

Supplementary Information

Human papillomavirus in oral potentially malignant disorders: a systematic review and meta-analysis of lesion-associated specimens with a framework for clinical interpretation

BIOHORIZONS | Volume 1

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Contents: Online Resources 1–9 (four tables and five supplementary figures). Materials are presented in the order in which they are cited in the manuscript.

Online Resource 1. Complete search strategy by information source

Reproducible strategy					
Source	Last search	Executed/documented search string	Field or interface	Limits and procedure	Records incorporated
PubMed (including MEDLINE)	June 3, 2026	((("human papillomavirus" OR "HPV" OR "HPV DNA" OR "HPV genotype") AND ("oral potentially malignant disorders" OR "oral premalignant lesions" OR "oral leukoplakia" OR "oral lichen planus" OR "oral epithelial dysplasia" OR "oral submucous fibrosis" OR "erythroplakia" OR "oral mucosa")) AND (english[Language] OR spanish[Language])) AND ("2019/01/01"[Date - Publication] : "2026/06/03"[Date - Publication])	Untagged terms with PubMed automatic term mapping; language and date filters	English or Spanish; publication date from January 1, 2019 to June 3, 2026	194
Google Scholar	June 3, 2026	("human papillomavirus" OR HPV) AND ("oral potentially malignant disorders" OR "oral premalignant lesions" OR "oral leukoplakia" OR "oral lichen planus" OR "oral epithelial dysplasia" OR "oral submucous fibrosis" OR erythroplakia)	Web interface; search of text and metadata; results ranked by relevance	Years 2019–2026; first 200 results by relevance; language verified during screening	200
OpenAlex	June 3, 2026	search=("human papillomavirus" OR HPV) AND ("oral potentially malignant disorder" OR "oral potentially malignant disorders" OR "oral premalignant lesion" OR "oral premalignant lesions" OR "oral leukoplakia" OR "oral lichen planus" OR "oral epithelial dysplasia" OR "oral submucous fibrosis" OR erythroplakia); filter=from_publication_date:2019-01-01,to_publication_date:2026-06-03,type:article	Works search: title, abstract, and available full text	Type: article; publication date from January 1, 2019 to June 3, 2026; language verified during screening	161
Cochrane Library/CENTRAL	June 3, 2026	("human papillomavirus" OR HPV OR "HPV DNA" OR "HPV genotype") AND ("oral potentially malignant disorder*" OR "oral premalignant lesion*" OR "oral leukoplakia" OR "oral lichen planus" OR "oral epithelial dysplasia" OR "oral submucous fibrosis" OR erythroplakia)	Title, abstract, and keyword	January 2019 to June 3, 2026; variations enabled; language verified during screening	3
Total before duplicate removal	—	—	—	—	558

Counts correspond to records incorporated on June 3, 2026 and may change if searches are rerun because of updates in platform indexing.

For Google Scholar, 200 refers to the first 200 results screened in relevance order, not the total number of results displayed by the platform.

The direct restriction to English or Spanish was applied in PubMed. For sources that did not permit an equivalent reproducible filter, language was verified during screening.

The total of 558 corresponds to the four electronic sources before removal of 164 duplicates and matches Fig. 1. Reference-list screening was supplementary and did not alter this documented total.

