ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Comparative analysis of diagnostic methods for high-risk HPV detection: A scoping review and meta-analysis

Supplementary file

Table S1. Search string for each database

Database	Search string	Date and time	Total obtained
PubMed	((((((human papillomavirus[Title/Abstract]) AND (Point-Of-Care[Title/Abstract])) AND (p16[Title/Abstract])) OR (Immunohistochemistry[Title/Abstract])) OR (Recombinase polymerase amplification[Title/Abstract])) OR (CRISPR[Title/Abstract])) OR (Loop-mediated Isothermal Amplification[Title/Abstract])	July 16, 2025 (8.53 pm)	2,661
Science Direct	(human papillomavirus) AND (Point-Of-Care) AND (p16 OR Immunohistochemistry OR (Recombinase polymerase amplification) OR CRISPR OR (Loop-mediated Isothermal Amplification))	July 16, 2025 (8.40 pm)	829
Scopus	(TITLE-ABS-KEY (human papillomavirus) AND TITLE-ABS-KEY (Point-Of-Care) AND TITLE-ABS-KEY (protein p16) OR TITLE-ABS-KEY (Immunohistochemistry) OR TITLE-ABS-KEY (Recombinase polymerase amplification) OR TITLE-ABS-KEY (CRISPR) OR TITLE-ABS-KEY (Loop-mediated Isothermal Amplification)) AND (LIMIT-TO (DOCTYPE, "ar")) AND (LIMIT-TO (LANGUAGE, "English")) AND (LIMIT-TO (EXACTKEYWORD, "Human") OR LIMIT-TO (EXACTKEYWORD, "Humans"))	July 16, 2025 (8.49 pm)	53

Table S2. Characteristics of included studies

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/ agreement	Benefit	Limitation
Petry *et al.*[1]	2011; Germany	Observational prospective	Sample characteristics: (i) Sample type: HR-HPV (ii) Organ: Cervix (iii) Tumor type: Not applicable (iv) Source of specimens: Liquid-based cytology vials collected during initial Pap/HPV co-testing screening visits Sample distribution: (i) Total samples = 425 (ii) Cytology positive = 108 (< CIN2 = 74; CIN2+ = 34) (iii) Cytology negative = 317 (< CIN2 = 314; CIN2+ = 3)	(i) HPV DNA testing: digene HC2 High-Risk HPV DNA Test (Qiagen, Germany) (ii) Dual-stained cytology: CINtec® PLUS Kit (REF 9531, mtm laboratories, Germany)	p16/Ki-67 dual-stained cytology	PCR	Dual-stained cytology vs. PCR For CIN2+: (i) Sensitivity = 91.89%; (ii) Specificity = 82.11% (iii) LR+ = 5.14 (iv) LR− = 0.10 (v) PPV = 66.67% (vi) NPV = 96.30% (vii) Accuracy = 84.85% For CIN3+: (i) Sensitivity = 96.43% (ii) Specificity = 76.92% (iii) LR+ = 4.18 (iv) LR− = 0.05 (v) PPV = 52.94% (vi) NPV = 98.77% (vii) Accuracy = 81.06%	Dual-stained cytology was performed from residual cellular material out of the liquid-based cytology vial collected at the initial screening visit. Histologic diagnoses were fully adjudicated using H&E-stained cervical biopsy specimens obtained during colposcopy follow-up, serving as the reference standard	Incomplete disease ascertainment among women with Pap negative/ HPV-positive screening test results

