methods in the subgroup of patients with ASCUS cytology (n=56)

Parameter	Standalone E6/E7 mRNA	Combined HPV Test
Contingency Table		
True Positive	13	14
False Positive	3	15
True Negative	38	26
False Negative	2	1
Performance Metrics, % (95% CI)		
Sensitivity	86.7 (62.1-96.3)	93.3 (69.8-99.2)
Specificity	92.7 (80.6-97.5)	63.4 (47.9-76.8)
Positive Predictive Value	81.3 (57.0-93.4)	48.3 (30.8-66.2)
Negative Predictive Value	95.0 (83.5-98.9)	96.3 (81.7-99.3)

Notes: This analysis includes 56 patients with ASCUS cytology, with 15 confirmed ≥CIN2+ and 41 <CIN2 on histology. Abbreviations: TP, true positive; FP, false positive; TN, true negative; FN, false negative; CI, confidence interval.

Table 9. Follow-up outcomes for HPV DNA-positive/E6/E7 mRNA-negative women with initial <CIN2 diagnosis (n=65)

Follow-up Outcome	n	%
Regression to Normal/HPV Clearance	35	53.8%
Persistent ASCUS/LSIL (without progression)	28	43.1%
Progression to ≥CIN2	2	3.1%
Total	65	100%
NPV for CIN2+ over follow-up		96.9%

Notes: ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; NPV, negative predictive value. The NPV was calculated as the proportion of patients who did not progress to CIN2+ during the follow-up period (63/65).

tice. Our results show that Test Method A (E6/E7 mRNA) achieved a specificity of 90.3% compared to 71.0% for Test Method B (combined DNA test), a statistically significant difference that translates into a substantial reduction in false-positive results. This key finding is consistent with a growing body of literature that positions E6/E7 mRNA as a marker of active oncogenic transformation rather than merely viral presence [23]. Crucially, the reproducibility of this performance profile was confirmed in an independent validation cohort, where the mRNA assay again demonstrated robust accuracy (AUC= 0.895) and maintained its superior specificity (89.1% vs. 70.3%), underscoring the generalizability of our findings. While the sensitivity of the mRNA test (87.7%) was slightly lower than that of the DNA test (94.7%), this difference was not statistically significant, suggesting that the gain in specificity does not come at the cost of a significant loss in detection capability for high-grade lesions. The overall diagnostic capacity, as measured by the AUC, was excellent and similar for both assays, confirming their comparable ability to distinguish between patients with and without significant disease.

The enhanced specificity of the E6/E7 mRNA assay is rooted in its biological underpinnings. HPV DNA tests detect the presence of the virus, which is a necessary but not sufficient condition for cervical carcinogenesis. Many of these