Online Resource 2. JBI critical appraisal by study and domain

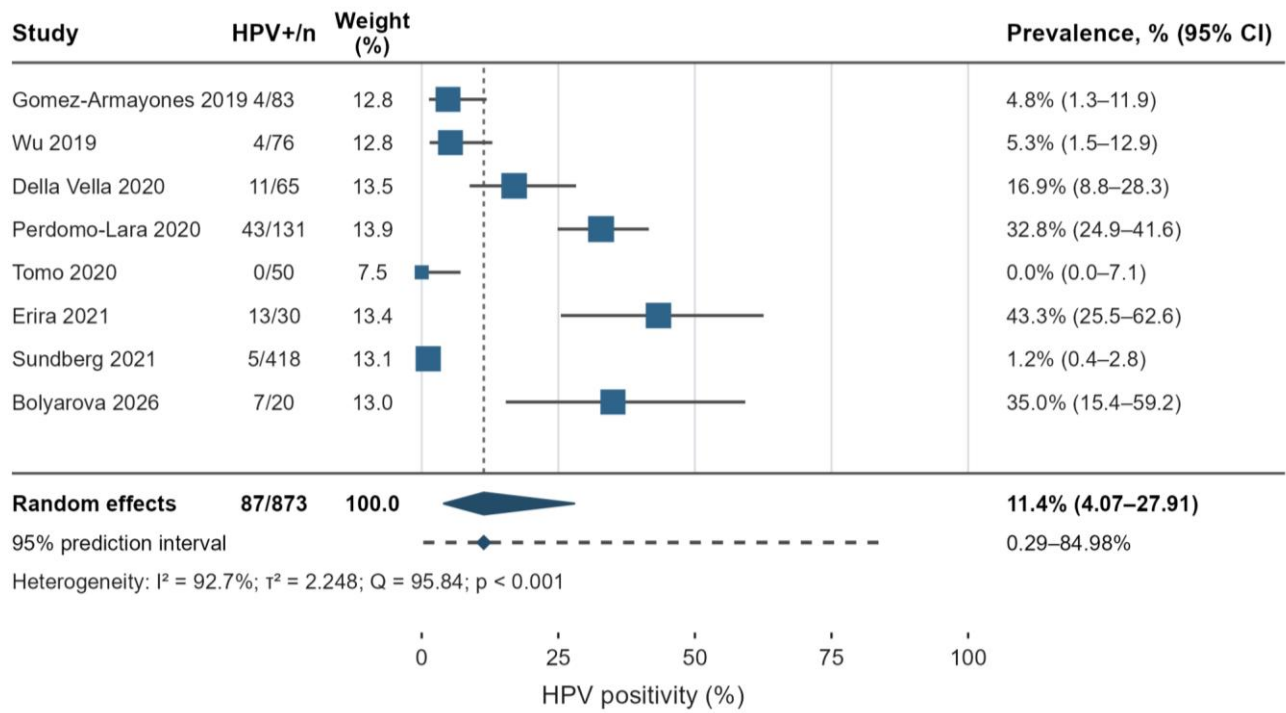
Authors and year	Analytical use	Decision after JBI appraisal	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
Della Vella et al., 2020	Primary meta-analysis	Include	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gomez-Armayones et al., 2019	Primary meta-analysis	Include	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
Mosmann et al., 2019	Primary meta-analysis	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Unclear
Sundberg et al., 2019	Non-pooled quantitative	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Unclear
Wu et al., 2019	Primary meta-analysis	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Farhadi et al., 2020	Non-pooled quantitative	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Hendawi et al., 2020	Non-pooled quantitative	Include	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
Perdomo-Lara et al., 2020	Primary meta-analysis	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sundberg et al., 2021	Primary meta-analysis	Include	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tomo et al., 2020	Primary meta-analysis	Include	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear
Buenahora et al., 2021	Narrative	Include	Yes	Unclear	No	Yes	Unclear	Yes	Yes	Yes	Unclear
Erija et al., 2021	Primary meta-analysis	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Ghosh et al., 2021	Non-pooled quantitative	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Unclear
Kaewmaneeuan et al., 2021	Non-pooled quantitative	Include	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Runow Stark et al., 2021	Non-pooled quantitative	Include	Unclear	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear
Sivakumar et al., 2021	Non-pooled quantitative	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Rathore et al., 2022	Non-pooled quantitative	Seek further information	Unclear	Unclear	No	Unclear	No	Unclear	Unclear	Unclear	Unclear
Vijayan et al., 2022	Non-pooled quantitative	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Fantozzi et al., 2024	Non-pooled quantitative	Include	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
Gilligan et al., 2023	Primary meta-analysis	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Mohammadi et al., 2023	Non-pooled quantitative	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Petrović et al., 2023	Non-pooled quantitative	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Parchami et al., 2024	Non-pooled quantitative	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Buttà et al., 2025	Non-pooled quantitative	Include	Unclear	Unclear	No	Yes	Unclear	Yes	Yes	Yes	Unclear
Duś-Ilnicka et al., 2025	Narrative	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Bolyarova et al., 2026	Primary meta-analysis	Include	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Unclear
Elsahn et al., 2026	Non-pooled quantitative	Include	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Unclear