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/ agreement	Benefit	Limitation
Mirghani *et al.*[2]	2016; France	Observational retrospective	Sample characteristics: (i) Sample type: HR-HPV (ii) Organ: Oropharyngeal (iii) Tumor type: Squamous cell carcinoma (iv) Source of specimens: Gustave Roussy Cancer Center tissue bank Sample distribution: (i) Total samples = 105 (male = 73; female = 32)	(i) p16-IHC = CINtec p16 Histology Kit (Ref. 9511, Roche mtm laboratories AG, Germany) (ii) p16-ISH = ISH I View Blue Plus Detection Kit (Ventana Medical System, Inc., USA) (iii) p16-PCR = INNO-LiPA HPV Genotyping Extra II kit (Fujirebio, Japan) (iv) RNAscope HPV-test = RNAscope 2.0 BROWN assay kit and HPV-HR18 probe cocktail (Advanced Cell Diagnostics Inc., USA) (v) INNO-LiPA HPV Genotyping Extra II kit (Fujirebio, Japan)	RNAscope HPV test	(i) Algorithm-1 = p16-IHC combined with HPV-DNA identification by ISH (ii) Algorithm-2 = p16-IHC combined with HPV-DNA identification by PCR (iii) Genotyping	RNA scope vs. algorithm-1: (i) Sensitivity = 53.45% (ii) Specificity = 51.14% (iii) LR+ = 1.09 (iv) LR− = 0.91 (v) PPV = 59.05% (vi) NPV = 45.45% (vii) Accuracy = 52.45% RNA scope vs. algorithm-2: (i) Sensitivity = 49.60% (ii) Specificity = 48.19% (iii) LR+ = 0.96 (iv) LR− = 1.05 (v) PPV = 59.05% (vi) NPV = 38.83% (vii) Accuracy = 49.04% RNA scope vs. genotyping: (i) Sensitivity = 43.66% (ii) Specificity = 35.82% (iii) LR+ = 0.68 (iv) LR− = 1.57 (v) PPV = 59.05% (vi) NPV = 23.08% (vii) Accuracy = 41.15%	(i) RNAscope HPV-test and combination algorithms (p16-based) perform better than p16 alone in identifying truly HPV-driven OPC (ii) RNAscope HPV-test has the advantage of being a single test	(i) Comparison method (algorithm) was not a gold standard (ii) Lack of fresh sample

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/ agreement	Benefit	Limitation
Brady *et al.*[3]	2019; Finland	Cohort	(i) Sample type: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82 (ii) Organ: Oropharyngeal (iii) Tumor type: Oropharyngeal squamous cell carcinoma; (iv) Source: Tissue microarray block (v) Number of samples = 357	(i) RNAscope® 2.5 HD Reagent Kit (Advanced Cell Diagnostics, Inc., USA): Used for high-risk HPV E6/E7 mRNA ISH (ii) Ventana Inform HR-HPV ISH Assay (Benchmark XT series stainer): Used for HPV DNA ISH (iii) Multiplex HPV Genotyping Kit® (DiaMex GmbH, Germany): Used for HPV DNA PCR	(i) HPV mRNA ISH: Detects transcriptionally active HPV using RNAscope® probes for E6/E7 mRNA. (ii) HPV DNA ISH: Detects HPV DNA directly in tissue using Ventana Inform HR-HPV ISH (iii) HPV DNA PCR: Amplifies HPV DNA from FFPE samples using DiaMex kit and Luminex LX-100 analyzer. (iv) p16 IHC: Immunohistochemistry using CINtec Histology Kit to detect p16INK4a protein as a surrogate marker	p16 IHC	mRNA ISH vs. p16 IHC: (i) Sensitivity = 93.40% (ii) Specificity = 92.4% (iii) LR+ = N/A (iv) LR− = N/A (v) PPV = 95.5% (vi) NPV = 89% (vii) Accuracy = 92.89% DNA ISH vs. p16 IHC: (i) Sensitivity = 86.3% (ii) Specificity = 95.3% (iii) LR+ = N/A (iv) LR− = N/A (v) PPV = 96.2% (vi) NPV = 83.5% (vii) Accuracy = 90.57% DNA PCR vs. p16 IHC: (i) Sensitivity = 83.5% (ii) Specificity = 89.1% (iii) LR+ = N/A (iv) LR− = N/A (v) PPV = 94.7% (vi) NPV = 69.5% (vii) Accuracy = 86.21%	ISH for high-risk HPV E6/E7 mRNA is a highly sensitive and specific method for detecting transcriptionally active HPV in oropharyngeal squamous cell carcinoma, making it a valuable complement to p16 IHC for accurate HPV status determination, especially in treatment planning	The study was limited by differing HPV DNA detection methods across patient cohorts, lack of matched fresh frozen tissue for qRT-PCR comparison, and insufficient sample material to apply all techniques uniformly

