Archives of Academic Emergency Medicine. 2026; 14(1): e37

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REVIEW ARTICLE

Diagnostic Accuracy of DNA Methylation Markers for Detecting Cervical Precancer and Cervical Cancer: A Systematic Review and Meta-analysis

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Received: June 2026; Accepted: July 2026; Published online: 15 August 2026

Abstract: **Background:** DNA methylation markers have been proposed as molecular triage tools for detecting cervical precancer and cancer, particularly among HPV-positive women. However, diagnostic performance varies across markers, assays, specimens, and clinical settings. This study aimed to evaluate the marker-specific diagnostic accuracy of DNA methylation markers and methylation panels for detecting CIN2+ and CIN3+ in cervical cancer screening, triage, and related diagnostic contexts. **Methods:** This systematic review and diagnostic test accuracy meta-analysis was reported according to PRISMA-DTA. Marker-specific analyses were performed separately by endpoint to avoid double counting. Pooled sensitivity, specificity, diagnostic odds ratio, clinical utility estimates, risk of bias, publication bias, and certainty of evidence were assessed using diagnostic test accuracy methods, QUADAS-2, Deeks' test, and GRADE-DTA. **Results:** The broad main diagnostic meta-analysis included 74 eligible studies, contributing 260 diagnostic test accuracy comparisons across 130 marker-endpoint-sample analyses. A strict sensitivity dataset included 164 comparisons from 51 studies. Seven marker analyses were available for CIN2+ and seven for CIN3+. Diagnostic performance varied by marker and endpoint. For CIN2+, S5 EPB41L3 plus HPV16/18/31/33 methylation showed the highest sensitivity but lower specificity, whereas PAX1, JAM3, SOX1, PAX1/JAM3, and Six-gene/GynTect showed higher specificity. For CIN3+, S5 showed the highest sensitivity, while PAX1/JAM3, JAM3, SOX1, PAX1, and Six-gene/GynTect had more balanced performance. Deeks' test did not show strong evidence of publication bias for most analyses, although borderline findings were observed in selected marker-endpoint analyses. GRADE-DTA certainty was low or very low across marker-endpoint analyses. **Conclusions:** DNA methylation markers showed heterogeneous diagnostic accuracy for detecting CIN2+ and CIN3+. Some markers demonstrated potentially useful sensitivity- or specificity-oriented profiles, but certainty of evidence was low or very low. Further standardized prospective validation is required before routine clinical implementation.

Keywords: Cervical Cancer; DNA Methylation; Diagnostic Test Accuracy; Meta-Analysis

Cite this article as: Horamee STA, Hosseini M, Arabpour M, Modarressi MH. Diagnostic Accuracy of DNA Methylation Markers for Detecting Cervical Precancer and Cervical Cancer: A Systematic Review and Meta-analysis. Arch Acad Emerg Med. 2026;14(1): e37. https://doi.org/10.22037/aaem.v14i1.3062.

1. Introduction:

Cervical cancer remains a major global public health problem despite being largely preventable through human papillomavirus (HPV) vaccination, screening, and treatment of precancerous lesions. Recent global estimates indicate that cervical cancer continues to cause substantial morbidity and mortality, with the greatest burden in low- and middle-income countries [1]. The World Health Organization has therefore launched a global strategy to eliminate cervical cancer as a public health problem, supported by vaccination, screening, and treatment targets [2]. Achieving these targets requires accurate screening and triage strategies that identify women at greatest risk of clinically relevant disease while minimizing unnecessary referral and overtreatment.

Persistent infection with high-risk HPV is the central causal event in cervical carcinogenesis [3,4]. HPV-based screening is more sensitive than cytology and provides greater protection against invasive cervical cancer when implemented in organized screening programs [5]. However, HPV infection is common and often transient, especially in younger women. As a result, primary HPV testing has limited specificity for clinically significant cervical precancer, and many HPV-positive women do not have CIN2+ or CIN3+ lesions requiring treatment. This has created a need for molecular triage tests that can distinguish transient HPV infections from transforming infections associated with progression to high-grade cervical intraep-ithelial neoplasia and cancer [6].

Current triage strategies, including cytology and HPV genotyping, have important limitations. Cytology is subjective, requires laboratory infrastructure, and may have variable reprodu-cibility across settings. HPV16/18 genotyping identifies women at increased risk but does not fully capture the biological transition from productive infection to transforming disease. The increasing use of HPV self-sampling further reinforces the need for objective molecular triage tests that can be applied to clinician-collected and self-collected specimens. Self-sampling can improve screening participation, particularly among underscreened women, but its value depends on reliable downstream triage strategies [7].

DNA methylation has emerged as a biologically plausible and clinically promising biomarker for cervical precancer and cancer. During HPV-mediated transformation, genetic and epigenetic alterations accumulate in host cells, and methylation changes can help distinguish productive HPV infections from transforming lesions with higher malignant potential [8]. Host-cell DNA methylation markers and HPV DNA methylation

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markers have therefore been investigated as tools for detecting advanced cervical intraep-ithelial neoplasia and cervical cancer, particularly among HPV-positive women [9].

A wide range of host-cell and viral methylation markers has been evaluated in cervical screening and triage studies, including single-gene markers such as PAX1, SOX1, JAM3, EPB41L3, MAL, CADM1, ZNF671, and miR124-2, as well as multigene or combined host/viral panels such as FAM19A4/miR124-2, CADM1/MAL-based panels, S5 EPB41L3 plus HPV16/18/31/33 methylation classifiers, PAX1/JAM3 panels, and Six-gene/GynTect-type assays. Nevertheless, studies differ substantially in design, clinical setting, specimen type, HPV status, methylation assay platform, diagnostic threshold, target endpoint, and reference standard. These sources of heterogeneity make it difficult to determine which methylation markers have the most reliable diagnostic performance for CIN2+ and CIN3+.

Therefore, this systematic review and meta-analysis aimed to evaluate the marker-specific diagnostic accuracy of DNA methylation markers and methylation panels for detecting cervical precancer and cervical cancer.

2. Methods:

2.1. *Study design and reporting*

This systematic review and diagnostic test accuracy meta-analysis was designed to evaluate the diagnostic performance of DNA methylation markers for detecting cervical precancer and cervical cancer. The review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy studies (PRISMA-DTA) [10]. The PRISMA-DTA checklist was used to guide reporting of the review question, eligibility criteria, index tests, target conditions, reference standards, search strategy, study selection, data extraction, risk-of-bias assessment, synthesis, and interpretation of findings.

The primary quantitative endpoints were CIN2+ and CIN3+. Analyses were conducted separately by marker and endpoint to avoid double counting and to preserve clinically meaningful distinctions between diagnostic targets. Non-pooled evidence was summarized narratively, risk of bias was assessed using QUADAS-2 [11], certainty of evidence was evaluated using GRADE-DTA [12,13], and potential clinical interpr-etability was examined through likelihood ratios and post-test probability analyses.

2.2. *Eligibility criteria*

Studies were eligible if they included human participants evaluated for cervical precancer, cervical cancer, or related cervical disease endpoints; assessed at least one DNA methylation marker, methylation panel, or methylation classifier as an index test; reported diagnostic accuracy data for CIN2+, CIN3+, HSIL+, cervical cancer, or a clearly defined related endpoint; and provided sufficient information to extract or reconstruct a 2 x 2 diagnostic table. Original diagnostic accuracy, cohort, cross-sectional, nested case-control, case-control, discovery/validation, and clinically relevant assay-validation studies were eligible.

Studies were excluded from quantitative synthesis if they did not provide extractable or reconst-ructable TP, FP, FN, and TN data; reported only methylation frequency, AUC, p-values, or association estimates without a diagnostic threshold; evaluated prognostic, progression, recurrence, post-treatment, or treatment-selection endpoints rather than diagnostic detection of CIN2+ or CIN3+; used cancer-only or sensitivity-only designs without non-disease controls; or focused only on analytical validation without clinical diagnostic accuracy data. Studies excluded from quantitative synthesis but relevant to methylation biomarkers were retained for narrative synthesis when appropriate.

2.3. *Information sources, search strategy, and study selection*

A comprehensive electronic search was conducted in PubMed/MEDLINE, Embase, Scopus, and Web of Science from database inception to 28 February 2026. The search strategy was designed to identify diagnostic test accuracy studies evaluating DNA methylation markers or methylation panels for cervical precancer or cervical cancer screening and triage. Search terms combined controlled vocabulary and free-text terms related to human papillomavirus infection, cervical cancer or cervical intraep-ithelial neoplasia, DNA methylation or epigenetic biomarkers, and diagnostic accuracy or triage. Animal-only studies were excluded using database-specific filters or exclusion terms. The complete database-specific search strategies are provided in Supplementary Appendix 1.

Records retrieved from the searches were deduplicated, screened by title and abstract, and then assessed in full text against the predefined eligibility criteria. Title-and-abstract screening and full-text assessment were performed independently by two reviewers, with disagreements resolved by discussion or consultation with a third reviewer. Studies relevant to the review question but unsuitable for quantitative pooling were retained for narrative synthesis when they provided clinically or methodo-logically informative evidence.

2.4. *Data extraction*

Data were extracted independently by two reviewers using a structured diagnostic test accuracy extraction form, and discrepancies were resolved by consensus. Extracted items included first author, year, country, study design, clinical setting, population charact-eristics, specimen type, HPV assay or HPV status, methylation marker or panel, methylation assay platform, index-test threshold, target endpoint, comparator group, reference standard, verification approach, TP, FP, FN, TN, sensitivity, specificity, DOR, AUC when reported, and information relevant to risk of bias and applicability.

For each index test and endpoint, true positives were participants with the target condition who tested methylation-positive; false negatives were participants with the target condition who tested methylation-negative; false positives were participants without the target condition who tested methylation-positive; and true negatives were participants without the target condition who tested methylation-negative. Sensitivity was calculated as TP/(TP + FN), specificity as TN/(TN + FP), DOR as (TP x TN)/(FP x FN), LR+ as sensitivity/(1 - specificity), and LR- as (1 - sensitivity)/specificity.

Index tests included single-gene methylation markers, multigene host methylation panels, combined host/viral methylation classifiers, and commercial methylation assays. The principal pooled marker groups included PAX1, SOX1, JAM3, PAX1/JAM3 panel, FAM19A4/miR124-2, S5 EPB41L3 plus HPV16/18/31/33 methylation, and Six-gene/GynTect-type panels. The main target conditions were CIN2+ and CIN3+,

which were analyzed separately. HSIL+, invasive cervical cancer, and other endpoints were retained for systematic review and narrative synthesis but were not pooled with CIN2+ or CIN3+ unless clinically and analytically comparable.

The preferred reference standard was histology-based verification, including colposcopy-directed biopsy, LLETZ/cone histology, registry-confirmed histology, or histopathology. Studies using partial verification, follow-up verification, mixed clinical verification, cytology-based endpoints, p16/Ki-67 endpoints, or non-histologic reference standards were not automatically excluded, but these features were assessed for risk of bias and applicability and considered in sensitivity analyses, narrative synthesis, and GRADE-DTA certainty assessments.

2.5. *Risk of bias and applicability assessment*

Risk of bias and applicability concerns were assessed independently by two reviewers using QUADAS-2 [11], with disagreements resolved by consensus. The four QUADAS-2 domains were patient selection, index test, reference standard, and flow/timing. Risk of bias was judged for all four domains, and applicability concerns were assessed for patient selection, index test, and reference standard. Judgments were classified as low, high, or unclear. Overall risk-of-bias and applicability judgments were derived from domain-level assessments recorded in the extraction checklist.

2.6. *Statistical analysis*

Quantitative diagnostic test accuracy analyses were performed in Python. For each marker-endpoint analysis, 2 × 2 diagnostic tables were used to calculate study-level sensitivity, specificity, diagnostic odds ratio, positive likelihood ratio, and negative likelihood ratio. Analyses were conducted separately for each methylation marker or methylation panel and diagnostic endpoint. CIN2+ and CIN3+ were analyzed separately.

Within each marker-specific analysis, only one eligible dataset from each study was included to avoid double counting. When a study reported multiple different methylation markers, each marker was retained in its corresponding marker-specific analysis. However, when a study reported multiple datasets for the same marker and endpoint, one primary dataset was selected for the main analysis according to predefined criteria, prioritizing validation cohorts, prespecified thresholds, clinically relevant endpoints, histology-based reference standards, larger or more representative cohorts, and non-overlapping populations. Secondary datasets were retained for sensitivity analysis or narrative synthesis when informative.

For each marker-endpoint group, pooled sensitivity and specificity were estimated using logit-transformed proportions with inverse-variance weighting under a random-effects model. Between-study variance was estimated using a random-effects approach. Pooled estimates were back-transformed to the probability scale and reported with 95% confidence intervals. Diagnostic odds ratios were pooled on the natural logarithmic scale and then exponentiated to obtain pooled DORs with 95% confidence intervals. When a 2 × 2 table contained zero cells, a continuity correction was applied only as required for logarithmic calculations.

A strict sensitivity dataset was analyzed to evaluate the robustness of the main estimates under more restrictive eligibility criteria. Stability of pooled sensitivity, specificity, and DOR was compared between the broad main analysis and the strict sensitivity analysis. Results were classified as robust when estimates remained directionally similar and clinically consistent. Moderate change was assigned when sensitivity, specificity, or DOR changed enough to suggest sensitivity to study selection. Marker-endpoint analyses that could not be pooled in the strict dataset were classified as not comparable.

Threshold-related heterogeneity and between-study variability were assessed descriptively by comparing sensitivity and specificity across studies, inspecting ROC-space patterns, and comparing broad and strict sensitivity analyses. Because several marker-endpoint analyses included relatively few studies, formal subgroup and threshold-effect analyses were interpreted cautiously.

Publication bias was assessed using Deeks' funnel plot asymmetry test for marker-endpoint analyses included in the overall analysis [14]. Because several analyses included fewer than 10 studies, Deeks' test results were interpreted cautiously.

Exploratory clinical utility was evaluated by estimating likelihood ratios and post-test probabilities from pooled point estimates. Post-test probabilities were calculated by converting assumed pre-test probabilities to odds, multiplying by the relevant likelihood ratio, and converting post-test odds back to probability. Post-test probabilities were calculated at assumed pre-test probabilities of 5%, 10%, and 20%. These estimates were interpreted as clinical utility approximations rather than formal decision-analytic modelling.

2.7. *Certainty of evidence*

The certainty of evidence for marker-specific diagnostic accuracy estimates was assessed using the GRADE approach for diagnostic test accuracy [12,13]. Certainty was evaluated separately for each marker and endpoint. Domains considered were risk of bias, indirectness, inconsistency, imprecision, and publication bias. Certainty was rated as high, moderate, low, or very low. These ratings refer to certainty in diagnostic accuracy estimates and do not imply certainty in downstream clinical effectiveness.

3. Results

3.1. *Study selection and evidence base*

The database searches yielded 1,689 records, including 416 records from PubMed, 482 from Embase, 460 from Scopus, and 329 from Web of Science. After removal of 559 duplicates, 1,130 unique records were screened by title and abstract. Of these, 1,032 records were excluded, and 102 reports were assessed in full text. Sixteen full-text reports were excluded: four were review articles, eight focused on other cancers, and four did not report CIN2+ or CIN3+ as eligible diagnostic endpoints. Overall, 86 primary reports were included in the systematic review evidence base. Of these, 74 studies [15-88] were included in the quantitative diagnostic test accuracy synthesis, contributing 260 diagnostic accuracy comparisons across 130 marker-endpoint-sample analyses. The remaining 12 primary reports were retained for narrative-only synthesis because they were relevant to cervical methylation biomarkers but were not eligible for quantitative diagnostic test accuracy pooling. The study selection process is shown in Figure 1.

The included quantitative studies were published between 2011 and 2026 and were conducted across diverse geographic settings, with the largest contributions from China, the Nether-

lands, the United Kingdom, and Germany, and additional studies from the USA, Taiwan, Sweden, Russia, Canada, Kenya, Mexico, South Africa, Costa Rica, Papua New Guinea, Greece, Colombia, Portugal, and multicountry European or worldwide cohorts. The main target conditions were CIN2+ and CIN3+, whereas a smaller number of studies addressed HSIL+ or cervical cancer-related endpoints (Table 1).

Most quantitative studies evaluated clinician-collected cervical scrapings, exfoliated cervical cells, liquid-based cytology samples, or cervical smears. A smaller subset evaluated self-collected vaginal or cervicovaginal samples, paired clinician/self-collected specimens, or biopsy/FFPE material (Table 1). The evaluated index tests included single-gene methylation markers and multigene or combined host/viral methylation panels, including PAX1, SOX1, JAM3, FAM19A4/miR124-2, CADM1/MAL-based panels, S5 EPB41L3 plus HPV16/18/31/33 methylation classifiers, PAX1/JAM3 panels, and Six-gene/GynTect-type panels (Table 1).

Methylation testing was most commonly performed using bisulfite-based quantitative methylation-specific PCR, real-time methylation-specific PCR, pyrosequencing, droplet digital PCR, methylation arrays, or commercial methylation assays. Reference standards were mainly histology-based, including colposcopy-directed biopsy, LLETZ/cone histology, registry-based histology, or histopathology. Verification was complete in 29 studies, partial in 25 studies, and otherwise based on case-control, follow-up, mixed, or unreported verification approaches (Table 1). Study-level QUADAS-2 risk-of-bias and applicability judgments are presented in Table 2.

3.2. *Quantitative diagnostic accuracy meta-analysis*

- *Marker-specific diagnostic accuracy for CIN2+*

Seven marker analyses were available for CIN2+: FAM19A4/miR124-2, JAM3, PAX1, PAX1/JAM3 panel, S5 EPB41L3 + HPV16/18/31/33 methylation, SOX1, and Six-gene/GynTect panel. These marker groups represented a mixture of commercial methylation tests, qMSP-based assays, pyrosequencing-based classifiers, and combined host/viral methylation strategies. Marker-specific pooled estimates for CIN2+ and CIN3+ are summarized in Table 3 and Figure 2.