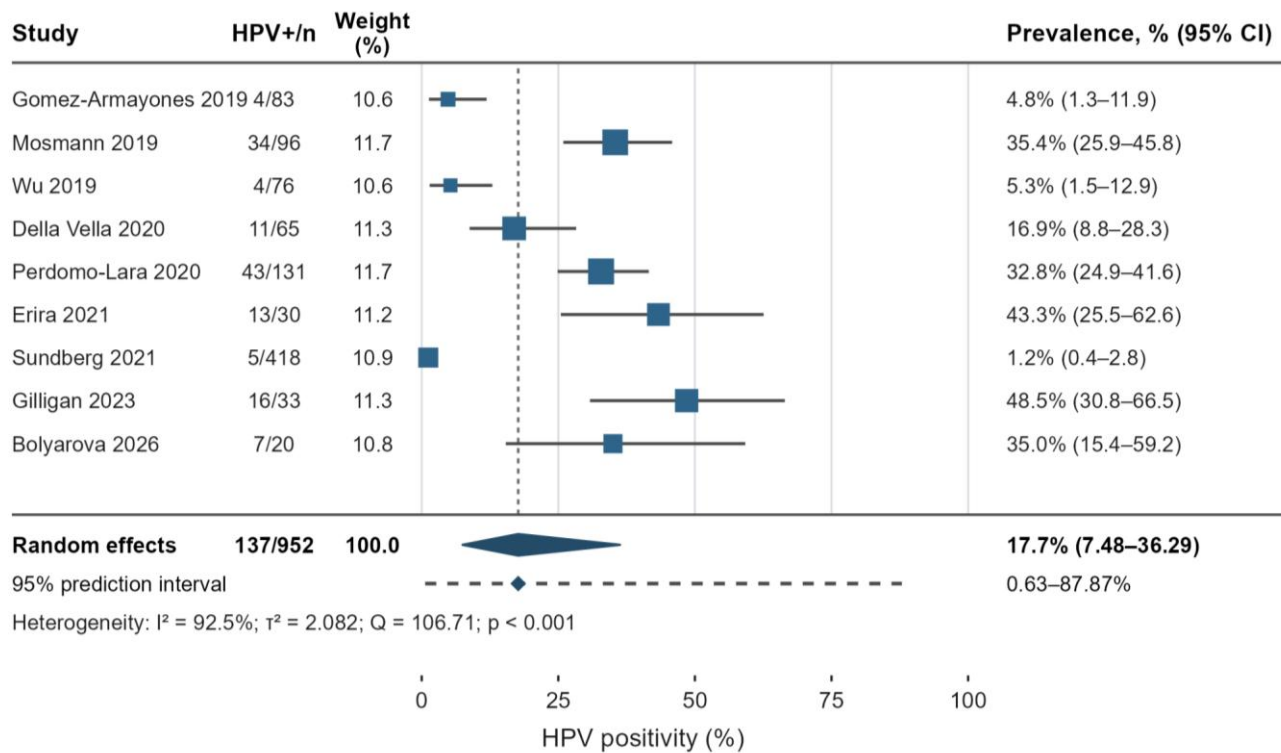
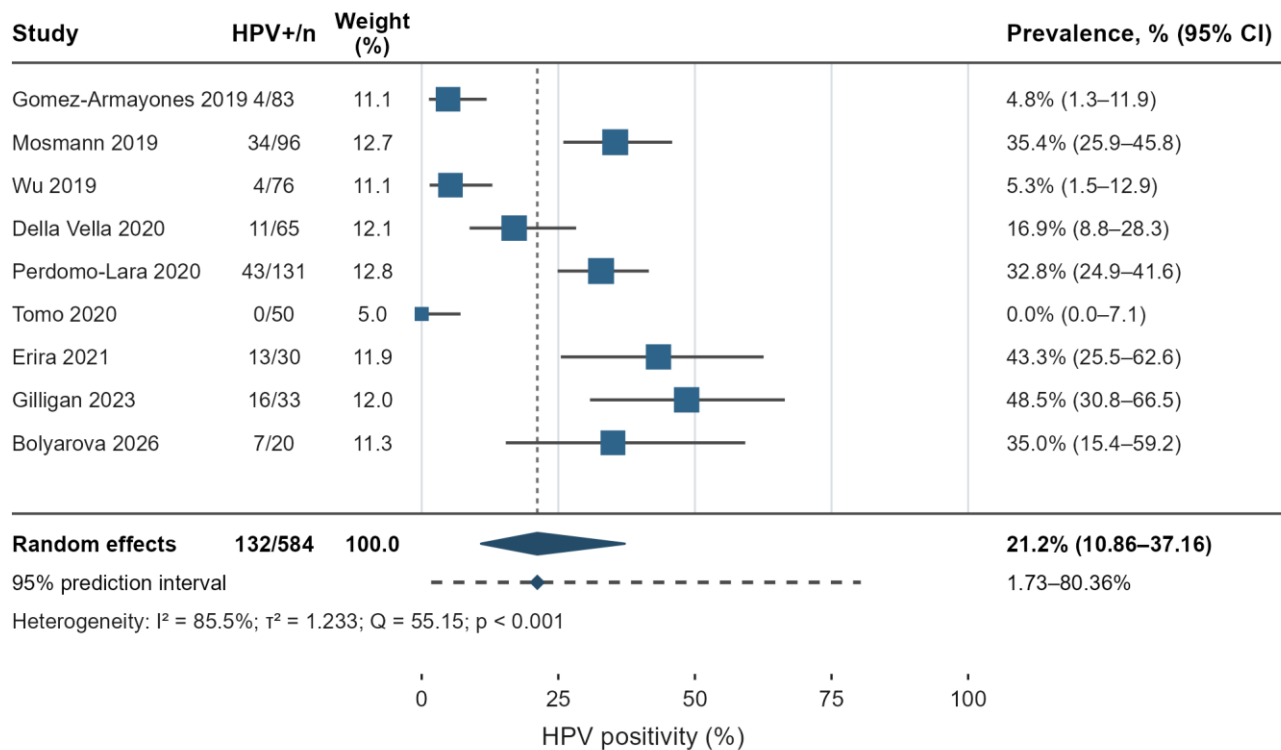
Q1: appropriate sampling frame; Q2: appropriate participant selection; Q3: adequate sample size; Q4: participants and setting described; Q5: sufficient sample coverage.