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/ agreement	Benefit	Limitation
El-Salem *et al.*[4]	2019; USA	Observational retrospective	Sample characteristics: (i) Sample type: HPV-16, 35, 33, 18, 45, 59 (ii) Organ: Head and neck (iii) Tumor type: Head and neck squamous cell carcinoma (iv) Source: Fine needle aspirations Sample distribution: (i) Total samples = 218 (ii) HPV-positive = 177 (iii) HPV-negative = 31 (iv) N/A = 10	(i) HPV genotyping: LightCycler 480 High Resolution Melting Master kit (ii) PCR: LightCycler 480 High Resolution Melting Master kit	Positive result will be tested by RT-PCR, whereas negative result will be tested by Sanger sequencing	p16 IHC	(i) Tissue-based staining (RT-PCR and Sanger Sequencing vs. p16 IHC): PPV = 98% NPV = 21% (ii) Fine-needle aspiration specimens using "any staining" as surrogate for HPV in (RT-PCR and Sanger Sequencing vs. p16 IHC): PPV = 94% NPV = 48%		
Shrestha *et al.*[5]	2019; South Korea	Observational retrospective	Sample characteristics: (i) Sample type: 24 HPV serotypes (15 high risk, 9 low risk) (ii) Organ: Ocular (iii) Tumor type: Ocular surface squamous neoplasia (iii) Source: FFPE tissue Sample distribution (i) Total samples = 38 (male = 27; female = 11)	(i) p16 IHC = CINtec Histology Kit (mtm Laboratories AG (Germany) (ii) HPV DNA Chip Test: MyGene Company's	IHC	HPV DNA chip test	IHC vs. DNA chip: (i) Sensitivity = 100% (ii) Specificity = 54.05% (iii) LR+ = 2.18 (iv) LR– = 0 (v) PPV = 5.56% (vi) NPV = 100% (vii) Accuracy = 55.26%	(i) Comprehensive genotyping: Simultaneous detection of 24 HPV serotypes (ii) Dual validation: Combines molecular and immunohistochemical approaches (iii) Clinical relevance: First study to apply DNA chip test to OSSN	(i) Low HPV detection: Only 2.6% positivity by DNA chip despite 31.6% p16 expression (ii) Retrospective design: Limited clinical data and follow-up (iii) Single-center study: May not generalize across populations (iv) Potential false negatives: DNA chip may miss low-copy HPV or rare serotypes

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Yin *et al.*[6]	2019; USA	Experimental laboratory study	Sample characteristics: (i) Sample type: HPV-16, 18, 31 (ii) Organ: Cervix (iii) Tumor type: Not applicable (iv) Source: Saliva, cervical swab (v) Total samples = 15	(i) Microfluidic chip: Custom PMMA-based chip (fabricated in house) (ii) Smart Cup platform: exothermic chemical reaction LAMP (fabricated in house)	Smart Cup LAMP	Real-time PCR	HPV-16: Smart Cup vs. PCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0 (v) PPV = 100% (vi) NPV = 100% (vii) Accuracy = 100% HPV-16: Smart Cup vs. Pap smear: (i) Sensitivity = 33.33% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0.67 (v) PPV = 100% (vi) NPV = 85.71% (vii) Accuracy = 86.67% HPV-16: PCR vs. Pap smear: (i) Sensitivity = 33.33% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0.67 (v) PPV = 100% (vi) NPV = 85.71% (vii) Accuracy = 86.67% HPV-18: Smart Cup vs. PCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0 (v) PPV = 100% (vi) NPV = 100% (vii) Accuracy = 100% HPV-18: Smart Cup vs. Pap smear: (i) Sensitivity = 33.33% (ii) Specificity = 100%	(i) Instrument-free: No need for electricity or lab equipment (ii) Low cost (iii) User-friendly: Smartphone app enables objective color analysis	(i) Limited genotyping: Only detects HPV-16, 18, and 31 (ii) Manual sample prep: Requires heat lysis and pipetting (iii) Environmental sensitivity: Colorimetric readout may vary with lighting if not app-assisted (iv) A limit of detection of 50 copies per test for HPV-16 can be achieved with the non-buffered LAMP solution, which is 10-fold higher than that of NEB LAMP reaction buffer (v) Limited positive HPV sample

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
							(iii) LR+ = NC (iv) LR− = 0.67 (v) PPV = 100% (vi) NPV = 85.71% (vii) Accuracy = 86.67%		
							HPV-18: PCR vs. Pap smear: (i) Sensitivity = 33.33% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0.67 (v) PPV = 100% (vi) NPV = 85.71% (vii) Accuracy = 86.67%		
							HPV-31: Smart Cup vs. PCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0 (v) PPV = 100% (vi) NPV = 100% (vii) Accuracy = 100%		
							HPV-31: Smart Cup vs. Pap smear: (i) Sensitivity = 33.33% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0.67 (v) PPV = 100% (vi) NPV = 85.71% (vii) Accuracy = 86.67%		
							HPV-31: PCR vs. Pap smear (i) Sensitivity = 33.33% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR− = 0.67 (v) PPV = 100% (vi) NPV = 85.71% (vii) Accuracy = 86.67%		