For FAM19A4/miR124-2, four studies were included in the main analysis (Table 3). The pooled sensitivity was 0.62 (95% CI: 0.49-0.74), specificity was 0.77 (95% CI: 0.74-0.80), and DOR was 5.15 (95% CI: 2.90-9.13). This analysis was pooled only in the broad main dataset and was not comparable in the strict sensitivity analysis.

For JAM3, seven studies were included in the main analysis and four in the strict sensitivity analysis (Table 3). The main pooled sensitivity was 0.66 (95% CI: 0.49-0.79), specificity was 0.93 (95% CI: 0.88-0.96), and DOR was 26.64 (95% CI: 11.07-64.09). In the strict sensitivity analysis, sensitivity decreased to 0.58 (95% CI: 0.40-0.75), specificity increased slightly to 0.95 (95% CI: 0.93-0.96), and DOR remained broadly similar at 23.47 (95% CI: 9.59-57.45), representing a moderate change.

For PAX1, six studies were included in the main analysis and four in the strict sensitivity analysis (Table 3). The pooled sensitivity was 0.73 (95% CI: 0.56-0.84), specificity was 0.92 (95% CI: 0.90-0.95), and DOR was 35.02 (95% CI: 14.47-84.76). Strict sensitivity estimates were similar or slightly stronger, with sensitivity of 0.78 (95% CI: 0.50-0.92), specificity of 0.92 (95% CI: 0.91-0.94), and DOR of 43.04 (95% CI: 14.28-129.71). These findings were classified as robust.

For the PAX1/JAM3 panel, five studies were included in the main analysis and four in the strict sensitivity analysis (Table 3). The main pooled sensitivity was 0.72 (95% CI: 0.58-0.83), specificity was 0.94 (95% CI: 0.92-0.95), and DOR was 43.73 (95% CI: 16.83-113.65). In the strict sensitivity analysis, sensitivity decreased to 0.64 (95% CI: 0.51-0.76), specificity remained high at 0.93 (95% CI: 0.91-0.95), and DOR was 29.37 (95% CI: 12.11-71.27), representing a moderate change.

For S5 EPB41L3 + HPV16/18/31/33 methylation, eight studies were included in the main analysis and five in the strict sensitivity analysis (Table 3). The pooled sensitivity was 0.84 (95% CI: 0.77-0.90), whereas specificity was lower at 0.47 (95% CI: 0.38-0.55). The pooled DOR was 4.67 (95% CI: 3.34-6.53). The strict sensitivity analysis showed similar sensitivity of 0.83 (95% CI: 0.74-0.89), specificity of 0.45 (95% CI: 0.33-0.57), and DOR of 3.84 (95% CI: 2.56-5.75). This analysis was classified as robust.

For SOX1, six studies were included in the main analysis and four in the strict sensitivity analysis (Table 3). The pooled sensitivity was 0.67 (95% CI: 0.55-0.77), specificity was 0.90 (95% CI: 0.87-0.92), and DOR was 20.21 (95% CI: 9.93-41.15). The strict sensitivity analysis yielded similar estimates, with sensitivity of 0.68 (95% CI: 0.54-0.78), specificity of 0.90 (95% CI: 0.87-0.92), and DOR of 18.13 (95% CI: 9.01-36.49). These findings were classified as robust.

For the Six-gene/GynTect panel, seven studies were included in the main analysis and six in the strict sensitivity analysis (Table 3). The pooled sensitivity was 0.64 (95% CI: 0.57-0.69), specificity was 0.93 (95% CI: 0.86-0.97), and DOR was 24.10 (95% CI: 9.34-62.17). In the strict sensitivity analysis, sensitivity was 0.62 (95% CI: 0.56-0.67), specificity was 0.92 (95% CI: 0.83-0.96), and DOR was 18.12 (95% CI: 7.27-45.18). This analysis was classified as robust.

- *Marker-specific diagnostic accuracy for CIN3+*

Seven marker analyses were also available for CIN3+: FAM19A4/miR124-2, JAM3, PAX1, PAX1/JAM3 panel, S5 EPB41L3 + HPV16/18/31/33 methylation, SOX1, and Six-gene/GynTect panel (Table 3).

For FAM19A4/miR124-2, four studies were included in both the main and strict sensitivity analyses (Table 3). The pooled sensitivity was 0.70 (95% CI: 0.59-0.79), specificity was 0.74 (95% CI: 0.71-0.78), and DOR was 6.59 (95% CI: 3.66-11.88). Estimates were unchanged in the strict sensitivity analysis, and the result was classified as robust.

For JAM3, eight studies were included in the main analysis and six in the strict sensitivity analysis (Table 3). The pooled sensitivity was 0.74 (95% CI: 0.65-0.82), specificity was 0.90 (95% CI: 0.85-0.94), and DOR was 27.44 (95% CI: 16.55-45.49). In the strict sensitivity analysis, sensitivity was 0.70 (95% CI: 0.60-0.78), specificity was 0.91 (95% CI: 0.84-0.95), and DOR was 23.22 (95% CI: 12.80-42.10). This analysis was classified as robust.

For PAX1, 11 studies were included in the main analysis and nine in the strict sensitivity analysis (Table 3). The pooled sensitivity was 0.76 (95% CI: 0.70-0.81), specificity was 0.85 (95% CI: 0.80-0.89), and DOR was 18.82 (95% CI: 10.98-32.27). The strict sensitivity analysis produced similar estimates, with sensitivity of 0.74 (95% CI: 0.67-0.79), specificity of 0.85 (95% CI:

0.80-0.90), and DOR of 18.54 (95% CI: 9.98-34.44). These findings were classified as robust.

For the PAX1/JAM3 panel, five studies were included in the main analysis and four in the strict sensitivity analysis (Table 3). The main pooled sensitivity was 0.80 (95% CI: 0.69-0.88), specificity was 0.88 (95% CI: 0.86-0.90), and DOR was 31.59 (95% CI: 16.69-59.78). In the strict sensitivity analysis, sensitivity was 0.76 (95% CI: 0.66-0.83), specificity was 0.89 (95% CI: 0.88-0.90), and DOR was 24.77 (95% CI: 14.56-42.15). The analysis was classified as robust.

For S5 EPB41L3 + HPV16/18/31/33 methylation, seven studies were included in the main analysis and four in the strict sensitivity analysis (Table 3). The pooled sensitivity was high at 0.90 (95% CI: 0.82-0.94), while specificity was 0.52 (95% CI: 0.44-0.61). The pooled DOR was 9.88 (95% CI: 3.73-26.12). In the strict sensitivity analysis, sensitivity decreased to 0.86 (95% CI: 0.78-0.91), specificity decreased to 0.43 (95% CI: 0.32-0.56), and DOR decreased to 5.17 (95% CI: 2.53-10.55). This was classified as a moderate change.

For SOX1, eight studies were included in the main analysis and seven in the strict sensitivity analysis (Table 3). The pooled sensitivity was 0.82 (95% CI: 0.72-0.88), specificity was 0.85 (95% CI: 0.82-0.88), and DOR was 26.80 (95% CI: 12.35-58.16). In the strict sensitivity analysis, sensitivity decreased to 0.75 (95% CI: 0.69-0.81), specificity remained similar at 0.85 (95% CI: 0.81-0.88), and DOR decreased to 17.92 (95% CI: 10.05-31.95). This was classified as a moderate change.

For the Six-gene/GynTect panel, eight studies were included in both the main and strict sensitivity analyses (Table 3). The pooled sensitivity was 0.73 (95% CI: 0.65-0.80), specificity was 0.86 (95% CI: 0.81-0.90), and DOR was 17.33 (95% CI: 10.03-29.93). Estimates were unchanged in the strict sensitivity analysis, and the result was classified as robust.

- ***Comparative diagnostic performance across markers***

Diagnostic performance varied substantially across markers and endpoints. For CIN2+, PAX1/JAM3 panel, PAX1, JAM3, SOX1, and the Six-gene/GynTect panel showed relatively high specificity, whereas S5 EPB41L3 + HPV16/18/31/33 methylation showed higher sensitivity but lower specificity (Table 3; Figure 2). This pattern indicates that S5 may identify a larger proportion of CIN2+ cases, but at the cost of more false-positive results.

For CIN3+, S5 EPB41L3 + HPV16/18/31/33 methylation showed the highest pooled sensitivity, followed by SOX1 and the PAX1/JAM3 panel. However, S5 again showed lower specificity than most other markers. PAX1/JAM3 panel, JAM3, SOX1, PAX1, and the Six-gene/GynTect panel showed more balanced sensitivity-specificity profiles (Table 3; Figures 2 and 4). Direct comparisons between markers should be interpreted cautiously because the contributing studies differed in population, clinical setting, sample type, methylation assay, diagnostic threshold, and target endpoint. The sensitivity-specificity trade-off across marker-endpoint analyses is displayed in the diagnostic performance map (Figure 4), and the study-level ROC-space distributions for CIN2+ and CIN3+ are shown in Figure 5 and Figure 6, respectively.

- ***Sensitivity analysis***

The strict sensitivity analysis generally supported the direction of the main findings. For CIN2+, robust results were observed for PAX1, S5 EPB41L3 + HPV16/18/31/33 methylation, SOX1, and the Six-gene/GynTect panel, while JAM3 and the PAX1/JAM3 panel showed moderate changes (Table 3; Figure 3).

For CIN3+, robust results were observed for FAM19A4/miR124-2, JAM3, PAX1, PAX1/JAM3 panel, and the Six-gene/GynTect panel. S5 EPB41L3 + HPV16/18/31/33 methylation and SOX1 showed moderate changes in the strict sensitivity analysis, with reduced DOR estimates and, for S5, lower specificity (Table 3; Figure 3). Overall, the strict sensitivity analysis indicated that several marker-endpoint estimates were stable, but some analyses remained sensitive to study selection and eligibility restrictions.

- ***Publication bias***

Publication bias was assessed using Deeks' funnel plot asymmetry test for the marker-endpoint analyses included in the overall analysis. In total, 14 pooled marker-endpoint analyses were evaluated, including seven analyses for CIN2+ and seven analyses for CIN3+. The number of studies per analysis ranged from 4 to 11; therefore, the results of Deeks' test should be interpreted cautiously, particularly for analyses with fewer than 10 studies. Deeks' funnel plots are presented in Figure 7.

For CIN2+, most marker analyses did not show statistically significant evidence of funnel plot asymmetry. Deeks' test was non-significant for FAM19A4/miR124-2, JAM3, PAX1/JAM3 panel, S5 panel, SOX1, and the Six-gene/GynTect panel. However, PAX1 showed borderline evidence of funnel plot asymmetry with a Deeks' test p-value of 0.050. In addition, JAM3 and SOX1 showed borderline trends toward asymmetry, with p-values of 0.087 and 0.081, respectively.

For CIN3+, Deeks' test did not show statistically significant evidence of publication bias in any marker analysis. The lowest p-value was observed for the PAX1/JAM3 panel ($p = 0.089$), suggesting a borderline trend toward funnel plot asymmetry, but this did not reach conventional statistical significance. The remaining CIN3+ analyses showed no evidence of significant asymmetry, with p-values ranging from 0.132 to 0.909.

Overall, the Deeks' funnel plot analysis did not provide strong evidence of publication bias across most methylation marker analyses. Nevertheless, the borderline findings observed for PAX1 in CIN2+, and to a lesser extent for JAM3, SOX1, and PAX1/JAM3 panel, should be interpreted with caution because several analyses included a small number of studies. Therefore, these findings are best regarded as exploratory rather than definitive evidence of small-study effects or publication bias.

3.3. *Narrative synthesis*

- ***Overview of narrative-only and non-pooled evidence***

In addition to the quantitative evidence base, 12 primary reports from the earlier extraction dataset were incorporated into the narrative synthesis because they addressed cervical methylation biomarkers but were not suitable for quantitative DTA pooling [89-100]. These reports included discovery or validation studies without reconst-ructable 2x2 data, prognostic or post-treatment studies, cancer-only or sensitivity-only reports, assay reprodu-cibility studies, tissue-based classifiers, and studies in special populations such as vaccinated women or women living with HIV (Supplementary Table S1).

Several primary studies evaluated relevant methylation markers but could not be pooled because they did not provide a fixed binary threshold with TP, FP, FN, and TN counts for CIN2+ or CIN3+. Clarke et al. evaluated candidate host DNA methylation markers, including SOX1, in a discovery and validation framework, but no fixed binary threshold or reconst-ructable 2x2 table was available for quantitative pooling [90]. Hesselink et al. assessed MAL-m1/miR124-2 and related markers in self-collected cervicovaginal lavage specimens from hrHPV-positive women, but the main report provided threshold-dependent sensitivity ranges rather than a single unique operating point with complete 2x2 counts [92]. Liu et al. evaluated an HPV16 methylation classifier and reported AUC-based discrimination for HSIL and squamous cell carcinoma comparisons, but sensitivity, specificity, and 2x2 data were not available for pooling [93]. Zheng et al. developed tissue-based DNA methylation classifiers for cervical cancer risk prediction among HIV-positive Nigerian women; this was a tissue-based discovery and machine-learning classification study rather than a routine cervical screening or triage DTA study, and exact 2x2 denominators were not extractable [100].

A separate group of studies addressed prognostic, post-treatment, or treatment-selection questions rather than primary diagnostic accuracy. Louvanto et al. evaluated methylation for predicting progression of untreated CIN2 during active surveillance [94]. Dübbel et al. assessed ZNF671 methylation in relation to progressive, persistent, recurrent, or regressive cervical intraep-ithelial neoplasia using repeated FFPE tissue samples [89]. Wu et al. evaluated a six-methylation-marker assay for predicting LEEP specimen histology among women with biopsy-diagnosed HSIL [99]. These studies provide clinically relevant information on progression or risk stratification, but their endpoints differ from the screening or triage detection of CIN2+ and CIN3+ and were therefore not pooled.

Two studies contributed cancer-related or sensitivity-only evidence. Vink et al. evaluated FAM19A4/miR124-2 methylation in invasive cervical cancer and reported methylation positivity among cancer cases, but the absence of a non-disease control group prevented estimation of specificity [98]. Kinkorova Lunackova et al. assessed FAM19A4/miR124-2 methylation as an ancillary test in HPV-associated AIS or endocervical adenocarcinoma after severe glandular cytology and reported sensitivity-only evidence [96]. These studies were not eligible for DTA pooling because FP and TN counts were not available, but they support the broader relevance of methylation positivity in glandular and invasive cervical cancer contexts.

Some excluded studies were technically informative rather than clinical DTA studies. Floore et al. evaluated intra- and inter-laboratory agreement of the FAM19A4/miR124-2 methylation test and reported high reprodu-cibility, but no CIN2+ or CIN3+ clinical 2x2 accuracy data were provided [91]. Snellenberg et al. developed and validated a multiplex methylation-specific PCR assay for CADM1, MAL, and hsa-miR124-2 and reported analytical agreement between multiplex and singleplex qMSP assays, but no clinical 2x2 diagnostic accuracy data were available [97].

Evidence from HPV-vaccinated populations remains limited. Louvanto et al. evaluated viral and host-cell methylation markers among HPV-vaccinated women with cervical squamous intraep-ithelial lesions. Methylation levels were generally low, and individual viral or host-cell markers did not differ significantly between LSIL/HSIL cases and controls; however, a combined viral-gene/EPB41L3 methylation measure differed significantly between HSIL cases and controls [95]. No diagnostic 2x2 table suitable for the present CIN2+/CIN3+ pooling was available. This study was therefore summarized narratively and may be relevant to future evaluations of methylation triage in vaccinated screening cohorts.

Two evidence syntheses were identified in the earlier dataset. Salta et al. conducted a systematic review and meta-analysis of DNA methylation as a triage marker in HPV-based cervical cancer screening [101], and Zhang et al. provided a narrative review of host-cell and HPV DNA methylation markers for cervical cancer triage [102]. These records were not counted as primary studies and were not included in quantitative pooling or the primary evidence base, but they may be cited in the Introduction or Discussion for background context.

Overall, the narrative evidence shows that the methylation biomarker literature for cervical precancer and cervical cancer is broader than the set of studies eligible for quantitative DTA synthesis. Many non-pooled markers and classifiers showed potentially relevant diagnostic, prognostic, or technical signals, but most could not be pooled because of insufficient replication, non-comparable thresholds, non-diagnostic endpoints, tissue-based or special-population designs, sensitivity-only reporting, or absence of complete 2x2 diagnostic data. These findings support the need for standardized reporting of TP, FP, FN, and TN; prespecified diagnostic thresholds; clear separation of CIN2+ and CIN3+ endpoints; and prospective validation in clinically relevant screening and triage populations.

3.4. *Clinical utility and post-test probability analysis*

Clinical utility was estimated from pooled point estimates using likelihood ratios. LR+ was calculated as sensitivity/(1 - specificity), and LR- as (1 - sensitivity)/specificity. For assumed pre-test probabilities of 5%, 10%, and 20%, post-test probabilities were calculated by converting probability to odds, multiplying by the relevant likelihood ratio, and converting odds back to probability.

To evaluate the potential clinical interpr-etability of the pooled diagnostic accuracy estimates, post-test probabilities were calculated using pooled likelihood ratios derived from the overall analysis. This analysis included 14 pooled marker-endpoint analyses, comprising seven analyses for CIN2+ and seven analyses for CIN3+. Post-test probabilities were estimated at three clinically plausible pre-test probabilities: 5%, 10%, and 20% (Table 4; Supplementary Table S2).

For CIN2+, methylation testing substantially increased the probability of disease after a positive result for most marker analyses. At a pre-test probability of 10%, the median post-test probability after a positive test was 50.8%, whereas the median post-test probability after a negative test was 3.9%. Among CIN2+ analyses, the PAX1/JAM3 panel produced one of the highest post-test probabilities after a positive test, increasing the probability from 10% to 55.4%. PAX1 and the PAX1/JAM3 panel also showed the lowest post-test probability after a negative test, reducing the probability from 10% to approximately 3.2%.