Q6: valid method for identifying the condition; Q7: standardized and reliable measurement; Q8: appropriate statistical analysis; Q9: adequate response rate or appropriate management of a low response.

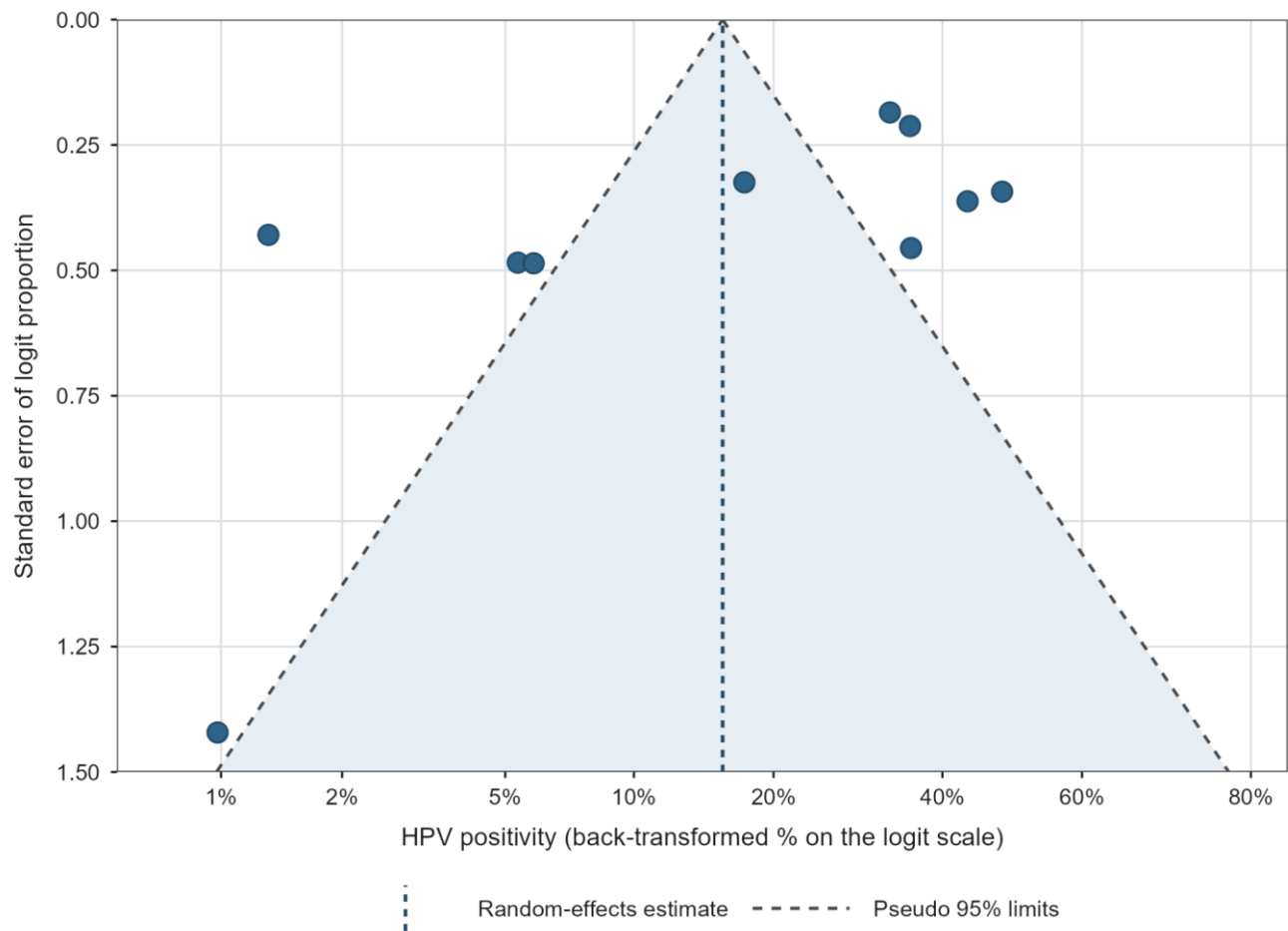
Responses: Yes, No, Unclear, or Not applicable. Post-appraisal decision: Include, Exclude, or Seek further information. This decision is not an overall risk-of-bias classification; limitations remain visible by domain.