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Li *et al.*[7]	2020; China	Cross-sectional diagnostic accuracy study	Sample characteristics: (i) Sample type: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (ii) Organ: Cervix (iii) Tumor type: Not applicable (iv) Source: Exfoliated cervical cells collected in PreservCyt medium Sample distribution: (i) Total samples = 1,122 (positive = 610; negative = 572)	(i) Roche Cobas: CINtec PLUS Cytology Kit (Roche Tissue Diagnostics/ Ventana Medical Systems, Inc., Tucson, AZ). (ii) BD Onclarity: OnclarityTM HPV assay (Onclarity; Becton, Dickinson and Company—BD Diagnostics, USA). (iii) Cytology: ThinPrep® Pap Test (Marlborough, MA, USA).	PCR method with Onclarity	PCR method with Roche Cobas	HPV-16: Onclarity vs. Cobas: (i) Sensitivity = 87.65% (ii) Specificity = 99.47% (iii) LR+ = 166.88 (iv) LR− = 0.12 (v) PPV = 96.75% (vi) NPV = 97.83% (vii) Accuracy = 97.68% HPV-18: Onclarity vs. Cobas: (i) Sensitivity = 66.67% (ii) Specificity = 99.82% (iii) LR+ = 368 (iv) LR− = 0.33 (v) PPV = 85.71% (vi) NPV = 99.46% (vii) Accuracy = 99.29% Other HPV: Onclarity vs. Cobas: (i) Sensitivity = 82.63% (ii) Specificity = 98.03% (iii) LR+ = 41.94 (iv) LR− = 0.18 (v) PPV = 92.64% (vi) NPV = 94.95% (vii) Accuracy = 94.47%	BD Onclarity is comparable screening in detection cervical lesion in Chinese women compared to Cobas	The study's referral criteria, prioritizing Cobas positivity and excluding negatives, introduced selection bias and reduced assay discordance, while incomplete follow-up and its cross-sectional design limited the clinical performance assessment

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Nuovo[8]	2020; USA	Observational retrospective	Sample characteristics: (i) Sample type: No description of DNA HPV specific type (ii) Organ: Cervix (iii) Tumor type: Cervical intraepithelial neoplasia (iv) Source: FFPE, cervical biopsy Sample distribution: (i) Total FFPE samples = 150 (ii) Test FFPE cases (n = 100): CIN = 62; control = 38 (iii) Total biopsy samples = 50 (CIN = 25; control = 25)	(i) ISH: Enzo Life Sciences HPV DNA screening probe cocktail (PATHO-GENE AP HPV in situ screening and typing assays, Enzo Life Sciences HPV in situ kit, Farmingdale, NY, Enz-32884). (ii) IHC: p16 (Abcam, Cambridge MA); Ki67 (Abcam, Cambridge MA); Importin-β (antibody, optimal dilution 1:750); Exportin-5 (antibody, optimal dilution 1:750); Mcl1 (Enzo Life Sciences, Farmingdale, NY, optimal dilution 1:750); HPV consensus protein L1 (Biocare, Pacheco, CA, optimal dilution 1:7,500)	ISH and IHC	-	Detection for CIN: (i) False positive ISH = 38/125 (30.4%) (ii) False positive IHC = 36/125 (28.8%)	Specific biomarker (HPV DNA L1 capsid) may distinct between CIN	IHC for several biomarker can show high background that may lead to false positive

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Weeramange *et al.*[9]	2021; Australia	Cohort	Sample characteristics: (i) Sample type: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 53, 66, 67, 73, 6, 11 (ii) Organ: Head and neck (iii) Tumor type: Head and neck cancer (iv) Source: Saliva Sample distribution: (i) Total samples = 501 (ii) Primary HNC = 491 (iii) Recurrent HNC =10	(i) p16 IHC: CINtec p16INK4a (E6H4 clone) (ii) qPCR: PowerUp SYBR Green Master Mix (Applied biosystems) (iii) MassARRAY: iPlex Pro Reagent Set (Agena Bioscience)	MassARRAY	p16 IHC	MassARRAY vs. p16 IHC: (i) Sensitivity = 99.48% (ii) Specificity = 38.03% (iii) LR+ = 1.61 (iv) LR− = 0.01 (v) PPV = 81.43% (vi) NPV = 96.43% (vii) Accuracy = 83.02%	(i) Noninvasive: Saliva-based testing is patient-friendly and suitable for screening (ii) High predictive value: Salivary HR-HPV DNA had a 99.5% positive predictive value for tumor p16 status (iii) Prognostic utility: Strong correlation with improved survival in OPC patients (iv) Surveillance potential: Effective in detecting recurrence in HPV-HNC	(i) Tumor HPV status: Assessed only via p16 IHC, not direct HPV DNA testing (ii) NPV: Only 38% for salivary HR-HPV DNA (iii) Limited recurrent cohort: Small sample size (n = 10) for recurrence analysis 4. No co-infection analysis: Other HR-HPV types not assessed in HPV-16-positive samples