For CIN3+, the clinical utility pattern was generally similar, although the absolute post-test probabilities after a positive test varied across markers. At a pre-test probability of 10%, the median post-test probability after a positive test was 36.5%, while the median post-test probability after a negative test was 3.1%. These findings indicate that positive methylation tests

may meaningfully enrich the probability of high-grade cervical disease, whereas negative tests may reduce the probability of CIN2+ or CIN3+ to a low residual risk, particularly for markers with lower negative likelihood ratios.

Overall, the post-test probability analysis supports the potential role of selected methylation markers as triage tools. Markers and panels with high positive likelihood ratios may be most useful for ruling in clinically relevant cervical lesions after a positive result, whereas markers with low negative likelihood ratios may be useful for reducing residual disease probability after a negative result. However, these estimates are based on pooled point estimates and should be interpreted as clinical utility approximations rather than formal decision-analytic modelling.

3.5. *GRADE-DTA certainty of evidence*

The certainty of evidence for each marker-specific diagnostic accuracy analysis was assessed using the GRADE approach for diagnostic test accuracy. Certainty was evaluated separately for each methylation marker or panel and target endpoint, considering risk of bias, indirectness, inconsistency, imprecision, and publication bias. Risk of bias and applicability were informed by QUADAS-2 judgments, and inconsistency was assessed using the robustness of pooled estimates in strict sensitivity analyses.

The certainty of evidence was assessed using the GRADE approach for diagnostic test accuracy for 14 marker-endpoint analyses, including seven analyses for CIN2+ and seven analyses for CIN3+. Overall certainty was low for seven analyses and very low for seven analyses; none of the marker-specific analyses reached high or moderate certainty. The main reasons for downgrading were risk of bias and indirectness. Risk-of-bias concerns were mainly related to patient selection, index-test conduct or threshold derivation, and flow/timing, including partial verification or enriched case-control sampling in several analyses. Indirectness was mainly driven by heterogeneity in clinical context, including screening, triage, referral, and case-control populations; differences in specimen type, assay platform, and cut-off strategy; and variation in reference verification (Table 5).

For CIN2+, the certainty of evidence was low for SOX1 and the Six-gene/GynTect panel, and very low for FAM19A4/miR124-2, JAM3, PAX1, the PAX1/JAM3 panel, and S5 EPB41L3 plus HPV16/18/31/33 methylation. S5 showed the highest pooled sensitivity for CIN2+ (0.84, 95% CI 0.77-0.90), but specificity was low (0.47, 95% CI 0.38-0.55), indicating a high false-positive burden. PAX1, JAM3, the PAX1/JAM3 panel, SOX1, and the Six-gene/GynTect panel showed higher specificity (0.90-0.94), but sensitivity was moderate and certainty was limited by risk of bias and indirectness. Strict sensitivity analyses supported the robustness of PAX1, S5, SOX1, and the Six-gene/GynTect panel for CIN2+, whereas JAM3 and the PAX1/JAM3 panel showed moderate changes and FAM19A4/miR124-2 could not be compared because it was pooled only in the broad main analysis.

For CIN3+, the certainty of evidence was low for FAM19A4/miR124-2, JAM3, PAX1, the PAX1/JAM3 panel, and the Six-gene/GynTect panel, and very low for S5 EPB41L3 plus HPV16/18/31/33 methylation and SOX1. The PAX1/JAM3 panel showed a relatively favorable balance of sensitivity and specificity for CIN3+ (sensitivity 0.80, 95% CI 0.69-0.88; specificity 0.88, 95% CI 0.86-0.90), but certainty remained low because of serious risk of bias and indirectness. S5 showed the highest sensitivity for CIN3+ (0.90, 95% CI 0.82-0.94), but specificity was low (0.52, 95% CI 0.44-0.61) and the strict sensitivity analysis showed a moderate change, leading to very low certainty. PAX1, JAM3, and the Six-gene/GynTect panel had robust strict sensitivity analyses with low certainty, whereas SOX1 showed moderate change in strict sensitivity analysis and was rated as very low certainty.

Publication bias was not downgraded as a separate domain in the marker-specific GRADE-DTA assessments. Deeks' funnel plot asymmetry tests were conducted for all 14 pooled marker-endpoint analyses, but several analyses included fewer than 10 studies; therefore, absence of significant asymmetry was interpreted cautiously. A concise rationale for GRADE-DTA downgrading is provided in Supplementary Table S3, and expected clinical consequences at a moderate assumed prevalence are summarized in Supplementary Table S4.

Overall, the highest certainty level reached in this evidence base was low certainty; no marker-endpoint analysis was rated as high or moderate certainty.

PAX1, JAM3, the PAX1/JAM3 panel, and the Six-gene/GynTect panel for CIN3+ had low certainty and relatively robust strict sensitivity analyses.

S5 EPB41L3 plus HPV16/18/31/33 methylation showed high sensitivity but low specificity, especially for CIN2+ and CIN3+, implying a high false-positive burden despite detecting more cases.

The dominant reasons for downgrading were risk of bias and indirectness, followed by inconsistency and imprecision in selected marker-endpoint analyses.

Publication bias was not downgraded separately, but it could not be reliably assessed for most analyses because too few studies contributed.

4. Discussion:

This systematic review and diagnostic test accuracy meta-analysis provides a comprehensive synthesis of DNA methylation biomarkers for detecting cervical precancer and cervical cancer. Across the included studies, methylation markers showed variable diagnostic performance depending on the marker, target endpoint, specimen type, assay platform, and clinical setting. The quantitative synthesis focused primarily on CIN2+ and CIN3+, with marker-specific analyses performed to avoid double counting and to preserve the independence of diagnostic comparisons.

Overall, several methylation markers and panels demonstrated potentially useful diagnostic accuracy. For CIN2+, PAX1, JAM3, SOX1, the PAX1/JAM3 panel, and the Six-gene/GynTect panel generally showed high specificity, whereas S5 EPB41L3 plus HPV16/18/31/33 methylation showed higher sensitivity but substantially lower specificity. For CIN3+, S5 showed the highest pooled sensitivity, while PAX1/JAM3, JAM3, SOX1, PAX1, and the Six-gene/GynTect panel provided more balanced sensitivity-specificity profiles. These findings suggest that different methylation markers may serve different clinical roles: some may be more suitable for identifying a larger proportion of high-grade lesions, while others may be more useful for reducing unnecessary referrals through higher specificity.

However, the certainty of evidence was limited. GRADE-DTA

assessment indicated that no marker-endpoint analysis reached high or moderate certainty (Table 5). Certainty was low for several CIN3+ analyses and low or very low for CIN2+ analyses, mainly because of risk of bias, indirectness, and, in selected analyses, inconsistency or imprecision. Therefore, although methylation markers may have potential as triage tools, the present evidence should be interpreted as supportive but not definitive.

PAX1 showed one of the most consistent diagnostic profiles across endpoints. For CIN2+, PAX1 demonstrated relatively high specificity and moderate sensitivity, and the strict sensitivity analysis supported the robustness of the main findings. For CIN3+, PAX1 also showed a stable balance between sensitivity and specificity, with little change in the strict sensitivity analysis. This consistency suggests that PAX1 is among the more reproducible methylation markers in the current evidence base, although certainty remained limited because contributing studies differed in design, population, assay methods, and clinical context.

JAM3 also demonstrated relatively high specificity, particularly for CIN2+ and CIN3+. The diagnostic odds ratio was high in both endpoint analyses, indicating meaningful discrimination between disease and non-disease groups. However, for CIN2+, the strict sensitivity analysis showed a moderate change, suggesting that pooled estimates may be influenced by study selection or differences in eligibility criteria. For CIN3+, the findings were more robust, supporting JAM3 as a potentially useful marker for higher-grade disease detection.

The PAX1/JAM3 panel showed strong diagnostic performance, particularly for CIN3+, where it provided a favorable balance of sensitivity and specificity. For CIN2+, the panel showed high specificity but a moderate change in strict sensitivity analysis. This finding suggests that combined PAX1/JAM3 testing may improve diagnostic discrimination compared with some single markers, but its performance remains dependent on assay implementation, threshold selection, and population characteristics. Further direct comparative studies are needed to determine whether the combined panel consistently outperforms its individual components.

SOX1 showed moderate sensitivity and high specificity for CIN2+ and relatively favorable performance for CIN3+. However, the strict sensitivity analysis for CIN3+ showed a moderate change, indicating that the pooled estimate was somewhat sensitive to study selection. These findings support SOX1 as a potentially useful marker, but the evidence remains heterogeneous and should be interpreted cautiously.

S5 EPB41L3 plus HPV16/18/31/33 methylation showed a distinct diagnostic profile. It had the highest pooled sensitivity for both CIN2+ and CIN3+, especially for CIN3+, but specificity was substantially lower than that of most host-gene markers and panels. This pattern suggests that S5 may be useful when the clinical priority is to minimize missed high-grade lesions, but it may also increase false-positive referrals. The clinical value of S5 therefore depends on the intended use: it may be better suited to sensitive triage or risk stratification rather than highly specific rule-in testing.

FAM19A4/miR124-2 showed moderate diagnostic performance. For CIN3+, the strict sensitivity analysis was robust, but the number of contributing studies was limited. For CIN2+, the analysis was available only in the broad main dataset and could not be compared in the strict sensitivity analysis. These findings suggest that FAM19A4/miR124-2 remains clinically relevant, particularly given its established use in some methylation testing platforms, but the pooled evidence in this review was not strong enough to support high-certainty conclusions.

The Six-gene/GynTect panel showed relatively high specificity and robust sensitivity analyses for both CIN2+ and CIN3+. Its sensitivity was moderate, suggesting that it may be more useful as a rule-in or referral-reduction strategy than as a highly sensitive standalone test. The stability of the strict sensitivity analyses supports the reliability of the direction of effect, although the overall certainty remained low because of risk of bias and indirectness.

The post-test probability analysis provided clinically interpretable estimates of how methylation test results may change disease probability. Across CIN2+ analyses, positive methylation results substantially increased the probability of disease for several markers, especially those with high positive likelihood ratios, such as PAX1/JAM3, PAX1, JAM3, and the Six-gene/GynTect panel. At a moderate assumed pre-test probability of 10%, these markers increased the probability of CIN2+ to approximately 50% or higher after a positive result. In contrast, negative results generally reduced residual probability, especially for markers with lower negative likelihood ratios.

For CIN3+, the clinical utility pattern was similar. Positive tests increased post-test probability most clearly for markers with higher specificity, whereas markers with high sensitivity and low negative likelihood ratios reduced residual risk after a negative result. S5, despite its low specificity, showed a low post-test probability after a negative test for CIN3+, consistent with its sensitivity-oriented profile. These findings highlight that methylation markers should not be judged solely by pooled sensitivity, specificity, or DOR. Their clinical role depends on whether the intended purpose is to rule in high-grade disease, reduce unnecessary colposcopy, or minimize missed CIN3+.

Nevertheless, these post-test probability estimates should be interpreted as approximations. They were derived from pooled point estimates and assumed pre-test probabilities rather than from prospective decision-analytic models. Clinical implementation would require validation in specific screening or triage pathways, including consideration of disease prevalence, HPV status, cytology results, vaccination status, feasibility, cost, and patient-level consequences.

The narrative synthesis showed that the methylation biomarker literature is broader than the subset of studies eligible for quantitative pooling. Several additional markers and panels, including CADM1/MAL-based panels, ANKRD18CP/LHX8/EPB41L3, ASCL1/LHX8, ZNF671, MAL, miR124-2, HPV16 methylation, WID-CIN or WID-qCIN classifiers, careME, CervicalMethDx, GYPC, SEPTIN9, ZIC1, GATA4, POU4F3, and other exploratory classifiers, were identified but could not be pooled reliably.

The main reasons for non-pooling were insufficient replication, lack of extractable 2 x 2 diagnostic data, non-comparable thresholds, different target endpoints, non-histologic reference standards, cancer-only designs, prognostic or post-treatment endpoints, technical assay-validation designs, and tissue-based discovery classifiers. These studies remain important because they show the continuing development of methylation biomarkers and may identify candidates for future validation. However, they cannot currently provide stable pooled diagnostic accuracy estimates for clinical decision-making.

The inclusion of narrative-only studies strengthens the interpr-etability of this review by preventing potentially relevant evidence from being ignored simply because it was unsuitable for meta-analysis. At the same time, separating narrative-only evidence from quantitative synthesis avoids inappropriate pooling of non-comparable tests, endpoints, and study designs.

Publication bias was formally assessed using Deeks' funnel plot asymmetry test where sufficient marker-specific data were available. For CIN3+ analyses in the overall analysis, most Deeks' test p-values did not indicate clear evidence of funnel plot asymmetry: FAM19A4/miR124-2, p = 0.890; JAM3, p = 0.132; PAX1, p = 0.194; S5, p = 0.796; SOX1, p = 0.158; and Six-gene/GynTect, p = 0.909. The PAX1/JAM3 panel showed a lower p-value, p = 0.089, suggesting a borderline trend toward asymmetry but not reaching conventional statistical significance.

For CIN2+, most marker analyses did not show statistically significant evidence of funnel plot asymmetry. PAX1 showed borderline evidence of funnel plot asymmetry with a Deeks' test p-value of 0.050, and JAM3 and SOX1 showed borderline trends with p-values of 0.087 and 0.081, respectively. These findings should be interpreted cautiously because several marker-specific analyses included fewer than 10 studies.

Overall, these findings do not provide strong evidence of publication bias across most methylation marker analyses. However, absence of statistically significant asymmetry should not be interpreted as definitive absence of publication bias. Small numbers of studies reduce the power of funnel plot-based tests and make the assessment of selective publication or small-study effects uncertain. Publication bias was therefore assessed, but its interpretation remains cautious.

Risk of bias was an important limitation across the evidence base. Many studies had concerns related to patient selection, index-test conduct, reference-standard verification, or flow and timing. Common issues included enriched case-control designs, referral-based populations, data-driven or unclear thresholds, incomplete or partial verification, and heterogeneity in reference standards. These issues can exaggerate diagnostic performance or reduce applicability to real-world screening and triage settings.

Indirectness was also a major concern. Studies differed in clinical setting, population risk, HPV status, specimen type, methylation assay, cut-off strategy, and target endpoint. Some studies were conducted in screening populations, whereas others used colposcopy/referral populations, case-control designs, or selected high-risk cohorts. Similarly, clinician-collected cervical samples, self-collected vaginal samples, tissue specimens, and other specimen types were sometimes evaluated in contexts that may not be clinically interchangeable.

The GRADE-DTA assessment summarized these concerns. The highest certainty level reached was low, and several marker-endpoint analyses were rated as very low certainty. This does not mean that methylation markers lack diagnostic value. Rather, it indicates that confidence in the exact pooled estimates is limited and that the true diagnostic performance may differ from the summary estimates when the tests are applied in different populations or clinical pathways.

This review has several strengths. First, it provides a broad and updated synthesis of methylation biomarkers for cervical precancer and cancer, including both single-gene markers and multigene panels. Second, the analysis was structured by marker and endpoint, which reduced inappropriate pooling and minimized double counting. Third, CIN2+ and CIN3+ were analyzed separately, acknowledging their different clinical implications. Fourth, a strict sensitivity dataset was used to assess the robustness of marker-specific estimates. Fifth, narrative synthesis was used to summarize relevant studies that were unsuitable for quantitative pooling, thereby preserving clinically important evidence without compromising the validity of the meta-analysis.

Additional strengths include the use of QUADAS-2 to assess risk of bias and applicability, GRADE-DTA to assess certainty of evidence, and clinical utility analyses to translate pooled diagnostic accuracy estimates into post-test probabilities. Publication bias was also formally examined using Deeks' funnel plots where data permitted, which strengthens the transparency of the evidence assessment.

Several limitations should be considered. First, many included studies were at high or unclear risk of bias. Concerns were particularly common in-patient selection, index-test threshold selection, and flow/timing. Enriched case-control designs and referral-based cohorts may not fully represent screening or routine HPV-positive triage populations.

Second, substantial clinical and methodological heterogeneity was present. Studies differed in population charact-eristics, HPV status, clinical setting, specimen type, methylation assay, threshold definition, reference standard, and endpoint. These differences limit direct comparison between markers and reduce confidence in pooled estimates.

Third, some marker-specific analyses included relatively few studies. Although quantitative synthesis was possible for the main markers, several analyses had small k values, especially in strict sensitivity datasets. This affects the precision of estimates and limits the reliability of subgroup or publication bias assessments.

Fourth, publication bias was assessed using Deeks' funnel plots and regression-based tests, and most marker analyses did not show clear evidence of asymmetry. However, interpretation remains limited because several analyses included fewer than 10 studies. PAX1 in CIN2+ showed borderline evidence of funnel plot asymmetry with a Deeks' test p-value of 0.050, and JAM3, SOX1, and PAX1/JAM3 panel showed borderline trends in selected analyses. Therefore, publication bias cannot be fully excluded even though it was formally evaluated.

Fifth, not all potentially relevant studies could be included in the quantitative synthesis. Some studies reported only AUCs, methylation frequencies, sensitivity-only data, prognostic endpoints, cancer-only case series, or technical validation outcomes. These were summarized narratively but could not contribute to pooled estimates of sensitivity and specificity.

Sixth, clinical utility analyses were based on pooled point estimates and assumed pre-test probabilities. They provide useful interpretation but do not replace prospective clinical utility studies, cost-effectiveness analyses, or decision-analytic models. Finally, the available evidence does not establish whether methylation testing improves downstream patient outcomes, reduces overtreatment, or safely replaces existing triage strategies. The findings therefore support further validation rather than immediate widespread implementation.