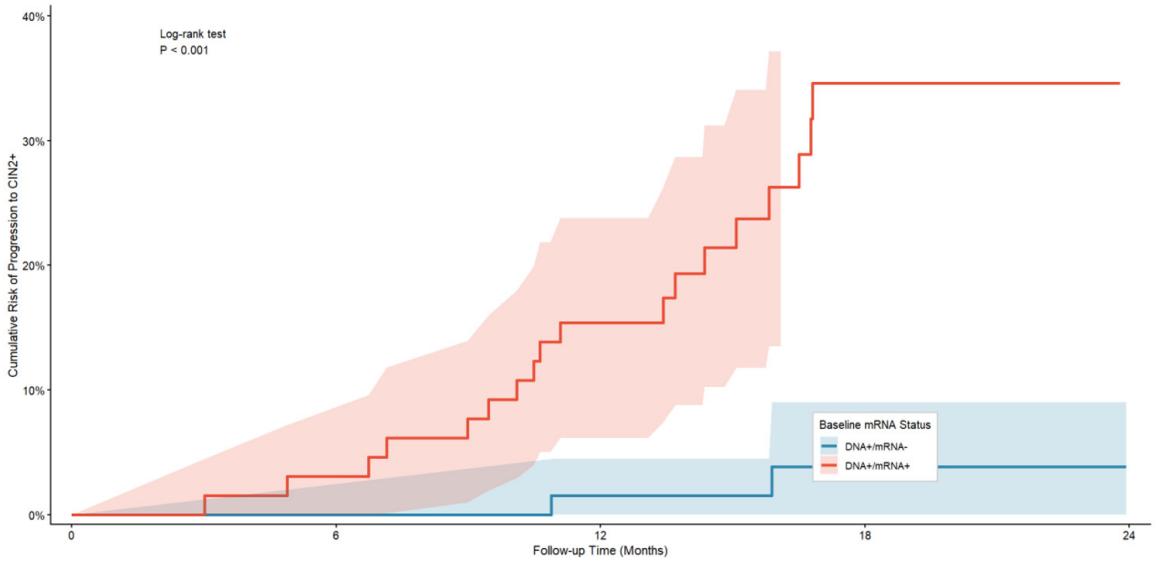

Figure 5. Cumulative risk of progression to CIN2+ stratified by baseline E6/E7 mRNA status in HPV DNA-positive women. Kaplan-Meier curves depicting the cumulative incidence of progression to CIN2+ during follow-up (median 18 months, IQR: 14-24 months) in HPV DNA-positive women stratified by baseline E6/E7 mRNA status. The DNA+/mRNA- group (blue, n=65) consisted of women with an initial histopathological diagnosis of CIN2. A matched DNA+/mRNA+ group (red) served as the comparison cohort. Shaded areas represent 95% confidence intervals. Statistical comparison was performed using the log-rank test.

infections are transient and will be cleared by the immune system without causing clinically significant lesions [24]. In contrast, the detection of E6/E7 mRNA indicates the active transcription of viral oncogenes, which are directly responsible for disrupting cell cycle control by targeting tumor suppressor proteins such as p53 and pRb. This process is a critical step in the malignant transformation of cervical cells [24]. Our study provides direct mechanistic support for this rationale by demonstrating a strong, dose-dependent correlation between E6/E7 mRNA levels and the severity of cervical histopathology (Spearman's ρ=0.72). In contrast, the correlation for DNA viral load was considerably weaker (ρ=0.38), with significant overlap across lower-grade lesions. This fundamental difference explains why mRNA testing, by quantifying the driver of oncogenesis itself, offers a clearer biological separation between inconsequential infections and progressive disease. Our findings align with recent comparative studies, such as the one by Benvari et al. (2025), which reported a specificity of 75.4% for mRNA testing versus 52.3% for DNA testing in detecting CIN2+ lesions [25]. The higher specificity observed in our study (90.3%) may be attributable to differences in the patient population, which consisted of a referral cohort with a high prevalence of disease, or the specific assays used. Nonetheless, the consistent trend across studies validates the principle that measuring oncogenic activity provides a more accurate assessment of immediate cancer risk.

One of the most significant clinical applications of this improved specificity is in the triage of women with ASCUS cytology. Our subgroup analysis revealed a striking difference in performance in this challenging population. The E6/E7 mRNA test maintained a very high specificity of 92.7%, whereas the combined DNA test's specificity dropped to 63.4%. This led to a dramatic improvement in the PPV from 48.3% for the DNA test to 81.3% for the mRNA test. This is particularly crucial, as ASCUS is the most common abnormal cytological finding, and a majority of these women do not have underlying high-grade disease. The poor specificity of DNA testing in this group leads to a high number of colposcopy referrals, causing patient anxiety and burdening healthcare resources. Our results are strongly supported by studies such as Zhu et al. (2019), who found that in an ASCUS cohort, mRNA testing had a specificity of 42.7% compared to just 17.5% for DNA testing, significantly improving the PPV [26]. By

more accurately identifying which ASCUS patients harbor a CIN2+ lesion, the E6/E7 mRNA test can serve as a powerful tool to reduce unnecessary colposcopies and focus resources on those who need them most.

The value of enhanced specificity is further magnified in young women, who have a high prevalence of transient HPV infections. Our age-stratified analysis revealed that the superiority of the mRNA assay was most pronounced in women under 30 years, where its specificity (96.7%) was a remarkable 36.7 percentage points higher than that of the DNA test (60.0%). This translates to a PPV of 93.3% for the mRNA assay, effectively eliminating a vast majority of false alarms in this population where avoiding unnecessary procedures and anxiety is a paramount concern.