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Wormald *et al.*[10]	2021; UK	Observational prospective	Sample characteristics: (i) Sample type: HPV-16, HPV-18 (ii) Organ: Cervix (iii) Tumor type: Stage I cervical cancer (iv) Source: cervical swab Sentence distribution: (i) Total samples = 41 (ii) Cervical cancer (stage I) = 27 (iii) Non-cancer, HPV-negative = 14	GenoID assay kit (PCR-based for HPV L1 gene) and PreTect HPV-Proofer (E6/E7 mRNA NASBA assay)	LAMP assay and endovaginal MRI	(i) qPCR (GenoID, Norchip) (ii) Histopathology	LAMP HPV-16 DNA/mRNA vs. GenoID and Norchip test (i) Sensitivity = 90.9% (ii) Specificity = 88.9% (iii) PPV = 76.9% (iv) NPV = 96.0% (v) Accuracy = 89.88% LAMP HPV-18 DNA/mRNA vs. GenoID and Norchip test: (i) Sensitivity = 100% (ii) Specificity = 22.2% (iii) PPV = 47.1% (iv) NPV = 100% (v) Accuracy = 36.05% LAMP-hTERT vs. histopathology: (i) Sensitivity = 31.3% (ii) Specificity = 77.8% (iii) PPV = 71.4% (iv) NPV = 38.9% (v) Accuracy: 44.62% LAMP-TERC vs. histopathology: (i) Sensitivity = 40% (ii) Specificity = 100% (iii) PPV = 100% (iv) NPV = 50% (v) Accuracy = 57.14% LAMP-cMYC vs. histopathology (i) Sensitivity = 43.8% (ii) Specificity = 100% (iii) PPV = 100% (iv) NPV = 50% (v) Accuracy = 60.91%	(i) Rapid, multiplexed detection of HPV and tumor markers (ii) Point-of-care potential via lab-on-a-chip compatibility (iii) Improved diagnostic accuracy when combined with MRI (up to 88%)	(i) Control group composition: no HPV-positive non-cancer controls were included, limiting assessment of HPV as a standalone biomarker for tumor presence (ii) PCR validation issues: Low RNA yield from cytology samples restricted the number of repeat experiments DNA purity was suboptimal, likely due to contaminants and low cellular content, affecting PCR performance PCR assays for TERC and MYC showed high variability and poor repeatability, preventing reliable comparison with LAMP results (iii) Sample volume constraints: limited nucleic acid availability led to single-run assays and hindered precision estimation (iv) Reproducibility: lack of repeated testing means LAMP assay reproducibility remains unverified (v) False positives: HPV-18 DNA LAMP assay showed primer–dimer formation, causing false positives in clinical samples (vi) Sensitivity vs specificity trade-off: Tumor markers had high specificity but low sensitivity HPV markers had high sensitivity but lower specificity Combined testing improved diagnostic accuracy but still requires optimization (vii) Extraction methodology: improved DNA/RNA extraction protocols are needed for better assay performance, especially in low-cellularity samples

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Mattox *et al.*[11]	2022; USA	Observational retrospective	Sample characteristics: (i) Sample type: HPV-16 (ii) Organ: Oropharyngeal (iii) Tumor type: HPV-16–positive oropharyngeal cancer (iv) Source: Plasma and oral rinse samples Sample distribution (i) Total samples = 358 Plasma = 212 Oral rinse =146	(i) p16 IHC (MTM Laboratories, Heidelberg, Germany) (ii) HPV-16 E6/E7 RNA in situ hybridization (ISH; RNAscope, Advanced Cell Diagnostics, Hayward, CA) (iii) NGS: MiSeq (Illumina, San Diego, CA) (iv) ddPCR: QX200 droplet generator (Bio-Rad, Hercules, CA) with SYBR Green (v) qPCR: PowerUp SYBR Green Master Mix on QuantStudio 3 (Applied Biosystems, Foster City, CA)	Plasma and oral rinse: NGS, ddPCR, and qPCR	Oropharyngeal cancer: p16 IHC and ISH	Plasma: NGS vs. imaging and clinical (IHC and ISH): (i) Sensitivity = 57.14% (ii) Specificity = 37.50% (iii) LR+ = 0.91 (iv) LR− = 1.14 (v) PPV = 13.79% (vi) NPV = 83.33% (vii) Accuracy = 40.43% Oral rinse: NGS vs. imaging and clinical (IHC and ISH): (i) Sensitivity = 100% (ii) Specificity = 37.93% (iii) LR+ = 1.61 (iv) LR− = 0 (v) PPV = 25% (vi) NPV = 100% (vii) Accuracy = 48.57% Plasma: ddPCR vs. imaging and clinical (IHC and ISH): (i) Sensitivity = 71.43% (ii) Specificity = 22.50% (iii) LR+ = 0.92 (iv) LR− = 1.27 (v) PPV = 13.89% (vi) NPV = 81.82% (vii) Accuracy = 29.79% Oral rinse: ddPCR vs. imaging and clinical (IHC and ISH): (i) Sensitivity = 33.33% (ii) Specificity = 93.10% (iii) LR+ = 4.83 (iv) LR− = 0.72 (v) PPV = 50% (vi) NPV = 87.10% (vii) Accuracy = 82.86% Plasma and oral rinse for qPCR: N/A	NGS and ddPCR were similarly sensitive for detecting HPV oropharyngeal cancer	Sample limited with early of HPV oropharyngeal cancer disease. The bank samples were not collected at defined intervals