No total score was calculated and no cutoffs were applied for low, moderate, or high risk. "Include" does not imply low risk; limitations in selection and precision remain visible in Q1–Q3.

Online Resource 3. Forest plot restricted to tissue or biopsy specimens

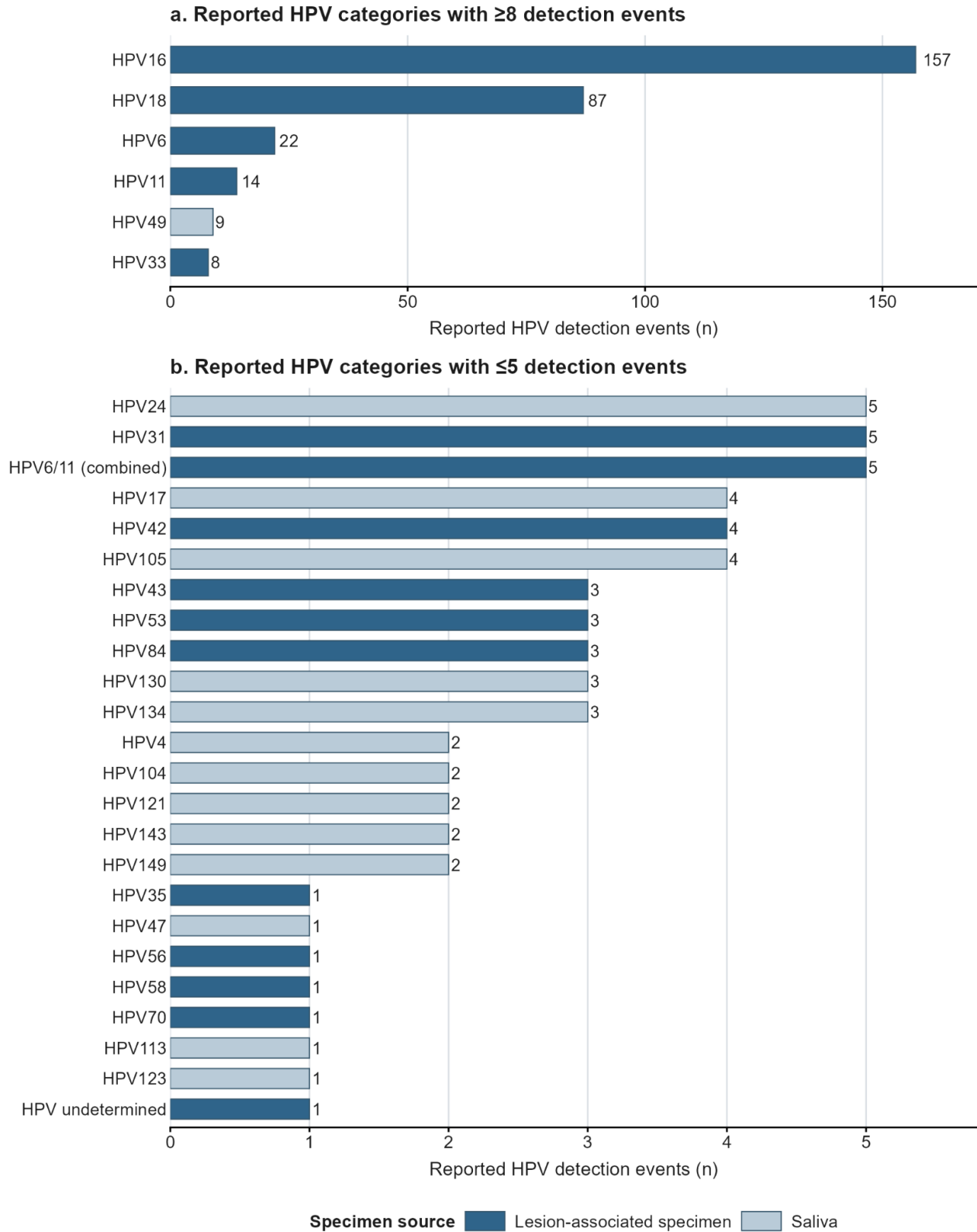
Online Resource 4. Forest plot excluding the zero-event study**Online Resource 5.** Forest plot after exclusion of the largest study