Further illustrating its clinical utility, the E6/E7 mRNA assay served as an effective secondary triage tool for patients stratified by the DNA test. Within the high-risk "Other hr-HPV" group (Type 2 Positive), the mRNA test significantly clarified individual risk. The initial PPV for this group was only 38.2%; however, among those who were also mRNA-positive, the PPV rose to 75.0%. Perhaps more importantly, for the 56.4% of patients in this group who tested negative for mRNA, the NPV was 90.3%, effectively downgrading their risk and suggesting that immediate colposcopy could be safely avoided. This demonstrates that E6/E7 mRNA testing can refine risk stratification beyond what is possible with genotyping and viral load alone. These findings are echoed by Sørbye et al. (2025), who found that mRNA triage of HPV DNA-positive women improved specificity from 53.0% (cytology) to 72.3% and reduced the number of required colposcopies by 31% while maintaining comparable sensitivity [27]. The superior specificity of mRNA testing, as confirmed in our study, has direct and meaningful clinical implications for resource utilization within screening programs. By significantly reducing false-positive rates compared to DNA-based strategies, mRNA testing can substantially decrease the number of unnecessary colposcopy referrals. This reduction translates into potential cost savings for healthcare systems by avoiding the direct costs of colposcopy procedures, associated pathological examinations, and follow-up visits. Furthermore, it alleviates patient anxiety, minimizes the risk of overtreatment, and allows healthcare resources to be concentrated on individuals at highest risk, thereby improving the overall efficiency and positive predictive value of the screening cascade. This risk refinement is crucial for managing the large group of women infected with non-16/18 HPV types, where the risk of progression is lower and the need for a more specific biomarker is greater [28].

Most importantly, our short-term follow-up data provide the first longitudinal evidence supporting the safety of this triage strategy. Among HPV DNA-positive women who were E6/E7 mRNA-negative and had a baseline diagnosis of <CIN2, the 18-month cumulative risk of progression to CIN2+ was only 3.1% (NPV: 96.9%). This robust real-world outcome data strongly validates that deferring immediate colposcopy for this large subgroup is a safe and viable clinical pathway, directly addressing a key concern in the implementation of more specific triage algorithms [29].

The analysis of discordant results provided further insight into the distinct capabilities of each test. The most common discordant pattern was an mRNA-negative, DNA-positive result (24 cases), of which 75% were found to have no high-grade disease (<CIN2). This confirms that the mRNA assay correctly identifies a large proportion of the DNA test's false positives, which likely represent transient, non-transforming infections. Conversely, the rare occurrence of an mRNA-positive, DNA-negative result (2 cases) was highly significant, as both of these patients had confirmed CIN2+. This suggests that the mRNA test may, in rare instances, detect active oncogenic expression in cases missed by the combined DNA test, potentially due to low viral loads of integrated HPV that are still transcriptionally active [30]. The ability to detect oncogenic activity independent of high viral load could be a unique advantage of mRNA testing, although this requires further investigation.

Despite its strengths, this study has several limitations. Its retrospective design may introduce selection bias, and as a single-center study, its findings may not be generalizable to all populations or healthcare settings. Specifically, our cohort was derived from a single tertiary hospital in China. The observed perfor-

mance of both tests, particularly the prevalence of specific HPV genotypes and the spectrum of cervical lesions, may be influenced by local epidemiological factors, screening practices, and referral patterns. Consequently, the absolute values of specificity, PPV, and the magnitude of difference between tests might vary in populations with different demographic characteristics, HPV genotype distributions, or underlying disease prevalence. Although we included an independent validation cohort from the same institution to bolster internal validity and demonstrate reproducibility over time, broader multicenter validation across diverse geographical and demographic settings is still warranted to confirm the general applicability of our conclusions. The sample size, while sufficient for the primary analysis, was relatively small for subgroup analyses, particularly for discordant results. Future research should involve larger, prospective, multicenter studies to validate these findings and to assess the long-term outcomes of mRNA-negative, DNA-positive women to confirm the safety of using this marker for deferring colposcopy. Additionally, a cost-effectiveness analysis is needed to determine the economic feasibility of incorporating mRNA testing into routine screening algorithms, weighing the higher upfront cost of the assay against the potential savings from reduced colposcopy referrals [31]. Long-term follow-up studies are also crucial to establish the negative predictive value of mRNA testing over time and to better understand the natural history of HPV infections in the context of oncogene expression.

Conclusion

This study demonstrates that E6/E7 mRNA testing offers a clinically valuable advantage over combined HPV DNA testing by significantly improving specificity and positive predictive value for the detection of CIN2+ lesions, without a significant compromise in sensitivity. Its superior performance, validated in an independent cohort, is mechanistically supported by a strong correlation between mRNA levels and disease severity. The assay's clinical utility is most impactful in key subgroups, including the triage of women with ASCUS cytology and young women under 30, and it effectively refines risk stratification among HPV DNA-positive individuals. Critically, longitudinal follow-up data confirm the safety of using mRNA negativity to defer colposcopy, providing a robust evidence base for its implementation.

Disclosure of conflict of interest

None.

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