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Guan *et al.*[12]	2024; USA	Experimental laboratory study	Sample characteristics (i) Sample type: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (ii) Organ: Cervix (iii) Tumor type: - (iv) Source: Cervical swab (v) Total samples = 75	(i) RPA: TwistAmp® Basic kit (ii) qPCR: GoTaq® Probe qPCR kit (Promega)	CRISPR-Cas12a-based detection combined with RPA	qPCR	HPV-16: CRIPSR vs. PCR: (i) Sensitivity = 80% (ii) Specificity = 98.5% HPV-18: CRIPSR vs. PCR: (i) Sensitivity = 100% (ii) Specificity = 100% HPV3-9, 45, 56, 66, 68: CRIPSR vs. PCR: (i) Sensitivity = 77.8% (ii) Specificity = 100% HPV-33, 35, 52, 58: CRIPSR vs. PCR: (i) Sensitivity = 91.7% (ii) Specificity = 100% HPV-31, 51, 59: CRIPSR vs. PCR: (i) Sensitivity = 100% (ii) Specificity = 100%	(i) Multiplexed detection: simultaneous identification of 14 HR-HPV subtypes (ii) Low cost (iii) Point-of-care ready: paper-based chip with lyophilized reagents	Optimization needed: Further refinement of primer design and crRNA specificity

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Hartanti *et al.*[13]	2025; Indonesia	Observational prospective	Sample characteristics: (i) Sample type: HPV-16, 18, 52 (ii) Organ: Cervix (iii) Tumor type: - (iv) Source: Cervical swab (v) Total samples = 20 (cancer = 15; non-cancer = 5)	(i) mRPA: TwistAmp Basic kit (TwistDx, UK) (ii) PCR: MyTaq HS Red Mix (Bioline, UK)	mRPA	Conventional PCR	HPV-16: mRPA vs. PCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) PPV = 100% (iv) NPV = 100% (v) Accuracy = 100% HPV-18: mRPA vs. PCR: (i) Sensitivity = 80% (ii) Specificity = 100% (iii) PPV = 100% (iv) NPV = 83% (v) Accuracy = 88.88% HPV-52: mRPA vs. PCR: (i) Sensitivity = 60% (ii) Specificity = 100% (iii) PPV = 100% (iv) NPV = 71% (v) Accuracy = 75%	(i) Rapid amplification: minimal equipment required (no thermal cycler) (ii) Suitable for point-of-care and low-resource settings (iii) Cost-effective	(i) Lower sensitivity for HPV-18 (80%) and HPV-52 (60%) compared to PCR (100% for all) (ii) Performance highly dependent on primer optimization