The findings suggest that selected methylation markers may have a role as triage tools, particularly among HPV-positive

women. Markers with high specificity, such as PAX1, JAM3, SOX1, PAX1/JAM3, and Six-gene/GynTect-type panels, may help reduce unnecessary referral if validated in prospective screening settings. More sensitivity-oriented markers, such as S5, may be useful when the priority is to minimize missed CIN3+ lesions, but their lower specificity may increase false-positive referrals.

Future studies should prioritize prospective designs in screening and HPV-positive triage populations. They should use pre-specified thresholds, standardized methylation assays, histology-based reference standards, and complete reporting of TP, FP, FN, and TN. Direct head-to-head comparisons of leading markers in the same population are needed to determine comparative performance. Further work should also evaluate self-sampling contexts, vaccinated populations, cost-effectiveness, patient acceptability, and integration with HPV genotyping, cytology, or other molecular triage strategies.

5. Conclusions

DNA methylation markers showed heterogeneous diagnostic accuracy for detecting CIN2+ and CIN3+. PAX1, JAM3, SOX1, PAX1/JAM3 panels, S5 methylation classifiers, FAM19A4/miR124-2, and Six-gene/GynTect-type panels each demonstrated distinct sensitivity- and specificity-oriented profiles. Markers with higher specificity may be useful for ruling in clinically relevant disease and reducing unnecessary referrals, whereas highly sensitive markers may help reduce missed high-grade lesions; however, these potential roles require prospective validation.

6. Declarations

6.1. *Acknowledgments*

None.

6.2. *Conflict of interests*

There is no conflict of interest.

6.3. *Funding and supports*

None.

6.4. *Authors' contributions*

Study design: Mohammad Hossein Modarressi, Sham Tahir Abdulla Horamee; Data Gathering: Sham Tahir Abdulla Horamee, Mostafa Hosseini and Maedeh Arabpour; Analysis: Mostafa Hosseini; Interpretation of finding: All authors; Drafting: Sham Tahir Abdulla Horamee; Critically revised: Mohammad Hossein Modarressi, Mostafa Hosseini, Maedeh Arabpour.

6.5. *Ethical statement*

Not applicable.

6.6. *Informed consent*

Not applicable.

6.7. *Availability of supporting data*

All used data were presented in the manuscript.

6.8. *Using artificial intelligence chatbots statement*

AI chat was used to assist with editing and improving the English of the manuscript.

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Table 1: Characteristics of included quantitative diagnostic accuracy studies

Study	Country	Design & setting	N analyzed*	Age, years†	Specimen	Methylation index test (method)	HPV test	Target condition	Reference standard
Adcock 2022 [15]	USA	Cross-sectional DTA; population screening triage	798	33 (27-42)	Clinician cervical LBC	S5 DNA classifier (method: pyrosequencing)	Linear Array HPV Genotyping assay (Roche) on LBC samples.	CIN2+, CIN3+	Registry histology
Anisimova 2025 [16]	Russia	Case-control DTA; hospital diagnostic/triage pilot	99 (73 hrHPV+)	18-70	Cervical swab	CADM1; MAL; PAX1; CADM1+MAL panel (method: ddPCR)	NR (HPV16/18 status reported)	HSIL+, cervical cancer	Histology/cytology
Pun 2015 [17]	Taiwan	Case-control DTA; hospital hrHPV+ triage	200 (55 hrHPV+)	44.2 ± 13.3	Cervical scraping	AJAP1 methylation (M-index); HS3ST2 methylation (M-index); POU4F3 methylation (M-index) (method: qMSP)	Hybrid Capture II (HCII)	CIN3+	Colposcopy histology
Banila 2022 [18]	Multicountry	Case-control DTA; worldwide diagnostic sample set	974	NR	Cervical specimen/biopsy (varied); Cervical LBC ± biopsy	S5 classifier (cut-off 0.80); S5 classifier (cut-off 2.62); S5 classifier (cut-off 3.70) (method: pyrosequencing)	Multiple/varied by site (HPV genotyping performed; details varied)	CIN3+	Histology/cytology
Barrett 2022 [19]	NR	Nested case-control; screening triage/risk prediction	1,254	NR	LBC sample	WID-CIN (5000 CpG signature) (method: EPIC array)	NR	CIN2+, CIN3+	Histology/follow-up
Boers 2016 [20]	Netherlands	Cross-sectional DTA; abnormal-cytology referral	215 (152 hrHPV+)	37 (32-43)	Clinician cervical scraping	ANKRD18CP; Panel: C13ORF18/JAM3/ANKRD18CP; C13ORF18; CDH6; EPB41L3; GATA4; GFRA1; JAM3; Panel: JAM3/ANKRD18CP; Panel: JAM3/GFRA1/ANKRD18CP; KCNIP4; LHX8; ST6GALNAC5; TERT; ZSCAN1 (method: qMSP)	GP5+/6+ PCR; GP5+/6+ positive typed with COBAS® 4800 HPV test (HPV16/18 + 12 other hrHPV types)	CIN2+, CIN3+	Biopsy/LLETZ histology
Bonde 2021 [21]	Scotland, Denmark, Slovenia, Netherlands	Retrospective cross-sectional DTA; multicenter screening triage	2,384	29-76.3	Clinician cervical LBC (ThinPrep/SurePath/UCM)	QIAsure FAM19A4/miR124-2 test (method: qMSP)	Cobas 4800; HC2; Abbott RealTime High Risk HPV; BD Onclarity; CLART HPV2 (varies by center)	CIN2+, CIN3+	Histology/follow-up
Bowden 2025 [22]	UK/Greece	Case-control DTA; hospital colposcopy/referral	241	18-70	Clinician cervical LBC (ThinPrep/PreservCyt); Cervical LBC (GSE14375)	PAX1/NREP-AS1 methylation (cg16767801 + cg23642047) (method: EPIC array)	Abbott RealTime HR HPV assay (14 hrHPV types) and Anyplex HPV 28 (28 low/high-risk types)	CIN3+	Histopathology
De Strooper 2014 [23]	Netherlands	Retrospective cross-sectional DTA; population screening triage	250 (234 hrHPV+)	19-62	Clinician cervical scrape	CADM1/MAL panel (method: qMSP)	GP5+/6+ PCR	CIN2+, CIN3+	Histology/follow-up
De Strooper 2016 [24]	Netherlands	Model development/validation; self-sampling screening triage	1,049	NR	Self cervicovaginal lavage; Self vaginal brush	FAM19A4/miR124-2 methylation (predefined threshold) (method: qMSP)	GP5+/6+ PCR for HPV positivity	CIN2+, CIN3+	Histology/follow-up
DeVuyst 2015 [25]	Kenya	Prospective cohort; HIV+ screening/referral	248	18-55	Clinician cervical scrape	3-marker panel (CADM1/MAL/MIR124-2) (method: qMSP)	GP5+/6+ PCR with enzyme immunoassay for hrHPV; genotyping by reverse line blot hybridization.	CIN2+, CIN3+	Colposcopy/biopsy histology
de Waard 2023 [26]	Netherlands	Self-sampling screening triage	304	30-60	Self-sample (vaginal)	3-marker panel (ANKRD18CP/EPB41L3/LHX8) (method: qMSP)	Cobas 4800 HPV test	CIN3+	Dutch registry histology/cytology
de Waard	Netherlands	Cohort validation; self-	2,482	30-63	Self vaginal Evalyn Brush	3-marker panel	Cobas 4800 HPV test	CIN3+	Dutch registry

Study	Country	Design & setting	N analyzed*	Age, years†	Specimen	Methylation index test (method)	HPV test	Target condition	Reference standard
2025 [27]		sampling screening triage				(LHX8/EPB41L3/ANKRD18CP) (method: qMSP)			histology/cytology
Dick 2019 [28]	Netherlands	Prospective cohort; screening risk stratification	1,025	29-61	Cervical sample (clinician)	FAM19A4/miR124-2 (method: qMSP)	GP5+/6+ PCR-EIA with reverse line blot genotyping	CIN3+	Registry histology follow-up
Dick 2020 [29]	Netherlands	Case-control DTA; biomarker case-control evaluation	577	NR	Cervical samples (controls) and CIN3/cancer specimens	ASCL1/LHX8 panel (method: qMSP)	Clinically validated high-risk HPV DNA assays	CIN3+	Histology/clinical follow-up
Dippmann 2020 [30]	Germany	Cross-sectional DTA; diagnostic/triage cohort	195 (149 hrHPV+)	19-84	Cervical sample (clinician)	QIAsure test; GynTect® test (method: commercial methylation-specific PCR (QIAsure; GynTect))	GP5+/6+ PCR-EIA assay or Cobas HPV test	CIN2+, CIN3+	Histology/cytology
Bu 2023 [31]	China	Cross-sectional DTA; hospital hrHPV+ referral	248 (1,050 screened)	20-65	Cervical scrapings (clinician-collected)	Septin9 DNA methylation (qMS-PCR) (method: qMS-PCR)	Fluorescence quantitative PCR genotyping (Kaipu Bio, China; 14 hrHPV types)	CIN2+	Biopsy/surgical histology
Chang 2015 [32]	Taiwan	Paired cross-sectional DTA; hospital referral; paired sampling	136	47.9 ± 12.9	Physician cervical sample; Self vaginal sample	PAX1 methylation (QMSP); SOX1 methylation (QMSP); ZNF582 methylation (QMSP) (method: qMSP)	Not reported	CIN3+	Histology/cytology
Chang 2021 [33]	Taiwan	Prospective cohort; screening triage	470 (4,762 screened)	45.8 ± 11.3	Cervical specimen (clinician-collected)	PAX1 test (PAX1m) (method: qMSP-based assay)	EpiGene hrHPV DNA Kit (PCR; 16 HPV types including 16/18/31/33/35/39/45/51/52/56/58/59/66/68 + 6/11)	CIN3+	Histology
Chen 2025 [34]	China	Model development/validation; screening/colposcopy clinics	2,372 (2,497 total)	NR	Cervical exfoliated cells in PreservCyt (clinician-collected)	Triple-target methylation Panel 1; Triple-target methylation Panel 2 (method: MSRE-qPCR)	Not reported	CIN3+	Histopathology
Clarke 2018 [35]	USA	Nested case-control; population screening cohort	≤60/type; type-specific	NR	Cervical specimen in STM (clinician-collected)	12-type HPV DNA methylation assay (L1/L2 CpG sites) (method: bisulfite NGS)	Hybrid Capture 2 (HC2) in routine screening; typing by Cobas/Linear Array/Onclarity/MY09/11 PCR (varied over time)	CIN3+	Histology/follow-up
Cook 2019 [36]	Canada	Nested case-control; screening RCT	257	25-65	STM cervical sample (clinician-collected)	S5 DNA classifier (method: pyrosequencing)	Hybrid Capture 2 (HC2) high-risk HPV DNA test	CIN2+, CIN3+	Histology/follow-up
Fan 2025 [37]	China	Prospective cross-sectional DTA; opportunistic screening triage	549 (824 enrolled)	40.0 (33.0-51.0)	LBC / exfoliated cervical cells (clinician-collected)	GYPC methylation (GYPCm) (method: real-time methylation PCR)	HPV gene array test kit (HybriBio; 21 subtypes), restricted to 14 high-risk types.	CIN2+, CIN3+	Colposcopy/biopsy histology
Brentnall 2014 [38]	UK	Colposcopy referral	1,493	NR	PreservCyt cervical sample (clinician-collected)	R classifier (S4 precursor) (method: pyrosequencing)	Linear Array/qPCR in P1 and BD HPV test in P2; HPV16/18/31 methylation assays selected by genotyping.	CIN2+	Centrally reviewed histology
Brentnall 2015 [39]	UK	Colposcopy referral	1,493	NR	PreservCyt cervical sample (clinician-collected)	S5 classifier with HPV33 (method: pyrosequencing)	Linear Array/qPCR in P1 and BD HPV test in P2; HPV33 and prior HPV16/18/31 methylation data.	CIN2+	Centrally reviewed histology
Fei 2024 [40]	China	Cross-sectional DTA; screening/colposcopy	334 (938 total)	36 (29-48)	Clinician-collected cervical exfoliated cells / PreservCyt LBC	JAM3 methylation (JAM3m); PAX1 methylation (PAX1m); CISCER PAX1/JAM3 panel (method: qMSP)	Cobas 4800 HPV test (Roche), differe-ntiating HPV16/18 and other hrHPV types.	CIN2+, CIN3+	Biopsy histology

Study	Country	Design & setting	N analyzed*	Age, years†	Specimen	Methylation index test (method)	HPV test	Target condition	Reference standard
Gao 2024 [41]	China	Cross-sectional DTA; colposcopy referral	420 (658 total)	18-75	Clinician cervical scrapings	PAX1/SOX1 panel (method: qMSP)	Digene HC2 High-Risk HPV DNA Test (QIAGEN); reported HPV16/18, other 12 hrHPV, mid/low-risk and negative categories.	CIN2+, CIN3+	Colposcopy histology
Gilham 2024 [42]	UK	Nested case-control; screening trial cohort	1,143	20-66	Archived LBC (clinician-collected)	S5 DNA classifier (method: pyrosequencing (S5))	Hybrid Capture 2 for hrHPV positivity; genotyping by Line Blot Assay, PapilloCheck, and Linear Array at various trial stages.	CIN3+	Histology/registry follow-up
Gradissimo 2023 [43]	USA	Nested case-control; population screening cohort	1,400	NR	Residual cervicovaginal specimen (clinician-collected/cotesting repository)	HPV/host DNA Methylation Score (method: next-generation bisulfite sequencing)	Hybrid Capture 2 (HC2) for cotesting; HPV genotyping performed on banked specimens.	CIN3+	Histology/follow-up
Hansel 2014 [44]	Germany	Cross-sectional DTA; colposcopy referral	217 (2,013 recruited)	18-81	Clinician cervical scrape	DLX1/ITGA4/RXFP3/SOX17/ZNF671 panel (method: qMSP)	GP5+/6+ PCR-EIA for hrHPV; multiplex PCR for seven major hrHPV types for genotyping.	CIN2+, CIN3+	Histopathology
Hernandez-Lopez 2019 [45]	Mexico	Nested case-control; screening cohort; referred subset	316 (29,759 screened)	37 (median)	Clinician-collected exfoliated cervical cells (ThinPrep aliquot)	S5 DNA classifier (method: pyrosequencing)	Cobas 4800 HPV test for hrHPV and HPV16/18 genotyping; HPV31/33 inferred from S5 PCR amplicons.	CIN2+, CIN3+	Standardized biopsy histology
Hesselink 2011 [46]	Netherlands	Population screening triage	236 (3,135 screened)	19-62	Clinician-collected cervical scrapes/scrapings	CADM1-m18/MAL-m1 panel (method: qMSP)	GP5+/6+ PCR-EIA for 14 high-risk HPV types; reverse line blot genotyping for hrHPV type information.	CIN3+	Histology/follow-up
Klischke 2021 [47]	Germany	Paired-sample DTA; colposcopy clinic; self-sampling	68 (89 recruited)	37 (mean)	Self dry cervicovaginal Evalyn Brush; Physician cervical LBC	GynTect six-marker methylation assay; GynTect among HPV-positive self-collected samples; GynTect among HPV-positive physician-taken samples (method: qMSP)	Abbott RealTime HighRisk HPV assay for 14 hrHPV genotypes.	CIN2+, CIN3+	Histology/cytology+HPV
Kong 2025 [48]	China	Prospective cohort; hrHPV+ screening triage	1,105 (20,456 screened)	42 (34-53)	Clinician-collected cervical cytology sample; residual LBC material used for methylation	JAM3 methylation alone; PAX1 methylation alone; PAX1m/JAM3m test; HPV16/18 OR PAX1m/JAM3m (method: qMSP)	Cobas 4800 HPV test detecting HPV16, HPV18 and pooled other 12 hrHPV types.	CIN2+, CIN3+	Colposcopy/surgical histology
Kremer 2018 [49]	South Africa	Prospective cohort; HIV screening/referral cohort	429	41 (35-46) / 44 (34-51)	Physician-taken cervical scrape	ASCL1; LHX8; ST6GALNAC5 (method: multiplex qMSP)	hrHPV status reported from parent study; exact assay details not fully described in the uploaded paper.	CIN3+	Biopsy/LLETZ histology
Larsson 2025 [50]	Sweden	Retrospective cohort; regional screening	1,915	30-70	Clinician residual LBC	QIAsure FAM19A4/miR-124-2 test; MONC/HONC genotyping AND QIAsure (method: methylation-specific real-time PCR (QIAsure))	Aptima HPV mRNA assay (Hologic), detecting 14 hrHPV types.	HSIL+	Histology/follow-up
Leeman 2018 [51]	Netherlands	Retrospective DTA; self-sampling non-responders	139 (456 total)	33-63	Self cervicovaginal sample	FAM19A4/miR124-2 test (method: qMSP)	GP5+/6+-PCR-EIA for hrHPV on self-samples; GP5+/6+ PCR-LMNX genotyping on self-samples; SPF10-PCR-DEIA-LiPA on whole tissue sections/LCM lesions.	CIN3+	Biopsy histology

