HPV

01

HPV screening

Screening methods, Pap smear interpretation and molecular test discrepancies

DNA / RNA testing • Genotyping • Pap smear

Why do false results occur?

False positive

A positive result when clinically significant HPV-related disease is not present.

False negative

A negative result despite infection or a lesion that the test should have detected.

Why it matters

Sampling, biology and technical factors can shift the balance between sensitivity and specificity.

Molecular testing: the trade-off

Molecular testing can reduce false negatives, but may slightly increase false positives.

↓ False negatives

↑ False positives

↔ Clinical balance

Pap smear result categories

Normal

Negative cytology

ASC-US / ASC-H

Borderline or concerning squamous atypia

LSIL / HSIL

Low- or high-grade squamous lesions

Pap Smear Result
Normal (Negative)
ASC-US (Atypical Squamous Cells of Undetermined Significance)
ASC-H (Atypical Squamous Cells – Cannot Exclude High-Grade Squamous Intraepithelial Lesion)
LSIL (Low-Grade Squamous Intraepithelial Lesion)
HSIL (High-Grade Squamous Intraepithelial Lesion)
SCC or Adenocarcinoma (Squamous Cell Carcinoma or Adenocarcinoma)
Endocervical Cells Present
Unsatisfactory (Inadequate Sample)

ASC / SIL ratio

1.5

Median reported ratio in the USA

≤ 3:1

Suggested ceiling in high-risk populations

Interpretation

A higher ratio may suggest overuse of the ASC category.

hrHPV positivity in ASC-US

50.6%

Experienced pathologists

40–50%

General practice

How reproducible is the Pap smear?

Agreement is stronger for well-defined abnormalities such as HSIL or CIN 2/3, and lower for mild or borderline categories such as ASC-US or LSIL.

κ 0.40–0.63

Inter-observer variability

κ 0.63–0.74

Intra-observer variability

Clinical implication

Borderline cytology is more vulnerable to interpretation differences.

Can a Pap smear miss glandular lesions?

Yes. Pap smears are less sensitive for adenocarcinoma or AIS than for squamous lesions.

~70–80%

Sensitivity for squamous lesions

~30–50%

Sensitivity for glandular lesions

> **Take-home point**
>
> A negative Pap does not completely exclude glandular disease.

Pap smear mimics of HSIL

- **Endometrial carcinoma**
- **Endocervical AIS or adenocarcinoma**
- **Metastatic cancers to the cervix (colon, breast, ovary)**
- **Small cell neuroendocrine carcinoma**
- **Ovarian or fallopian tube serous carcinoma**
- **Lymphoma / leukemia involving the cervix**

HPV molecular assays

Hybrid Capture 2 HPV DNA

2001 • Qiagen / Hilden, Germany

Cervista HPV HR

2009 • Hologic / Marlborough, Massachusetts

Cobas 4800 HPV

2011 • Roche / Basel, Switzerland

Aptima HPV assay

2011 • Gen-Probe; acquired by Hologic in 2012

BD Onclarity HPV

2018 • Becton Dickinson / Franklin Lakes, New Jersey

Alinity m HR HPV

2023 • Abbott Molecular / Alinity m System

False results in molecular HPV testing

Where biology, sampling and laboratory factors can change the result.

Causes of false-positive HPV results

Sample contamination during collection or laboratory processing

Contamination from equipment, other samples or the clinical environment

Transient infection detected during natural viral clearance

Cross-reactivity with non–high-risk HPV types or similar DNA sequences

Laboratory or clerical error: mislabeling, reporting issues or specimen mix-ups

False-positive results: residual or low-level DNA

Residual / latent HPV DNA

HPV DNA may persist at low levels after active infection has cleared, producing a positive result without active disease.

Low viral load

A test may amplify very small amounts of HPV DNA near its detection limit that are not clinically significant.

Interpret the molecular result in clinical and cytologic context.

Causes of false-negative HPV results

- Low viral load, especially in early infection
- Poor sample collection
- Insufficient cells, sampling outside the transformation zone, blood or mucus
- HPV infection deeper in tissue or viral integration
- HPV clearance in progress with waning viral load

False negatives: sampling & technical factors

- Sampling error when lesions are high in the endocervical canal
- PCR inhibitors, DNA degradation or transport problems
- Reagent failure or improper specimen storage
- Mutations in the HPV target gene region
- Atrophic cervix

DISCREPANCY

When Pap and HPV results disagree

Pap negative + HPV positive

HPV may be present before cytologic abnormalities appear.

Pap abnormal + HPV negative

Cell changes may be unrelated to the HPV types included in the test, or HPV may have cleared.

Why can Pap and molecular testing disagree?

Different samples or sampling sites for cytology and HPV testing

HPV infection is present, but cytologic abnormality has not developed yet

Low viral load during clearing, transient infection or early infection

Sampling error or insufficient sample

Cell changes not caused by HPV

HPV types not included in the assay (the source notes 14 high-risk types)

Pap changes may reflect low-risk HPV types or rare high-risk types not included

A key biological explanation

HPV often clears within 6–24 months.

1 Infection

HPV is detectable.

2 Clearance

Viral load falls as the infection clears.

3 Residual changes

Reactive cytologic changes may remain visible on Pap.

Both cytology and molecular testing can also produce false-positive or false-negative results because of technical or human factors.