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Thai *et al.*[14]	2025; USA	Experimental laboratory study	Sample characteristics: (i) Sample type: HPV-16, 18 (ii) Organ: Cervix (iii) Tumor type: - (iv) Source: Urine; (v) Total samples = 16	(i) DNA extraction: Quick-DNA/ RNA Viral Kit (Zymo Research) (ii) LAMP reagents: LAMP kit (New England Biolabs, Ipswich, MA)	SSMG-LAMP for triple mode (colorimetric, fluorometric, electrochemical)	PCR	Colorimetry: SSMG-LAMP vs. PCR: (i) Sensitivity = 66.67% (ii) Specificity = 84.62% (iii) LR+ = 4.33 (iv) LR− = 0.39 (v) PPV = 50% (vi) NPV = 91.67% (vii) Accuracy = 81.25% Fluorometry: SSMG-(i) Sensitivity = 83.33% (ii) Specificity = 84.62% (iii) LR+ = 5.42 (iv) LR− = 0.20 (v) PPV = 55.56% (vi) NPV = 95.65% (vii) Accuracy = 84.38% Electrochemistry: SSMG-LAMP vs. PCR: (i) Sensitivity = 66.67% (ii) Specificity = 96.15% (iii) LR+ = 17.33 (iv) LR− = 0.35 (v) PPV = 80% (vi) NPV = 92.59% (vii) Accuracy = 90.62%	(i) Triple-mode detection enhances accuracy and adaptability (ii) Rapid and portable: suitable for low-resource settings (iii) High sensitivity and specificity for HPV-16 and HPV-18 (iv) Visual and instrument-based readouts for flexible deployment	(i) Limited validation cohort (n = 16 urine samples) (ii) Potential inhibition from high dye concentrations (iii) Requires optimization for broader HPV subtype coverage

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
Yin *et al.*[15]	2025; China	Experimental laboratory study	Sample characteristics: (i) Sample type: HPV-16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 66, 68 (ii) Organ: Cervix (iii) Tumor type: - (iv) Source: Cervical swab Sample distribution (i) Total samples = 258 (ii) HPV positive = 200 (iii) HPV negative = 58	RPA: MIRA kit (AMP-Future Biotech)	(i) MIRA–CRISPR/Cas12a fluorescence assay (ii) TaqMan PCR	qPCR (Cobas HPV test)	HPV-16: CRISPR vs. qPCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR– = 0 (v) PPV = 100% (vi) NPV = 100% (vii) Accuracy = 100% HPV-18: CRISPR vs. qPCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR– = 0 (v) PPV = 100% (vi) NPV = 100% (vii) Accuracy = 100% Other HR-HPV: CRISPR vs. qPCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR– = 0 (v) PPV = 100% (vi) NPV = 100% (vii) Accuracy = 100% HPV-16: TaqMan PCR vs. qPCR: (i) Sensitivity = 100% (ii) Specificity = 96.67% (iii) LR+ = 30 (iv) LR– = 0 (v) PPV = 97.94% (vi) NPV = 100% (vii) Accuracy = 98.71%	(i) Ultra-sensitive: detection limit = 2 copies/μL (ii) Multiplex capability: simultaneous detection of 14 HR-HPV types (iii) Field-ready: visual readout possible without specialized equipment	(i) Cas12a optimization required: signal-to-background ratio sensitive to temperature, protein, and DTT concentration (ii) Limited genotyping efficiency: slightly reduced performance for HPV59 (iii) No longitudinal data: cross-sectional design limits prognostic insights (iv) Visual readout subjectivity: may vary under different lighting conditions

(*cont'd...*)

doi: 10.36922/EJMO025380407

Table S2. (*Continued*)

Study	Year; country	Study design	Sample	Diagnostic methods	Investigation method	Comparison method	Accuracy/agreement	Benefit	Limitation
							HPV-18: TaqMan PCR vs. qPCR: (i) Sensitivity = 100% (ii) Specificity = 98.31% (iii) LR+ = 59 (iv) LR– = 0 (v) PPV = 95.65% (vi) NPV = 100% (vii) Accuracy = 98.77% Other HR-HPV: TaqMan PCR vs. qPCR: (i) Sensitivity = 100% (ii) Specificity = 100% (iii) LR+ = NC (iv) LR– = 0 (v) PPV = 100% (vi) NPV = 100% (vii) Accuracy = 100%		

Abbreviations: ddPCR: Droplet digital polymerase chain reaction; HR-HPV: High risk human papillomavirus; hTERT: Human telomerase reverse transcriptase; IHC: Immunohistochemistry; ISH: In situ hybridization; LAMP: Loop-mediated isothermal amplification; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio; LR-HPV: Low risk human papillomavirus; MIRA: Multienzyme isothermal rapid amplification; MIRA-CRISPR: multienzyme isothermal rapid amplification-clustered regularly interspaced short palindromic repeats; mRPA: Multiplex recombinase polymerase amplification; NGS: Next Generation sequencing; NPV: Negative predictive value; PCR: Polymerase chain reaction; PPV: Positive predictive value; SSMG: Sequence specific magnetic gel.

doi: 10.36922/EJMO025380407

References

1. Petry KU, Schmidt D, Scherbring S, *et al*. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. *Gynecol Oncol*. 2011;121(3):505-509.