Study	Country	Design & setting	N analyzed*	Age, years†	Specimen	Methylation index test (method)	HPV test	Target condition	Reference standard
Li 2025 [52]	China	Hospital hrHPV+ triage	584	21-65	Cervical exfoliated cells (clinician-collected)	Six-gene methylation pattern (self-designed logistic model); Commercial six-gene methylation detection kit (method: real-time MSP)	hrHPV-positive status required; exact primary hrHPV assay not fully specified in the article. HPV16/18 testing performed by the hospital laboratory.	CIN2+, CIN3+	Histology
Lin 2025 [53]	China	Cross-sectional DTA; outpatient colposcopy	360	NR	Cervical smear / TCT	AJAP1; ASTN1; CDKN2A; DLX1; GATA4; HPV16/18 AND ITGA4; HPV16/18 AND all candidate gene; ITGA4; JAM3; LHX8; MAL; MIR124; PAX1; POU4F3; RXFP3; SOX1; SOX17; ST6GALNAC5; Combined 17-gene panel; ZNF671 (method: methylation sequencing)	Hybrebio hrHPV DNA test detecting 14 hrHPV types; 16/18 vs non-16/18 reported.	CIN2+	Histology
Liu 2023 [54]	China	Cross-sectional DTA; hospital lesion evaluation	156	26-72	Cervical scrape / ThinPrep	JAM3; LOC100289333; SOX1; ZIC1; MIR124-2 (method: MSP-qPCR)	Sansure-Biotech fluorescence quantitative PCR hrHPV assay detecting HPV16/18 and other hrHPV types.	CIN3+	Biopsy histology
Lorincz 2013 [55]	UK	RCT biomarker substudy; post-low-grade-cytology surveillance	84 (73 HPV16+)	NR	Exfoliated cervical cells / SurePath	HPV16 classifier S1; HPV16 classifier S2 (method: pyrosequencing)	PCR-EIA GP5+/6+ HR-HPV and HPV16 confirmation.	CIN2+	Histology at 6-12 mo
Lorincz 2016 [56]	UK	Nested validation study; routine screening	341 (710 total)	20-64	Residual LBC / PreservCyt	S5 DNA classifier; S4 DNA classifier (method: pyrosequencing)	Aptima used for primary hrHPV status; Abbott/BD genotyping available.	CIN2+, CIN3+	Histology within 12 mo
Luttmer 2016 [57]	Netherlands	Prospective cohort; multicenter referral	450 (532 total)	18-66	Physician cervical scrape; Cervicovaginal lavage (self/physician)	FAM19A4 (method: qMSP)	GP5+/6+ PCR-EIA; Luminex xMAP genotyping for hrHPV types	CIN2+, CIN3+	Biopsy/excision histology
Mirabello 2013 [58]	Costa Rica	Nested case-control; population cohort	196	NR	Swab-derived exfoliated cervical cell specimen	HPV16 DNA methylation CpG L1_6457 (method: pyrosequencing)	HPV16 genotyping and viral load testing; pyrosequencing methylation assays	CIN2+	Histology/clearance control
Molano 2024 [59]	Papua New Guinea	Paired-sample observational; low-resource screen-and-treat substudy	44	30-59	Clinician cervical sample; Self vaginal sample	CADM1; HPV16/18 OR CADM1; HPV16/18/31/33/45/52/58 OR CADM1; MAL OR CADM1; HPV16/18 OR MAL/CADM1; HPV16/18/31/33/45/52/58 OR MAL/CADM1; miR124-2 OR MAL OR CADM1; MAL; HPV16/18 OR MAL; HPV16/18/31/33/45/52/58 OR MAL; miR124-2; miR124-2 OR MAL; miR124-2 OR CADM1; HPV16/18 OR miR124-2; HPV16/18/31/33/45/52/58 OR miR124-2; HPV16/18 OR miR124-2/MAL; HPV16/18/31/33/45/52/58 OR miR124-2/MAL; HPV16/18 OR miR124-2/CADM1; HPV16/18/31/33/45/52/58 OR miR124-2/CADM1 (method: qMSP)	Xpert HPV point-of-care; Anyplex II HR HPV genotyping	HSIL+	LBC + p16/Ki-67
Mortaki 2025 [60]	Greece	Prospective cohort; colposcopy/LLETZ referral	170	26-70	ThinPrep cervical sample before LLETZ	QIAsure FAM19A4/miR124-2; Pap test 2 OR QIAsure (method: qMSP)	Roche COBAS 4800 HPV system	CIN2+	LLETZ/cone histology

Study	Country	Design & setting	N analyzed*	Age, years†	Specimen	Methylation index test (method)	HPV test	Target condition	Reference standard
Palmieri 2025 [61]	USA	Retrospective validation; laboratory/referral residual samples	527 (1,120 received)	NR	Clinician PreservCyt cervical cytology	CervicalMethDx precision classifier (method: qMSP (CervicalMethDx))	Aptima HPV assay; partial Aptima HPV16/18/45 genotyping in subset	CIN2+, CIN3+	Histology/normal cytology
Peng 2026 [62]	China	Cross-sectional DTA; postmenopausal colposcopy referral	216 primary cohort	57 (53-62)	Clinician-collected cervical exfoliated cells in PreservCyt	JAM3; LBC and hrHPV guideline strategy; PAX1; CISCER PAX1/JAM3 (method: qMSP)	Cobas 4800 HPV test	CIN2+, CIN3+	Consensus histopathology
Ramirez 2021 [63]	Colombia	Nested case-control; opportunistic screening trial	366 (2,661 trial)	20-69	Baseline exfoliated cervical cells in STM	S5 classifier (method: pyrosequencing)	Hybrid Capture 2 for hrHPV; CLART HPV4 genotyping	CIN2+, CIN3+	Biopsy histology follow-up
Ren 2025 [64]	China	Retrospective DTA; outpatient/referral; enriched cases	146 valid	21-75	Residual clinician-collected cervical cytology sample in PreservCyt	GynTect six-gene panel; ZNF671; HPV16/18 OR GynTect; HPV16/18 OR ZNF671m (method: qMSP)	YanengBio 14-type hrHPV DNA genotyping kit	CIN2+, CIN3+	Histology/clinical classification
Reuter 2021 [65]	UK	Paired method-comparison; archived colposcopy-referral samples	315 paired	17-72	Exfoliated cervical cells; FFPE biopsy	S5 classifier - exfoliated cells; S5 classifier - FFPE Zymo Std; S5 classifier - FFPE Zymo Std adjusted; S5 classifier - FFPE Zymo Std adjusted >=85% sensitivity; S5 classifier - FFPE Epitect Std; S5 classifier - FFPE Epitect Std adjusted; S5 classifier - FFPE Zymo Lightning; S5 classifier - FFPE Zymo Lightning adjusted; S5 classifier - FFPE Epitect Fast; S5 classifier - FFPE Epitect Fast adjusted (method: pyrosequencing)	Not a primary hrHPV triage study; S5 includes HPV type methylation targets	CIN2+	Histopathology
Rezhake 2024 [66]	China	Prospective cohort; rural population screening	233 (2,000 recruited)	40 (34-46)	Baseline clinician-collected cervical swab	careME EPB41L3 + HPV16/18; HPV16/18 OR careME (method: qPCR)	careHPV; GenPlex HPV genotyping	CIN2+	Colposcopy/biopsy histology
Rezhake 2025 [67]	China	Cross-sectional DTA; organized population screening	963 (8,638 screened)	49 (43-55)	Clinician cervical sample	hrHPV → careMe methylation triage (method: qPCR)	SureX HPV 25X PCR genotyping used for algorithm evaluation; DH2/careHPV hybrid-capture HPV tests also used in the screening program.	CIN2+	Histology/normal colposcopy
Salta 2025 [68]	Portugal	Regional screening triage	812	NR	Clinician cervical scrape	MAL methylation ddMSP; hsa-miR124-2 methylation ddMSP; hsa-miR124-2 OR MAL methylation ddMSP; hsa-miR124-2/MAL methylation OR HPV16/18/33 genotyping; hsa-miR124-2/MAL methylation OR HPV16/33 genotyping (method: ddMSP)	HrHPV testing in the regional screening program; genotyping strategies include HPV16/18, HPV16/18/33 and HPV16/33.	CIN3+, HSIL+	Clinical/histologic classification
Schmitz 2017 [69]	Germany	Cross-sectional DTA; outpatient colposcopy referral	306 (189 hrHPV+)	NR	Clinician cervical scrape (STM)	GynTect panel; GynTect panel in HPV-positive women; hrHPV positive AND GynTect positive triage (method: qMSP)	GP5+/6+ PCR-EIA for hrHPV.	CIN3+	Histopathology
Schmitz 2018 [70]	Germany	Retrospective DTA; screening/triage residual LBC	632	NR	LBC PreservCyt cervical scrape	GynTect panel (method: qMSP)	cobas HPV test; genotyping for HPV16/18.	CIN2+, CIN3+	Histology/normal cytology
Schreiberhuber 2024	Sweden	Prospective cohort; population HPV screening	2,377 (28,017	30-64	Clinician cervical smear	WID-qCIN; WID-qCIN OR HPV16/18	HPV test for 14 oncogenic types; HPV16/18 subtype available	CIN2+, CIN3+	Histology/follow-up

Study	Country	Design & setting	N analyzed*	Age, years†	Specimen	Methylation index test (method)	HPV test	Target condition	Reference standard
[71]			screened)			(method: quantitative real-time PCR on bisulfite-modified DNA (WID-qCIN))			
Shang 2025 [72]	China	Cross-sectional DTA; opportunistic screening	1,857 (1,796 hrHPV+)	42 (34-54)	LBC cervical cytology sample	CISCER PAX1/JAM3 (method: qMSP)	Cobas 4800 HPV; HPV16/18 and other hrHPV reported	CIN2+, CIN3+	Histology
Su 2025 [73]	China	Cross-sectional DTA; non-16/18 hrHPV+ triage	643 with histology	40 (33-52)	Clinician cervical cytology sample	CISCER PAX1/JAM3 (method: qMSP)	HybriBio 21-subtype HPV test; non-16/18 hrHPV only	CIN2+, CIN3+	Biopsy histology
Tian 2017 [74]	China	Case-control DTA; hospital hrHPV+ triage	173 non-16/18 hrHPV+	21.8-77.8	Residual liquid-based cytology cervical cells	NKX6.1; PAX1; PAX1m OR ZNF582m; PAX1m OR NKX6.1m; PAX1m OR SOX1m; SOX1; ZNF582m OR SOX1m; SOX1m OR NKX6.1m; ZNF582; ZNF582m OR NKX6.1m (method: MSP)	Nested multiplex PCR assay with type-specific primers	CIN3+	Colposcopy/biopsy histology
van Leeuwen 2019 [75]	Slovenia/Netherlands	Prospective cohort; population screening triage	253	30-64	Clinician ThinPrep cervical scrape	ANKRD18CP; ANKRD18CP/C13orf18/JAM3 panel; C13orf18; EPB41L3; JAM3; C13orf18/EPB41L3/JAM3 panel; SOX1; SOX1/ZSCAN1 panel; ZSCAN1 (method: qMSP)	Abbott RealTime High Risk HPV assay	CIN2+, CIN3+	Histology/screening follow-up
Vasiljevic 2014 [76]	UK	Colposcopy-referral cohorts	1,000 / 1,099	NR	Clinician PreservCyt cervical specimen	DPYS; EPB41L3; MAL (method: pyrosequencing)	Linear Array for P1; BD HPV test for P2	CIN2+	Centrally reviewed histology
Verhoef 2021 [77]	Netherlands	RCT cohort validation; primary HPV screening trial	715	29-60	Clinician cervical sample	ASCL1/LHX8 panel; ASCL1/LHX8 OR HPV16/18 (method: multiplex qMSP)	hrHPV testing in IMPROVE trial; genotyping HPV16/18/other	CIN2+, CIN3+	Registry/central histology
Verlaat 2017 [78]	Netherlands	Case-control validation; selected validation series	220 validation	NR	Clinician cervical scrape	GHSR; SST; ZIC1 (method: multiplex qMSP)	GP5+/6+ PCR-EIA or HPV-Risk assay	CIN3+	Histology
Verlaat 2018 [79]	Netherlands	Self-sampling screening cohorts	199 lavage / 287 brush	NR	Self lavage; Self brush	ASCL1/LHX8/ST6GALNAC5 3-gene classifier (method: multiplex qMSP)	hrHPV testing performed in PROHTECT trials	CIN3+	Histology/follow-up
Vink 2023 [80]	Europe	Retrospective multicenter DTA; multicenter HPV+ screening/referral	1,061	15-29	Clinician cervical scrape	FAM19A4/miR124-2 (method: qMSP)	Clinically validated HPV DNA assays across partner laboratories	CIN2+, CIN3+	Local pathology/follow-up
Wang 2023 [81]	China	Screening/clinic validation	692 specimens (605 scrapes)	25-73	Cervical scrape	PAX1/ST6GALNAC5 panel (method: qMSP)	HPV-positive status reported; assay details in supplementary material	HSIL+	Histopathology
Wang 2025 [82]	China	Cross-sectional DTA; hospital HPV+ triage	276 (255 HPV+)	20-75	Clinician cervical sample	GynTect six-gene test (method: PCR)	Fluorescent quantitative PCR for 15 HPV types	CIN3+	Biopsy histology
Yang 2025 [83]	China	Cross-sectional DTA; single-center colposcopy	119	21-74	Clinician cervical scrape	CISCER PAX1/JAM3; GynTect six-gene test (method: methylation PCR)	Aptima HPV mRNA assay and Aptima HPV16/18/45 genotyping	CIN2+, CIN3+	Biopsy histology
Yuan 2017 [84]	China	Case-control DTA; hospital colposcopy referral	259	NR	Clinician cervical exfoliated cells	C13ORF18; SLIT2/C13ORF18 panel; JAM3; JAM3/SLIT2 panel; JAM3/C13ORF18 panel; SLIT2; SOX1; JAM3/SOX1 panel; SLIT2/SOX1 panel; C13ORF18/SOX1 panel; TERT (method: pyrosequencing)	Hybrid Capture II (HCII), 13 high-risk HPV types	CIN2+, CIN3+	Histopathology

Study	Country	Design & setting	N analyzed*	Age, years†	Specimen	Methylation index test (method)	HPV test	Target condition	Reference standard
Zhai 2025 [85]	China	Cross-sectional DTA; rural screening triage	417 (3,000 screened)	45 (median)	Clinician cervical exfoliated cells	HPV16/18 OR E6/E7 mRNA for non-16/18 HPV+; PAX1; PAX1 and SOX1; PAX1 or SOX1; HPV16/18 OR PAX1/SOX1 methylation for non-16/18 HPV+; SOX1 (method: methylation qPCR)	Sansure HPV DNA test and GenPlex HPV test; HPV16/18 genotyping available; E6/E7 mRNA also evaluated	CIN2+, CIN3+	Histology/clinical follow-up
Zhang 2025 [86]	China	Cross-sectional DTA; screening-program colposcopy referral	640	35-65	Clinician cervical exfoliated cells	PAX1; PAX1 AND SOX1; PAX1/SOX1 methylation AND HPV16/18; PAX1/SOX1 methylation AND HPV positive; PAX1 OR SOX1; SOX1 (method: PCR-fluorescence probe methylation assay)	HPV12+2 / hrHPV genotyping; HPV16/18 and other 12 hrHPV types reported	CIN2+, CIN3+	Biopsy histology
Zhang J 2025 [87]	China	Prospective multicenter DTA; multicenter colposcopy triage	1,659	21-65	Clinician cervical brush/LBC	Methylation OR HPV16/18; Six-methylation marker assay (method: six-marker host DNA methylation assay; exact PCR platform NR in extracted data)	Fluorescence quantitative PCR HPV genotyping; HPV16/18 vs other 12 hrHPV	CIN2+, CIN3+	Colposcopy/biopsy histology
Zhu 2023 [88]	China	Screening/clinic validation	1,093 (672 hrHPV+)	45.3 ± 9.6 / 43.7 ± 11.1	Clinician-collected cervical scrapings	PAX1; HPV16/18 OR PAX1; ZNF671 OR PAX1; HPV16/18 OR ZNF671 OR PAX1; ZNF671; HPV16/18 OR ZNF671 (method: qMSP)	hrHPV testing and HPV16/18 genotyping reported	CIN3+	Colposcopy/biopsy histology

* N analyzed: the diagnostic/methylation analysis sample is shown first; the parent screened/recruited cohort is shown in parentheses only when informative. † Age hierarchy: a reported participant age range was used whenever available (an eligibility range was used only when no observed range was reported); otherwise median (IQR), then mean ± SD. Where only a central estimate without spread was available, it is explicitly labelled. A slash separates distinct cohorts/specimen groups.

Abbreviations: AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; CpG, cytosine-phosphate-guanine dinucleotide; DTA, diagnostic test accuracy; ddMSP, droplet digital methylation-specific polymerase chain reaction; ddPCR, droplet digital polymerase chain reaction; EPIC, Illumina Infinium MethylationEPIC array; FFPE, formalin-fixed paraffin-embedded; HC2, Hybrid Capture 2; HIV, human immunodeficiency virus; hrHPV, high-risk human papillomavirus; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LBC, liquid-based cytology; LLETZ, large loop excision of the transformation zone; MSP, methylation-specific polymerase chain reaction; MSP-qPCR, methylation-specific quantitative polymerase chain reaction; MSRE-qPCR, methylation-sensitive restriction enzyme quantitative polymerase chain reaction; NGS, next-generation sequencing; NR, not reported; PCR, polymerase chain reaction; qMS-PCR, quantitative methylation-specific polymerase chain reaction; qMSP, quantitative methylation-specific polymerase chain reaction; qPCR, quantitative polymerase chain reaction; RCT, randomized controlled trial; UK, United Kingdom; USA, United States of America. Gene symbols and proprietary/classifier names (e.g., S5, GynTect, QIAsure, CISCER, careME, WID-qCIN, CervicalMethDx) are retained as published.