The analysis included 9 studies, 584 evaluable units, and 132 positive units. Pooled positivity was 21.2% (95% CI 10.86–37.16), with a 95% prediction interval of 1.73–80.36% and $I^2 = 85.5\%$ ($\tau^2 = 1.233$).

Online Resource 6. Exploratory funnel plot

The plot includes the ten studies in the primary analysis. Visual asymmetry may reflect heterogeneity, sample size, and the relationship between a proportion and its standard error; it was not interpreted as evidence of publication bias.

Online Resource 7. Reported HPV categories: 357 events in 30 categories, including 316 (88.5%) from lesion-associated specimens and 41 (11.5%) from saliva. HPV16 and HPV18 accounted for 244 events (68.3%). No category had six or seven events. Counts depend on panel composition, allow codetection, and do not represent unique patients or genotype-specific prevalence.



Online Resource 8. HPV categories and reported detection events by study

Authors and year	Country	OPMD	Specimen	Method	Specimen source	Reported HPV categories	Reported detection events, n
Eirira et al., 2021	Colombia	Oral leukoplakia with dysplasia	FFPE biopsy	Conventional HPV16 E7 PCR	Lesion-associated	HPV16 (n=6)	6
Farhadi et al., 2020	Iran	Oral lichen planus	FFPE biopsy	Multiplex/genotype-specific PCR	Lesion-associated	HPV33 (n=7); HPV18 (n=1)	8
Sundberg et al., 2021	Sweden; Brazil; Romania	Oral leukoplakia	FFPE biopsy	TaqMan real-time PCR	Lesion-associated	HPV11 (n=1); HPV16 (n=2); HPV31 (n=1); HPV33 (n=1)	5
Gomez-Armayones et al., 2019	Spain	Oral lichen planus and oral dysplasia	FFPE biopsy	SPF10 PCR-DEIA/LiPA25	Lesion-associated	HPV16 (n=2); HPV18 (n=1); HPV undetermined (n=1)	4
Bolyarova et al., 2026	Bulgaria	Mixed OPMDs	Punch biopsy	Nested PCR + hybridization/chip	Lesion-associated	HPV6/11 (combined) (n=5); HPV42 (n=1); HPV43 (n=2); HPV56 (n=1)	9
Duś-Ilnicka et al., 2025	Poland	Oral lichen planus	Saliva	E7-MPG multiplex PCR + Luminex	Saliva	HPV17 (n=4); HPV24 (n=5); HPV47 (n=1); HPV49 (n=9); HPV104 (n=2); HPV105 (n=4); HPV113 (n=1); HPV143 (n=2); HPV4 (n=2); HPV121 (n=2); HPV123 (n=1); HPV130 (n=3); HPV134 (n=3); HPV149 (n=2)	41
Mosmann et al., 2019	Argentina	Mixed OPMDs	Lesion-directed swab	PCR MY09/MY11 + RFLP	Lesion-associated	HPV6 (n=9); HPV11 (n=8); HPV84 (n=2); HPV16 (n=6); HPV31 (n=3); HPV53 (n=2)	30
Elsahn et al., 2026	United Arab Emirates	Oral leukoplakia, oral submucous fibrosis, and oral lichen planus	Fresh tissue biopsy	HPV16 E6-specific PCR	Lesion-associated	HPV16 (n=11)	11
Della Vella et al., 2020	Italy	Oral leukoplakia	Lesional micro biopsy	RT-PCR Anyplex II HPV28	Lesion-associated	HPV6 (n=8); HPV11 (n=2); HPV16 (n=2); HPV35 (n=1); HPV42 (n=3); HPV43 (n=1); HPV53 (n=1)	18
Fantozzi et al., 2024	Italy	Histopathologically eligible OPMDs	Lesion-directed brushing	Real-time PCR/genotype-specific rtPCR	Lesion-associated	HPV16 (n=5); HPV18 (n=1); HPV58 (n=1); HPV6 (n=3); HPV11 (n=1); HPV70 (n=1)	12
Kaewmaneeuan et al., 2021	Thailand	Oral leukoplakia and oral lichen planus	FFPE biopsy	Qualitative HPV16/18 E6 PCR	Lesion-associated	HPV16 (n=3); HPV18 (n=30)	33
Gilligan et al., 2023	Argentina	Oral leukoplakia and proliferative verrucous leukoplakia	Lesion-directed brushing	PCR-RFLP	Lesion-associated	HPV16 (n=6); HPV31 (n=1); HPV6 (n=2); HPV11 (n=2); HPV84 (n=1)	12
Perdomo-Lara et al., 2020	Colombia	Oral epithelial dysplasia	FFPE biopsy	qPCR GP5+/GP6+ + Luminex xMAP	Lesion-associated	HPV16 (n=27); HPV18 (n=12)	39
Vijayan et al., 2022	India	Oral lichen planus	Biopsy/fresh tissue	Conventional HPV16/18 E6 PCR	Lesion-associated	HPV16 (n=64); HPV18 (n=39)	103
Mohammadi et al., 2023	Iran	Oral lichen planus	FFPE biopsy	Conventional HPV16/18 PCR	Lesion-associated	HPV16 (n=12); HPV18 (n=3)	15
Ghosh et al., 2021	Nepal	Oral epithelial dysplasia, oral lichen planus, and oral submucous fibrosis	FFPE biopsy	Conventional HPV16/18 PCR	Lesion-associated	HPV16 (n=2)	2
Sivakumar et al., 2021	India	Oral leukoplakia	Lesion-directed cytobrush	HPV16 E6-specific PCR	Lesion-associated	HPV16 (n=5)	5
Wu et al., 2019	China	Oral leukoplakia	FFPE biopsy	Reverse dot blot + HPV16 PCR	Lesion-associated	HPV16 (n=4)	4

HPV, human papillomavirus; OPMD, oral potentially malignant disorder; FFPE, formalin-fixed, paraffin-embedded tissue; PCR, polymerase chain reaction; qPCR, quantitative PCR.

Counts correspond to reported detection events rather than unique patients. Coinfections may contribute more than one event per participant.

A total of 357 events in 30 categories were recorded: 316 from lesion-associated specimens and 41 from saliva; these totals match Online Resource 7. Combined or undetermined categories were retained as reported and were not separated or reassigned artificially. Studies without separable category counts were not included in this table.