   doi: 10.1016/j.ygyno.2011.02.033

2. Mirghani H, Casiraghi O, Guerlain J, *et al*. Diagnosis of HPV driven oropharyngeal cancers: Comparing p16 based algorithms with the RNAscope HPV-test. *Oral Oncol*. 2016;62:101-108.

   doi: 10.1016/j.oraloncology.2016.10.009

3. Randén-Brady R, Carpén T, Jouhi L, *et al*. In situ hybridization for high-risk HPV E6/E7 mRNA is a superior method for detecting transcriptionally active HPV in oropharyngeal cancer. *Hum Pathol*. 2019;90:97-105.

   doi: 10.1016/j.humpath.2019.05.006

4. El-Salem F, Mansour M, Gitman M, *et al*. Real-time PCR HPV genotyping in fine needle aspirations of metastatic head and neck squamous cell carcinoma: Exposing the limitations of conventional p16 immunostaining. *Oral Oncol*. 2019;90:74-79.

   doi: 10.1016/j.oraloncology.2019.02.006

5. Shrestha T, Choi W, Kim GE, Yang JM, Yoon KC. Human papilloma virus identification in ocular surface squamous neoplasia by p16 immunohistochemistry and DNA chip test: A strobe-compliant article. *Medicine*. 2019;98(2):e13944.

   doi: 10.1097/MD.0000000000013944

6. Yin K, Pandian V, Kadimisetty K, *et al*. Synergistically enhanced colorimetric molecular detection using smart cup: a case for instrument-free HPV-associated cancer screening. *Theranostics*. 2019;9(9):2637-2645.

   doi: 10.7150/thno.32224

7. Li T, Wu Z, Jiang M, *et al*. Clinical performance of Onclarity HPV assay and Cobas HPV test in detection of cervical precancer and cancer in Chinese women. *Gynecol Oncol*. 2020;157(1):202-208.

   doi: 10.1016/j.ygyno.2020.01.011

8. Nuovo G. A broad-based approach to differentiate CIN from its mimics: The utility of in situ hybridization and immunohistochemistry. *Ann Diagn Pathol*. 2020;46:151515.

   doi: 10.1016/j.anndiagpath.2020.151515

9. Ekanayake Weeramange C, Liu Z, Hartel G, *et al*. Salivary High-Risk Human Papillomavirus (HPV) DNA as a Biomarker for HPV-Driven Head and Neck Cancers. *J Mol Diagn*. 2021;23(10):1334-1342.

   doi: 10.1016/j.jmoldx.2021.07.005

10. Wormald B, Rodriguez-Manzano J, Moser N, *et al*. Loop-Mediated Isothermal Amplification Assay for Detecting Tumor Markers and Human Papillomavirus: Accuracy and Supplemental Diagnostic Value to Endovaginal MRI in Cervical Cancer. *Front Oncol*. 2021;11:747614.

    doi: 10.3389/fonc.2021.747614

11. Mattox AK, D'Souza G, Khan Z, *et al*. Comparison of next generation sequencing, droplet digital PCR, and quantitative real-time PCR for the earlier detection and quantification of HPV in HPV-positive oropharyngeal cancer. *Oral Oncol*. 2022;128:105805.

    doi: 10.1016/j.oraloncology.2022.105805

12. Guan X, Yang R, Zhang J, *et al*. Programmable Multiplexed Nucleic Acid Detection by Harnessing Specificity Defect of CRISPR-Cas12a. *Adv Sci*. 2025;12(4):2411021.

    doi: 10.1002/advs.202411021

13. Hartanti MD, Amtha R, Wratsangka R, *et al*. Operational Feasibility and Performance of mRPA Versus PCR for the Detection of HPV Types 16, 18, and 52 in Clinical Samples: An Exploratory Study. *Int J Womens Health*. 2025;17:955-964.

    doi: 10.2147/IJWH.S496621

14. Thai DA, Liu J, Gutierrez Ruiz A, Zhang Y, Walther-Antonio M, Liu Y. Triple-mode point-of-care diagnostics for high-risk human papillomavirus in urine. *Biosens Bioelectron X*. 2025;26:100658.

    doi: 10.1016/j.biosx.2025.100658

15. Yin L, Zhao Z, Wang C, *et al*. Development and evaluation of a CRISPR/Cas12a-based diagnostic test for rapid detection and genotyping of HR-HPV in clinical specimens. *Microbiol Spectr*. 2025;13(1):e02253-24.

    doi: 10.1128/spectrum.02253-24