Table 2: Study-level QUADAS-2 risk-of-bias and applicability judgments

Study	Patient selection	Index test	Reference standard	Flow / timing	Overall	Applicability concerns
Adcock2022 [15]	High	Low	Unclear	Low	High	Unclear
Anisimova2025 [16]	High	High	Unclear	High	High	High
Pun2015 [17]	High	High	Unclear	Unclear	High	Unclear
Banila2022 [18]	High	Unclear	Unclear	High	High	High
Barrett2022 [19]	High	High	Low	High	High	Low/Unclear
Boers2016 [20]	Unclear	Unclear	Unclear	High	Unclear	Low
Bonde2021 [21]	Unclear	Low	Unclear	High	High	Low
Bowden2025 [22]	High	Unclear	Unclear	High	High	High
DeStrooper2014 [23]	Low	Unclear	Unclear	High	High	Low
DeStrooper2016 [24]	High	Unclear	Unclear	Unclear	High	Low
DeVuyst2015 [25]	Unclear	High	Unclear	Unclear	High	Low
deWaard2023 [26]	High	High	Unclear	Unclear	High	Low
deWaard2025 [27]	Low	High	Unclear	High	High	Low
Dick2019 [28]	Unclear	Unclear	Unclear	Unclear	Unclear	Low
Dick2020 [29]	High	High	Unclear	Unclear	High	Low
Dippmann2020 [30]	Unclear	Unclear	Unclear	Unclear	High	Low
Bu2023 [31]	Unclear	Unclear	Unclear	Unclear	High	Unclear
Chang2015 [32]	High	High	Unclear	Low	High	High
Chang2021 [33]	Unclear	Unclear	Unclear	Unclear	Unclear	Low
Chen2025 [34]	High	High	Unclear	Low	High	Unclear
Clarke2018 [35]	Unclear	Unclear	Unclear	Low	Unclear	Low
Cook2019 [36]	High	Low	Unclear	High	High	Low/Unclear
Fan2025 [37]	Low	High	Low	High	High	Unclear
Brentnall2014 [38]	Unclear	High	Unclear	Low	High	High
Brentnall2015 [39]	Unclear	High	Unclear	Low	High	High
Fei2024 [40]	High	Low	Unclear	High	High	Unclear
Gao2024 [41]	High	High	Unclear	Low	High	High
Gilham2024 [42]	High	Unclear	Low	High	High	Unclear
Gradissimo2023 [43]	High	High	Low	High	High	High
Hansel2014 [44]	High	Unclear	Unclear	High	High	High
Hernandez-Lopez2019 [45]	High	High	Low	Low	High	High
Hesselink2011 [46]	Low	Low	Unclear	High	High	Low
Klischke2021 [47]	Unclear	Unclear	Unclear	High	High	High
Kong2025 [48]	High	Low	Unclear	High	High	Low
Kremer2018 [49]	High	High	Unclear	Low	High	High
Larsson2025 [50]	High	Low	Unclear	High	High	High
Leeman2018 [51]	High	Unclear	Low	High	High	High
Li2025 [52]	Unclear	High	Unclear	Unclear	High	Unclear
Lin2025 [53]	High	High	Unclear	Low	High	High
Liu2023 [54]	High	High	Unclear	Low	High	High
Lorincz2013 [55]	High	Low	Unclear	Low	High	High
Lorincz2016 [56]	High	Low	Unclear	High	High	Unclear
Luttmer2016 [57]	High	Low	Unclear	High	High	High
Mirabello2013 [58]	High	High	Unclear	Unclear	High	High
Molano2024 [59]	High	High	High	High	High	High
Mortaki2025 [60]	High	Low	Unclear	Low	High	High
Palmieri2025 [61]	High	Unclear	Unclear	High	High	High

Study	Patient selection	Index test	Reference standard	Flow / timing	Overall	Applicability concerns
Peng2026 [62]	Unclear	Unclear	Low	Low	High	High
Ramirez2021 [63]	High	Low	Low	Low	High	High
Ren2025 [64]	High	Low	Unclear	High	High	High
Reuter2021 [65]	High	Low	Low	Low	High	High
Rezhake2024 [66]	Low	Low	Low	High	Unclear	Low
Rezhake2025 [67]	Low	Low	Unclear	High	High	Low
Salta2025 [68]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Schmitz2017 [69]	Unclear	Low	Unclear	Low	Unclear	High
Schmitz2018 [70]	High	Low	Unclear	High	High	High
Schreiberhuber2024 [71]	Low	Low	Low	High	High	Low
Shang2025 [72]	Unclear	Low	Low	Unclear	Unclear	High
Su2025 [73]	Unclear	Low	Low	High	High	Unclear
Tian2017 [74]	High	Unclear	Unclear	Low	High	High
vanLeeuwen2019 [75]	Low	Low	Unclear	High	Unclear	Low
Vasiljevic2014 [76]	Unclear	High	Unclear	Unclear	High	High
Verhoef2021 [77]	Low	Low	Low	High	Unclear	Low
Verlaat2017 [78]	High	High	Unclear	Unclear	High	High
Verlaat2018 [79]	High	High	Unclear	Unclear	High	High
Vink2023 [80]	High	Low	Unclear	High	High	High
Wang2023 [81]	High	High	Unclear	High	High	High
Wang2025 [82]	High	Unclear	Unclear	Low	High	High
Yang2025 [83]	Low	Low	Low	Low	Low/Unclear	High
Yuan2017 [84]	High	High	Unclear	Low	High	High
Zhai2025 [85]	Low	Low	Low	High	High/Unclear	Low/Unclear
Zhang2025PAX1SOX1 [86]	High	Unclear	Unclear	Low	High	High
ZhangJ2025 [87]	Unclear	Unclear	Unclear	Low	Unclear	Unclear
Zhu2023 [88]	High	Unclear	Unclear	Low	High	Unclear

Abbreviations: QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies 2.

Table 3: Marker-specific pooled diagnostic accuracy for CIN2+ and CIN3+

Marker / endpoint	k main	k strict	Main sensitivity	Main specificity	Main DOR	Strict sensitivity	Strict specificity	Strict DOR	Classification
CIN2+									
FAM19A4/miR124-2	4	Not pooled	0.62 (0.49-0.74)	0.77 (0.74-0.80)	5.15 (2.90-9.13)	Not available	Not available	Not available	Not comparable
JAM3	7	4	0.66 (0.49-0.79)	0.93 (0.88-0.96)	26.64 (11.07-64.09)	0.58 (0.40-0.75)	0.95 (0.93-0.96)	23.47 (9.59-57.45)	Moderate change
PAX1	6	4	0.73 (0.56-0.84)	0.92 (0.90-0.95)	35.02 (14.47-84.76)	0.78 (0.50-0.92)	0.92 (0.91-0.94)	43.04 (14.28-129.71)	Robust
PAX1/JAM3 panel	5	4	0.72 (0.58-0.83)	0.94 (0.92-0.95)	43.73 (16.83-113.65)	0.64 (0.51-0.76)	0.93 (0.91-0.95)	29.37 (12.11-71.27)	Moderate change
S5 EPB41L3 + HPV16/18/31/33 methylation	8	5	0.84 (0.77-0.90)	0.47 (0.38-0.55)	4.67 (3.34-6.53)	0.83 (0.74-0.89)	0.45 (0.33-0.57)	3.84 (2.56-5.75)	Robust
SOX1	6	4	0.67 (0.55-0.77)	0.90 (0.87-0.92)	20.21 (9.93-41.15)	0.68 (0.54-0.78)	0.90 (0.87-0.92)	18.13 (9.01-36.49)	Robust
Six-gene/GynTect panel	7	6	0.64 (0.57-0.69)	0.93 (0.86-0.97)	24.10 (9.34-62.17)	0.62 (0.56-0.67)	0.92 (0.83-0.96)	18.12 (7.27-45.18)	Robust
CIN3+									
FAM19A4/miR124-2	4	4	0.70 (0.59-0.79)	0.74 (0.71-0.78)	6.59 (3.66-11.88)	0.70 (0.59-0.79)	0.74 (0.71-0.78)	6.59 (3.66-11.88)	Robust
JAM3	8	6	0.74 (0.65-0.82)	0.90 (0.85-0.94)	27.44 (16.55-45.49)	0.70 (0.60-0.78)	0.91 (0.84-0.95)	23.22 (12.80-42.10)	Robust
PAX1	11	9	0.76 (0.70-0.81)	0.85 (0.80-0.89)	18.82 (10.98-32.27)	0.74 (0.67-0.79)	0.85 (0.80-0.90)	18.54 (9.98-34.44)	Robust
PAX1/JAM3 panel	5	4	0.80 (0.69-0.88)	0.88 (0.86-0.90)	31.59 (16.69-59.78)	0.76 (0.66-0.83)	0.89 (0.88-0.90)	24.77 (14.56-42.15)	Robust
S5 EPB41L3 + HPV16/18/31/33 methylation	7	4	0.90 (0.82-0.94)	0.52 (0.44-0.61)	9.88 (3.73-26.12)	0.86 (0.78-0.91)	0.43 (0.32-0.56)	5.17 (2.53-10.55)	Moderate change
SOX1	8	7	0.82 (0.72-0.88)	0.85 (0.82-0.88)	26.80 (12.35-58.16)	0.75 (0.69-0.81)	0.85 (0.81-0.88)	17.92 (10.05-31.95)	Moderate change
Six-gene/GynTect panel	8	8	0.73 (0.65-0.80)	0.86 (0.81-0.90)	17.33 (10.03-29.93)	0.73 (0.65-0.80)	0.86 (0.81-0.90)	17.33 (10.03-29.93)	Robust

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; DOR, diagnostic odds ratio; HPV, human papillomavirus; k main, number of studies in the main analysis; k strict, number of studies in the strict sensitivity analysis.

Table 4: Clinical utility estimates for CIN2+ and CIN3+

Marker	k	LR+	LR–	DOR (95% CI)	Post+ at 5%	Post– at 5%	Post+ at 10%	Post– at 10%	Post+ at 20%	Post– at 20%
CIN2+										
FAM19A4/miR124-2	4	2.73	0.49	5.15 (2.90–9.13)	12.6%	2.5%	23.3%	5.2%	40.6%	10.9%
JAM3	7	9.96	0.37	26.64 (11.07–64.09)	34.4%	1.9%	52.5%	3.9%	71.4%	8.4%
PAX1	6	9.58	0.30	35.02 (14.47–84.76)	33.5%	1.5%	51.6%	3.2%	70.5%	6.9%
PAX1/JAM3 panel	5	11.18	0.30	43.73 (16.83–113.65)	37.0%	1.5%	55.4%	3.2%	73.7%	6.9%
S5 EPB41L3 + HPV16/18/31/33 methylation	8	1.58	0.33	4.67 (3.34–6.53)	7.7%	1.7%	15.0%	3.6%	28.3%	7.7%
SOX1	6	6.74	0.37	20.21 (9.93–41.15)	26.2%	1.9%	42.8%	3.9%	62.8%	8.5%
Six-gene/GynTect panel	7	9.28	0.39	24.10 (9.34–62.17)	32.8%	2.0%	50.8%	4.2%	69.9%	8.9%
CIN3+										
FAM19A4/miR124-2	4	2.72	0.41	6.59 (3.66–11.88)	12.5%	2.1%	23.2%	4.3%	40.5%	9.2%
JAM3	8	7.54	0.29	27.44 (16.55–45.49)	28.4%	1.5%	45.6%	3.1%	65.3%	6.7%
PAX1	11	4.98	0.28	18.82 (10.98–32.27)	20.8%	1.5%	35.6%	3.1%	55.4%	6.6%
PAX1/JAM3 panel	5	6.76	0.22	31.59 (16.69–59.78)	26.2%	1.2%	42.9%	2.4%	62.8%	5.3%
S5 EPB41L3 + HPV16/18/31/33 methylation	7	1.89	0.20	9.88 (3.73–26.12)	9.0%	1.0%	17.3%	2.1%	32.1%	4.7%
SOX1	8	5.61	0.22	26.80 (12.35–58.16)	22.8%	1.1%	38.4%	2.3%	58.4%	5.1%
Six-gene/GynTect panel	8	5.17	0.32	17.33 (10.03–29.93)	21.4%	1.6%	36.5%	3.4%	56.4%	7.3%

Abbreviations: CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; DOR, diagnostic odds ratio; HPV, human papillomavirus; k, number of studies; LR+, positive likelihood ratio; LR-, negative likelihood ratio; Post+, post-test probability after a positive test; Post-, post-test probability after a negative test.

Table 5: GRADE-DTA evidence profile for marker-specific diagnostic accuracy analyses

Marker	Studies, n	Sensitivity (95% CI)	Specificity (95% CI)	DOR (95% CI)	RoB	Indirectness	Inconsistency	Imprecision	Pub. bias	Certainty
CIN2+										
FAM19A4/miR124-2	4	0.62 (0.49-0.74)	0.77 (0.74-0.80)	5.15 (2.90-9.13)	Very serious	Serious	Serious	Serious	Not serious	Very low
JAM3	7	0.66 (0.49-0.79)	0.93 (0.88-0.96)	26.64 (11.07-64.09)	Serious	Serious	Serious	Not serious	Not serious	Very low
PAX1	6	0.73 (0.56-0.84)	0.92 (0.90-0.95)	35.02 (14.47-84.76)	Serious	Serious	Not serious	Serious	Not serious	Very low
PAX1/JAM3 panel	5	0.72 (0.58-0.83)	0.94 (0.92-0.95)	43.73 (16.83-113.65)	Serious	Serious	Serious	Serious	Not serious	Very low
S5 EPB41L3 + HPV16/18/31/33 methylation	8	0.84 (0.77-0.90)	0.47 (0.38-0.55)	4.67 (3.34-6.53)	Very serious	Serious	Not serious	Not serious	Not serious	Very low
SOX1	6	0.67 (0.55-0.77)	0.90 (0.87-0.92)	20.21 (9.93-41.15)	Serious	Serious	Not serious	Not serious	Not serious	Low
Six-gene/GynTect panel	7	0.64 (0.57-0.69)	0.93 (0.86-0.97)	24.10 (9.34-62.17)	Serious	Serious	Not serious	Not serious	Not serious	Low
CIN3+										
FAM19A4/miR124-2	4	0.70 (0.59-0.79)	0.74 (0.71-0.78)	6.59 (3.66-11.88)	Serious	Not serious	Not serious	Serious	Not serious	Low
JAM3	8	0.74 (0.65-0.82)	0.90 (0.85-0.94)	27.44 (16.55-45.49)	Serious	Serious	Not serious	Not serious	Not serious	Low
PAX1	11	0.76 (0.70-0.81)	0.85 (0.80-0.89)	18.82 (10.98-32.27)	Serious	Serious	Not serious	Not serious	Not serious	Low
PAX1/JAM3 panel	5	0.80 (0.69-0.88)	0.88 (0.86-0.90)	31.59 (16.69-59.78)	Serious	Serious	Not serious	Not serious	Not serious	Low
S5 EPB41L3 + HPV16/18/31/33 methylation	7	0.90 (0.82-0.94)	0.52 (0.44-0.61)	9.88 (3.73-26.12)	Very serious	Serious	Serious	Serious	Not serious	Very low
SOX1	8	0.82 (0.72-0.88)	0.85 (0.82-0.88)	26.80 (12.35-58.16)	Serious	Serious	Serious	Not serious	Not serious	Very low
Six-gene/GynTect panel	8	0.73 (0.65-0.80)	0.86 (0.81-0.90)	17.33 (10.03-29.93)	Serious	Serious	Not serious	Not serious	Not serious	Low

Judgments were made separately for each marker and endpoint. Certainty refers to certainty in diagnostic accuracy estimates, not to clinical effectiveness. Abbreviations: CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; DOR, diagnostic odds ratio; GRADE-DTA, Grading of Recommendations Assessment, Development and Evaluation for diagnostic test accuracy; HPV, human papillomavirus; n, number of studies; Pub. bias, publication bias; RoB, risk of bias.

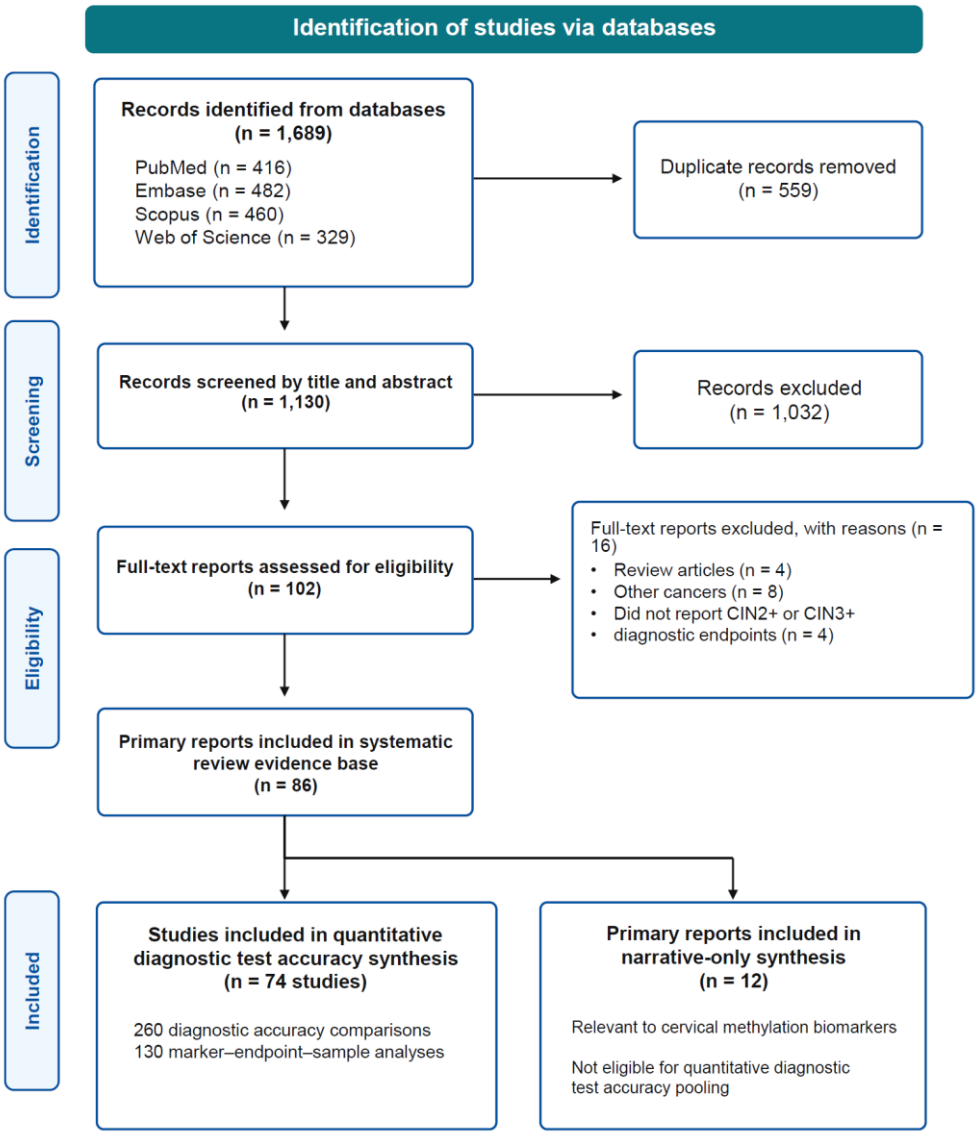

Figure 1: PRISMA-DTA flow diagram of study selection. The diagram summarizes database retrieval, duplicate removal, title-and-abstract screening, full-text assessment, exclusions, and allocation to quantitative diagnostic test accuracy synthesis or narrative-only synthesis.