Online Resource 9. Biomarkers, viral/tissue evidence, and epigenetic findings

Authors and year	Country	Population or lesion	Indirect or complementary biomarkers	Direct viral or tissue evidence	Epigenetic findings	Analytical interpretation
Hendawi et al., 2020	United Kingdom	Severe oral epithelial dysplasia (n=33)	p16 by IHC, any staining: 11/33 (33.3%)	HPV16/18 E6/E7 RNA by RNA-ISH: 6/33 (18.2%); E4 and L1 by IHC: 4/6 (66.7%) each among RNA-ISH-positive cases	—	RNA-ISH demonstrates viral transcription. E4/L1 support productive infection; p16 alone did not predict HPV status. Narrative synthesis; denominators were not pooled. p16 was assessed only in the four HPV DNA-positive cases; it does not estimate prevalence or confirm viral activity.
Gomez-Armayones et al., 2019	Spain	Oral lichen planus/dysplasia cases with positive HPV DNA (n=4)	High p16 expression: 2/4 (50.0%)	—	—	The results have different source populations. Only the case with >75% p16 positivity was also HPV16 positive; the two proportions were not combined.
Fantozzi et al., 2024	Italy	Oral mucosal diseases (n=99); histopathologically eligible OPMDs (n=59)	Any p16 positivity: 7/99 (7.1%); >75% positivity: 1/59 (1.7%)	—	—	All 50 cases were HPV DNA negative; this finding shows that isolated p16 is not a valid surrogate for HPV infection in this cohort.
Tomo et al., 2020	Brazil	Oral leukoplakia (n=50)	High p16 expression (score 2): 20/50 (40.0%)	—	—	PCR–p16 concordance was low. The combined definition is a sensitivity analysis and does not replace the primary PCR result (16/33).
Gilligan et al., 2023	Argentina	Oral leukoplakia/proliferative verrucous leukoplakia (n=33)	p16 grade III: 9/33 (27.3%); combined PCR+p16 definition: 5/33 (15.2%)	—	—	CISH localizes viral DNA and ChIP-PCR provides molecular detection, but neither assay quantifies transcripts. The 3/9 result was not extrapolated to the 54 cases or added to 5/54.
Parchami et al., 2024	Iran	Oral epithelial dysplasia (n=54); ChIP-PCR subset (n=9)	p16 score 1–2: 38/54 (70.4%); diffuse p16, score 2: 5/54 (9.3%)	CISH for HPV16/18 or HPV31/33: 5/54 (9.3%); high-risk HPV ChIP-PCR: 3/9 (33.3%) in a selected subset	—	The units are genomic regions, not patients. RRBS was performed in 12 selected specimens; region counts are neither a clinical proportion nor a prevalence estimate.
Buenahora et al., 2021	Colombia	Oral lesions/FFPE OPMDs (p16, n=41); RRBS subset (n=12; four per group)	p16 by IHC: 29/41 (70.7%)	—	270 differentially methylated regions: 142 hypermethylated and 128 hypomethylated; alterations in NOTCH1/NICD, interferon- γ , IL-6, and antiviral/innate-response pathways FAM19A4 and/or miR124-2	Exploratory oral-rinse result using cutoffs derived from cervical specimens. It does not demonstrate HPV dependence and was not incorporated into the prevalence meta-analysis.
Buttà et al., 2025	Italy	OPMDs assessed using oral rinse (n=17)	—	—	hypermethylation: 6/17 (35.3%); HPV positive: 2/7 (28.6%); HPV negative: 4/10 (40.0%)	p16 and Ki-67 are indirect markers; Ki-67 reflects proliferation. Absence of L1 does not exclude viral DNA or prove absence of a non-productive infection.
Sivakumar et al., 2021	India	Oral leukoplakia (n=25)	p16 by IHC: 4/25 (16.0%); high Ki-67 (>50%): 6/25 (24.0%)	HPV16 L1 protein by IHC: 0/25 (0.0%)	—	Coexistence of p16 and HPV16 in four cases is descriptive and does not establish causality. p16 was analyzed as a transformation biomarker rather than an HPV outcome.
Wu et al., 2019	China	Baseline oral leukoplakia (n=76); HPV16-positive cases (n=4)	p16 in the baseline specimen: 29/76 (38.2%); p16 among HPV16-positive cases: 4/4 (100%)	—	—	

HPV, human papillomavirus; OPMD, oral potentially malignant disorder; IHC, immunohistochemistry; RNA-ISH, RNA in situ hybridization; CISH, chromogenic in situ hybridization; ChIP-PCR, chromatin immunoprecipitation followed by PCR; RRBS, reduced representation bisulfite sequencing; DMR, differentially methylated region; FFPE, formalin-fixed, paraffin-embedded tissue.

p16 and Ki-67 are indirect or complementary biomarkers and were not considered direct HPV tests. RNA-ISH for E6/E7 provides direct evidence of transcription; CISH localizes viral DNA; ChIP-PCR provides molecular detection; and E4 and L1 support productive infection.

Denominators correspond to the populations or subsets actually assessed. Results from different tests were not summed and subsets were not extrapolated; therefore, no combined estimate was calculated.

DMR counts represent genomic regions rather than participants. Methylation findings are exploratory and are presented as a narrative synthesis without causal or prognostic inference.