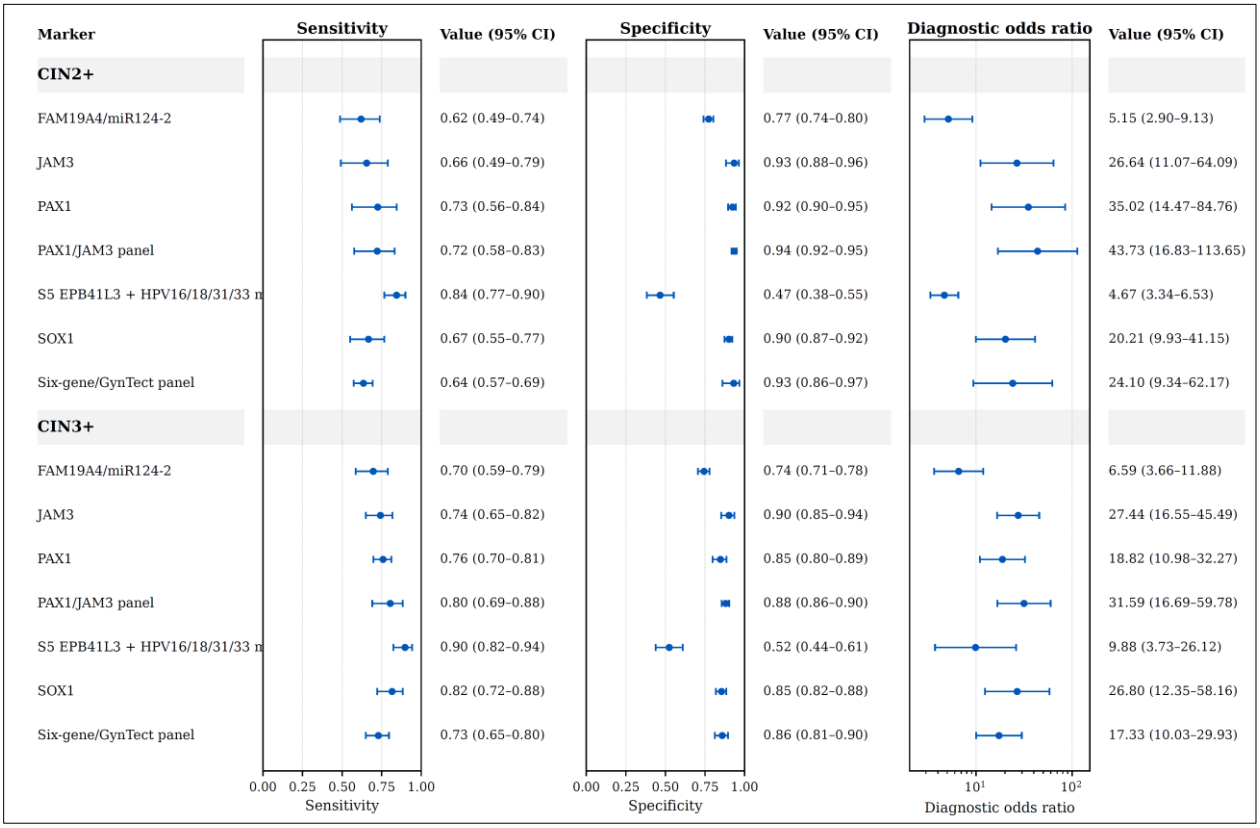

Figure 2: Overall pooled diagnostic accuracy estimates of methylation markers for CIN2+ and CIN3+. Pooled sensitivity, specificity, and diagnostic odds ratio (DOR) with 95% confidence intervals are shown for each marker-analysis in the overall analysis. Marker analyses are grouped by diagnostic endpoint, with CIN2+ shown first and CIN3+ shown subsequently. Each row represents one marker-analysis. Points indicate pooled estimates and horizontal lines indicate 95% confidence intervals.

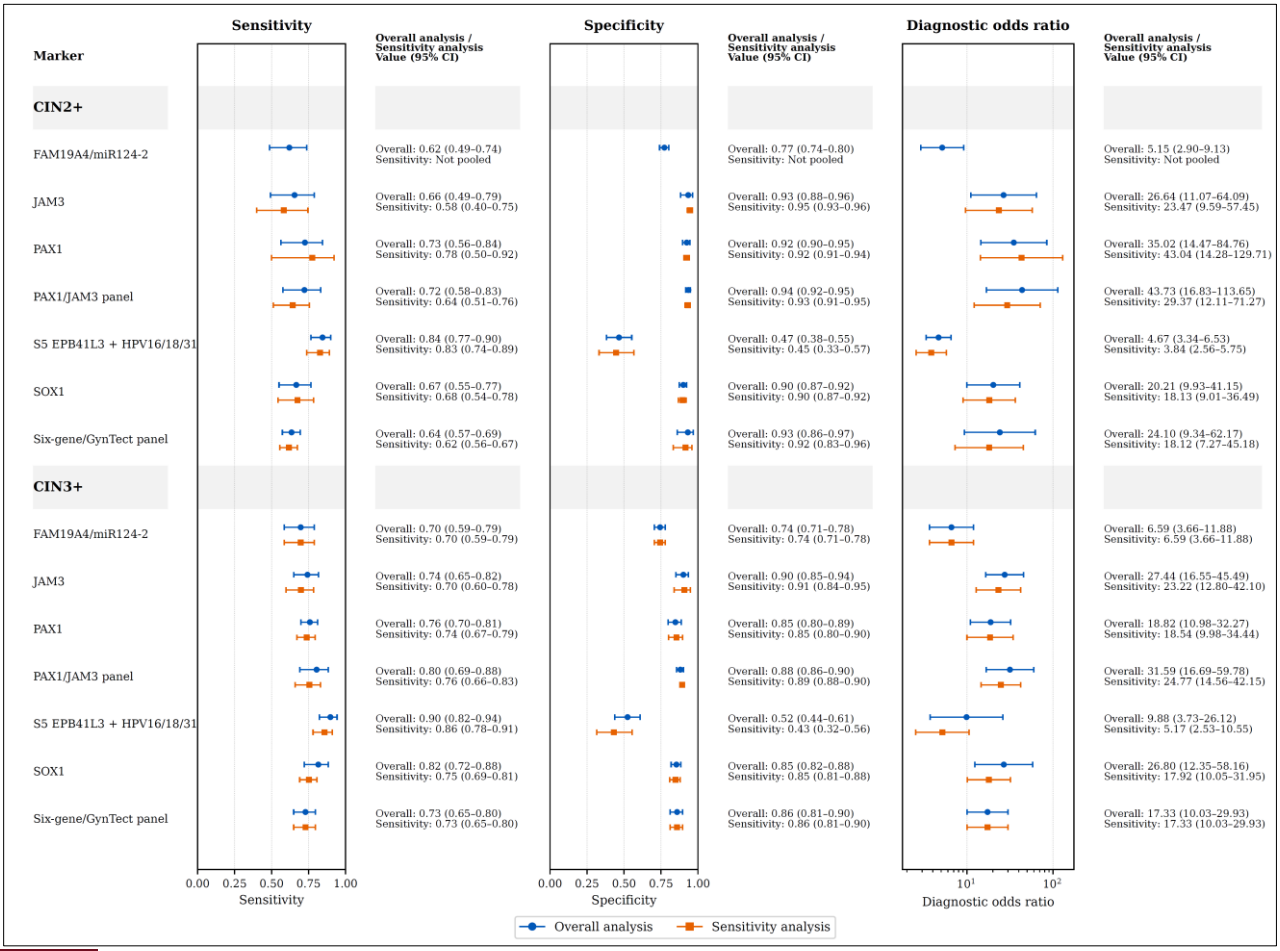

Figure 3: Sensitivity analysis comparing overall and sensitivity-analysis estimates. Pooled sensitivity, specificity, and diagnostic odds ratio (DOR) with 95% confidence intervals are compared between the overall analysis and the stricter sensitivity analysis for each marker-analysis. Marker analyses are grouped by endpoint into CIN2+ and CIN3+. Circles represent estimates from the overall analysis and squares represent estimates from the sensitivity analysis. One marker-analysis was not pooled in the sensitivity analysis because it did not meet the minimum number of eligible studies.

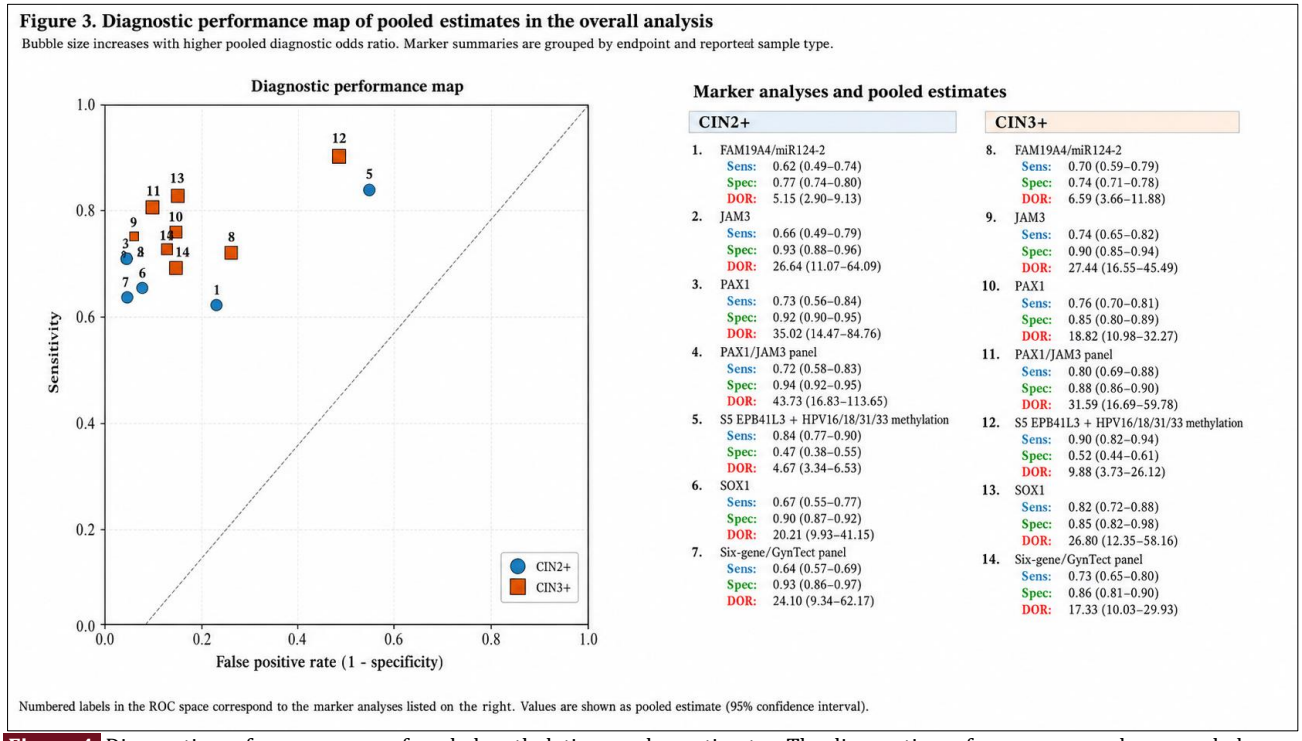

Figure 4: Diagnostic performance map of pooled methylation marker estimates. The diagnostic performance map shows pooled sensitivity against the false-positive rate, defined as 1 minus pooled specificity, for each marker-analysis in the overall analysis. Bubble size reflects the magnitude of the pooled diagnostic odds ratio (DOR), with larger bubbles indicating higher DOR. Numbered points correspond to the marker analyses listed on the right. Pooled sensitivity, specificity, and DOR with 95% confidence intervals are reported for each marker-analysis and grouped by endpoint as CIN2+ and CIN3+.

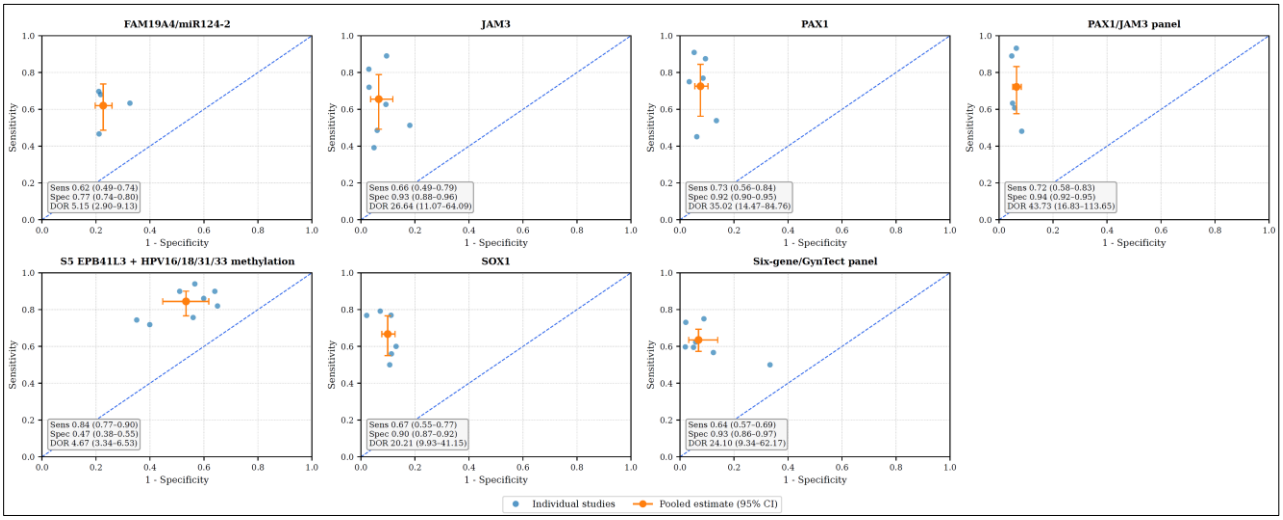

Figure 5: ROC-space plots for CIN2+ marker analyses in the overall analysis. Each panel shows the distribution of study-level diagnostic accuracy estimates for one methylation marker-analysis targeting CIN2+. The x-axis represents the false-positive rate, defined as 1 minus specificity, and the y-axis represents sensitivity. Blue points indicate individual studies, and the orange point represents the pooled estimate from the overall analysis. Error bars around the pooled point indicate 95% confidence intervals for pooled sensitivity and specificity. The inset reports pooled sensitivity, specificity, and diagnostic odds ratio (DOR) with 95% confidence intervals.

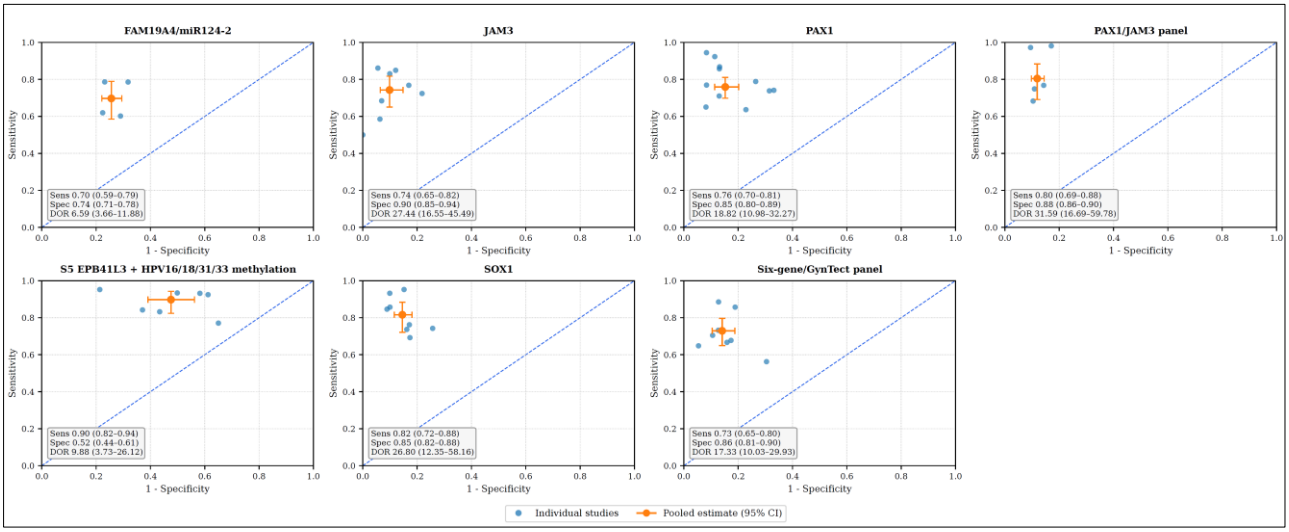

Figure 6: ROC-space plots for CIN3+ marker analyses in the overall analysis. Each panel shows study-level diagnostic accuracy estimates for one methylation marker-analysis targeting CIN3+. The x-axis represents the false-positive rate, defined as 1 minus specificity, and the y-axis represents sensitivity. Blue points indicate individual studies, and the orange point represents the pooled estimate from the overall analysis. Error bars around the pooled point indicate 95% confidence intervals for pooled sensitivity and specificity. The inset reports pooled sensitivity, specificity, and diagnostic odds ratio (DOR) with 95% confidence intervals.

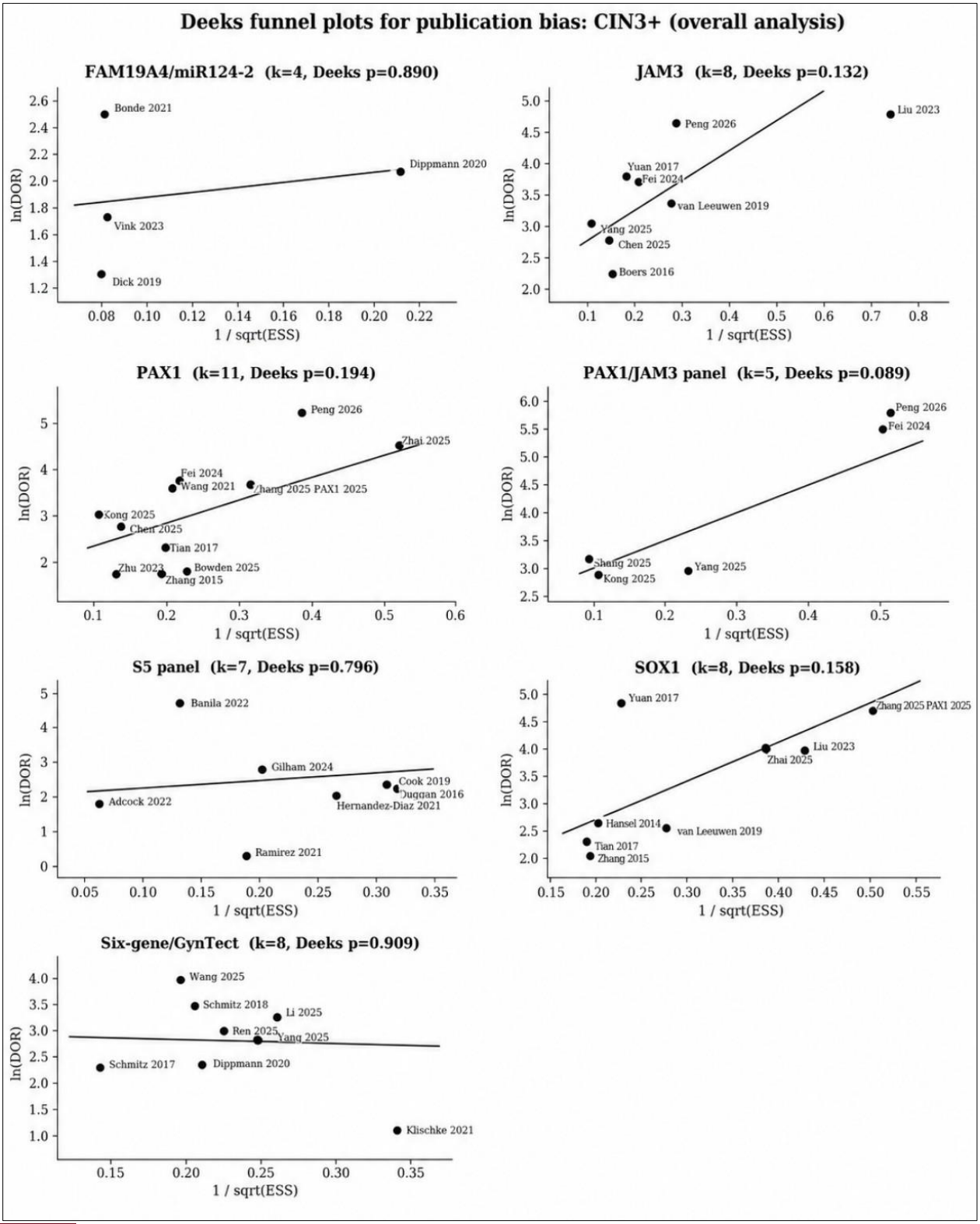

Figure 7: Deeks' funnel plots for publication bias: CIN3+ marker analyses in the overall analysis. Each panel shows the relationship between the log diagnostic odds ratio and 1/sqrt(effective sample size) for the marker-specific CIN3+ analysis. The regression line represents Deeks' funnel plot asymmetry assessment. Deeks' p-values are shown in each panel.

Appendix

Supplementary material 1: Complete database-specific search strategies used to identify studies evaluating DNA methylation markers for cervical precancer and cervical cancer screening or triage.

PubMed/MEDLINE: 416 records

("Papillomavirus Infections"[Mesh] OR "Papillo-maviridae"[Mesh] OR "human papillomavirus"[tiab] OR HPV[tiab] OR hrHPV[tiab] OR "high-risk HPV"[tiab] OR "high risk HPV"[tiab]) AND ("Uterine Cervical Neoplasms"[Mesh] OR "Cervical Intraep-ithelial Neoplasia"[Mesh] OR "Uterine Cervical Dysplasia"[Mesh] OR cervix[tiab] OR cervical[tiab] OR cervicovaginal[tiab] OR CIN[tiab] OR "CIN2+"[tiab] OR "CIN3+"[tiab]) AND ("DNA Methylation"[Mesh] OR methylation[tiab] OR hyperme-thylation[tiab] OR epigenetic*[tiab] OR epigenom*[tiab] OR "HPV methylation"[tiab] OR "methylation panel"[tiab] OR "methylation marker"[tiab] OR "methylation classifier"[tiab]) AND (triage[tiab] OR triaging[tiab] OR stratification[tiab] OR "risk stratification"[tiab] OR referral[tiab] OR screening[tiab] OR "primary screening"[tiab] OR "diagnostic accuracy"[tiab] OR sensitivity[tiab] OR specificity[tiab]) NOT (animals[Mesh] NOT humans[Mesh])

Embase: 482 records

('uterine cervix'/exp OR 'cervix cancer'/exp OR 'cervical intraep-ithelial neoplasia'/exp OR cervix:ti,ab OR cervical:ti,ab OR cervicovaginal:ti,ab) AND ('human papillomavirus infection'/exp OR 'human papillomavirus'/exp OR hpv:ti,ab OR 'human papillomavirus':ti,ab OR hrhpv:ti,ab OR 'high risk hpv':ti,ab OR 'hpv positive':ti,ab OR hpv-positive:ti,ab) AND ('dna methylation'/exp OR methylation:ti,ab OR hypermethylation:ti,ab OR epigenetic*:ti,ab OR 'methylation marker*':ti,ab OR 'methylation panel*':ti,ab OR 'methylation classifier':ti,ab) AND (triage:ti,ab OR triaging:ti,ab OR 'risk stratification':ti,ab OR 'diagnostic accuracy':ti,ab OR sensitivity:ti,ab OR specificity:ti,ab) NOT ('animal'/exp NOT 'human'/exp)

Scopus: 460 records

TITLE-ABS-KEY((cervix OR cervical OR cervicovaginal OR "cervical intraep-ithelial neoplasia" OR CIN OR "uterine cervix") AND (HPV OR "human papillomavirus" OR hrHPV OR "high-risk HPV" OR "high risk HPV" OR "HPV positive" OR HPV-positive) AND (methylation OR "DNA methylation" OR hyperme-thylation OR epigenetic* OR "methylation panel*" OR "methylation marker*" OR classifier) AND (triage OR triaging OR "risk stratification" OR "diagnostic accuracy" OR sensitivity OR specificity OR validation)) AND NOT TITLE-ABS-KEY(animals OR mouse OR mice OR murine OR rat)

Web of Science: 329 records

TS=((cervix OR cervical OR cervicovaginal OR "cervical intraep-ithelial neoplasia" OR CIN) AND (HPV OR "human papillomavirus" OR hrHPV OR "high-risk HPV" OR "high risk HPV" OR "HPV positive" OR HPV-positive) AND ("DNA methylation" OR methylation OR hyperme-thylation OR epigenetic* OR "methylation panel*" OR "methylation marker*" OR classifier) AND (triage OR triaging OR "risk stratification" OR "diagnostic accuracy" OR sensitivity OR specificity OR validation)) NOT TS=(animal* OR mouse OR mice OR murine OR rat*)

Supplementary Table 1: Narrative-only primary studies and background evidence syntheses not included in quantitative DTA pooling

Reference	Evidence type	Marker/test	Specimen or context	Endpoint/focus	Reason not pooled and narrative use
Duebbel et al., 2025 [89]	Primary prognostic study	ZNF671 methylation	Repeated FFPE cervical tissue samples	Progression, persistence, recurrence, or regression of CIN	Prognostic endpoint rather than screening/triage DTA. Retained as evidence for methylation and lesion behavior.
Clarke et al., 2017 [90]	Primary discovery/validation study	Candidate host methylation markers, including SOX1	FFPE discovery material and LBC validation samples	HSIL+ versus lower-grade or negative disease categories	No fixed binary threshold or reconst-ructable TP/FP/FN/TN for CIN2+ or CIN3+. Retained as candidate marker evidence.
Floore et al., 2019 [91]	Technical reprodu-cibility study	QIAsure FAM19A4/miR124-2	hrHPV-positive cervical specimens across laboratories	Intra- and inter-laboratory agreement	Assay reprodu-cibility study without clinical CIN2+/CIN3+ 2x2 data. Retained as technical validation evidence.
Hesselink et al., 2014 [92]	Primary DTA-related study not pooled	MAL-m1/miR124-2, CADM1-m18, MAL-m1, miR124-2	Self-collected cervicovaginal lavage from hrHPV-positive women	Triage for CIN3+	Reported AUCs and threshold-dependent sensitivity ranges, but no unique operating point with complete 2x2 counts. Retained as self-sampling evidence.
Liu et al., 2025 [93]	Primary discovery/validation study	Five-CpG HPV16 methylation classifier	Clinician-collected cervical exfoliated cells	HSIL and squamous cell carcinoma discrimination	AUC-based viral methylation classifier without complete sensitivity, specificity, or 2x2 data. Retained as viral methylation evidence.
Louvanto et al., 2020 [94]	Primary prognostic study	S5 DNA methylation classifier	Cervical brush samples in untreated CIN2 active surveillance	Regression, persistence, or progression to CIN3+	Prognostic endpoint rather than cross-sectional diagnostic detection. Retained as progression-risk evidence.
Louvanto et al., 2024 [95]	Primary special-population study	FAM19A4/miR124-2 and combined viral/EPB41L3 methylation	Cervical samples from HPV-vaccinated women	LSIL/HSIL in vaccinated cohort	No diagnostic 2x2 table. Retained as evidence relevant to future methylation triage in vaccinated populations.
Kinkorova Lunackova et al., 2025 [96]	Primary sensitivity-only study	FAM19A4/miR124-2 methylation	LBC after severe glandular cytology	HPV-associated AIS or endocervical adenocarcinoma	No non-disease control group; specificity not estimable. Retained as sensitivity-only glandular/invasive cancer evidence.
Salta et al., 2023 [101]	Systematic review/meta-analysis	DNA methylation triage markers	HPV-based cervical screening literature	Colposcopy referral triage	Evidence synthesis rather than primary study. Used for background or Discussion only; not counted as primary evidence.
Snellenberg et al., 2012 [97]	Technical assay validation study	Multiplex qMSP for CADM1, MAL, hsa-miR124-2	Clinician-collected cervical scrape	Analytical validation of multiplex methylation assay	Analytical validation without clinical DTA 2x2 data. Retained as assay-development evidence.
Vink et al., 2020 [98]	Primary cancer-only study	FAM19A4/miR124-2 methylation	Cervical scrapes and tissue specimens from invasive cervical cancer cases	Invasive cervical cancer methylation positivity	Cancer-only case series without non-disease controls; specificity not estimable. Retained as sensitivity-only cancer evidence.
Wu et al., 2025 [99]	Primary post-treatment/treatment-selection study	Six-gene/GynTect methylation assay and component markers	Cervical samples before LEEP	LEEP specimen histology among biopsy-diagnosed HSIL patients	Post-LEEP/treatment-selection endpoint rather than screening or triage DTA. Retained as risk-stratification evidence.
Zhang et al., 2022 [102]	Narrative review	Host-cell and HPV DNA methylation markers	Published cervical cancer triage literature	Review of methylation markers for triage	Review article rather than primary study. Used for background or Discussion only; not counted as primary evidence.

Reference	Evidence type	Marker/test	Specimen or context	Endpoint/focus	Reason not pooled and narrative use
Zheng et al., 2025 [100]	Primary tissue-based discovery/ML study	Tissue DNA methylation classifier	Cervical tissue biopsies from HIV-positive Nigerian women	Cervical cancer risk prediction and CIN/cancer classification	Tissue-based discovery and machine-learning classifier; exact 2x2 data not extractable for routine DTA pooling. Retained as special-population discovery evidence.

Abbreviations: AIS, adenocarcinoma in situ; AUC, area under the curve; CIN, cervical intraepithelial neoplasia; DNA, deoxyribonucleic acid; DTA, diagnostic test accuracy; FFPE, formalin-fixed paraffin-embedded; FN, false negative; FP, false positive; HIV, human immunodeficiency virus; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LBC, liquid-based cytology; LEEP, loop electrosurgical excision procedure; LSIL, low-grade squamous intraepithelial lesion; ML, machine learning; qMSP, quantitative methylation-specific PCR; TN, true negative; TP, true positive.

Supplementary Table 2: Endpoint-level summary of post-test probabilities

Endpoint	Pre-test probability	Median Post+	Median Post−	Best positive-test marker	Highest Post+	Best negative-test marker	Lowest Post−
CIN2+	5%	32.8%	1.9%	PAX1/JAM3 panel	37.0%	PAX1	1.5%
CIN2+	10%	50.8%	3.9%	PAX1/JAM3 panel	55.4%	PAX1	3.2%
CIN2+	20%	69.9%	8.4%	PAX1/JAM3 panel	73.7%	PAX1	6.9%
CIN3+	5%	21.4%	1.5%	JAM3	28.4%	S5 EPB41L3 + HPV16/18/31/33 methylation	1.0%
CIN3+	10%	36.5%	3.1%	JAM3	45.6%	S5 EPB41L3 + HPV16/18/31/33 methylation	2.1%
CIN3+	20%	56.4%	6.6%	JAM3	65.3%	S5 EPB41L3 + HPV16/18/31/33 methylation	4.7%

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, human papillomavirus; Post+, post-test probability after a positive test; Post-, post-test probability after a negative test.

Supplementary Table 3: Concise rationale for GRADE-DTA downgrading

Marker	Endpoint	Rationale
FAM19A4/miR124-2	CIN2+	Downgraded for: risk of bias, indirectness, inconsistency, imprecision; strict: Not comparable: pooled only in main broad.
JAM3	CIN2+	Downgraded for: risk of bias, indirectness, inconsistency; strict: Moderate change.
PAX1	CIN2+	Downgraded for: risk of bias, indirectness, imprecision; strict: Robust.
PAX1/JAM3 panel	CIN2+	Downgraded for: risk of bias, indirectness, inconsistency, imprecision; strict: Moderate change.
S5 EPB41L3 + HPV16/18/31/33 methylation	CIN2+	Downgraded for: risk of bias, indirectness; strict: Robust.
SOX1	CIN2+	Downgraded for: risk of bias, indirectness; strict: Robust.
Six-gene/GynTect panel	CIN2+	Downgraded for: risk of bias, indirectness; strict: Robust.
FAM19A4/miR124-2	CIN3+	Downgraded for: risk of bias, imprecision; strict: Robust.
JAM3	CIN3+	Downgraded for: risk of bias, indirectness; strict: Robust.
PAX1	CIN3+	Downgraded for: risk of bias, indirectness; strict: Robust.
PAX1/JAM3 panel	CIN3+	Downgraded for: risk of bias, indirectness; strict: Robust.
S5 EPB41L3 + HPV16/18/31/33 methylation	CIN3+	Downgraded for: risk of bias, indirectness, inconsistency, imprecision; strict: Moderate change.
SOX1	CIN3+	Downgraded for: risk of bias, indirectness, inconsistency; strict: Moderate change.
Six-gene/GynTect panel	CIN3+	Downgraded for: risk of bias, indirectness; strict: Robust.

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; GRADE-DTA, Grading of Recommendations Assessment, Development and Evaluation for diagnostic test accuracy; HPV, human papillomavirus.

Supplementary Table 4: Clinical consequences at moderate assumed prevalence

Marker	Endpoint	Assumed prevalence	True positives per 1000	False negatives per 1000	True negatives per 1000	False positives per 1000	Certainty of evidence
FAM19A4/miR124-2	CIN2+	10%	62	38	693	207	Very low
JAM3	CIN2+	10%	66	34	837	63	Very low
PAX1	CIN2+	10%	73	27	828	72	Very low
PAX1/JAM3 panel	CIN2+	10%	72	28	846	54	Very low
S5 EPB41L3 + HPV16/18/31/33 methylation	CIN2+	10%	84	16	423	477	Very low
SOX1	CIN2+	10%	67	33	810	90	Low
Six-gene/GynTect panel	CIN2+	10%	64	36	837	63	Low
FAM19A4/miR124-2	CIN3+	5%	35	15	703	247	Low
JAM3	CIN3+	5%	37	13	855	95	Low
PAX1	CIN3+	5%	38	12	808	143	Low
PAX1/JAM3 panel	CIN3+	5%	40	10	836	114	Low
S5 EPB41L3 + HPV16/18/31/33 methylation	CIN3+	5%	45	5	494	456	Very low
SOX1	CIN3+	5%	41	9	808	143	Very low
Six-gene/GynTect panel	CIN3+	5%	37	14	817	133	Low

For CIN2+, moderate prevalence was assumed to be 10%; for CIN3+, moderate prevalence was assumed to be 5%. Values are expected numbers per 1000 women tested and were rounded to the nearest whole number. Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, human papillomavirus.