Test Definition: HPV

HPV with Genotyping, PCR, ThinPrep

Overview

Useful For

Detection of high-risk (HR) genotypes associated with the development of cervical cancer

Aids in triaging women with abnormal Pap smear results

Individual genotyping of human papillomavirus (HPV)-16 and/or HPV-18, if present

Results of HPV-16 and HPV-18 genotyping can aid in triaging women with positive HR-HPV but negative Pap smear results

This test is **not recommended for** evaluation of suspected sexual abuse.

This test is **not intended for** use in determining the need for treatment (ie, excisional or ablative treatment of the cervix) in the absence of high-grade cervical dysplasia. Patients who are HPV16/18 positive should be monitored carefully for the development of high-grade cervical dysplasia according to current practice guidelines.

This test is **not intended for** women who have undergone hysterectomy.

This test is **not intended for** use with samples other than those collected by a clinician using an endocervical brush or spatula and placed in the ThinPrep Pap test PreservCyt solution.

Method Name

Real-Time Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type
Varies

Necessary Information
Specimen source is required.

Specimen Required
Submit only 1 of the following specimens:

Specimen Type: Cervical (endocervical or ectocervical)

Container/Tube: ThinPrep/PreservCyt solution vial

Specimen Volume: 3 mL of solution in ThinPrep/PreservCyt vial

Collection Instructions:

1. Bag ThinPrep specimens individually as they have a tendency to leak during transport.

2. Place labels on the vial and on the bag.

Specimen Type: Vaginal

Container/Tube: ThinPrep/PreservCyt solution vial

Specimen Volume: 3 mL of solution in ThinPrep/PreservCyt vial

Collection Instructions:

1. Bag ThinPrep specimens individually as they have a tendency to leak during transport.

2. Place labels on the vial and on the bag.

Additional Information: This assay is validated but **not FDA-approved** for vaginal source specimens.

Forms
If not ordering electronically, complete, print, and send Microbiology Test Request (T244) with the specimen.

Reject Due To

Specimen containing CytoRich Red preservative fluid	Reject

Specimen Minimum Volume

1 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)	42 days	
	Refrigerated	42 days	

Clinical & Interpretive

Clinical Information

Persistent infection with human papillomavirus (HPV) is the principal cause of cervical cancer. The presence of HPV has been implicated in more than 99% of cervical cancers worldwide, including both cervical squamous cell carcinoma and cervical adenocarcinoma. Before the development of invasive cancer, HPV infects the squamous mucosa cells and/or the glandular cells of the endocervix, leading to clonal expansion and morphologic changes. While the HPV-infected cells are restricted to their normal anatomic location, these changes are classified as cervical intraepithelial neoplasia (CIN). The severity of the morphologic changes and the degree to which those changes resemble the morphology of an invasive carcinoma are used to "grade" CIN. In general, high-grade CIN more closely resembles invasive carcinoma morphologically. HPV can also infect other mucosal cells in the anogenital region, such as the vaginal mucosa, leading to the development of HPV-associated intraepithelial neoplasia as well as invasive carcinoma not involving the cervix itself, although this is less common.

HPV is a small, nonenveloped, double-stranded DNA virus, with a genome of approximately 8,000 nucleotides. There are more than 118 different types of HPV and approximately 40 different HPVs can infect the human anogenital mucosa. Only a very small percentage of patients who are exposed to HPV will develop CIN. Of those patients who develop CIN, only a small percentage will progress to invasive cervical cancer. Sexually transmitted infection with HPV is extremely common, with estimates of up to 75% of all women being exposed to HPV at some point. However, almost all infected women will mount an effective immune response and clear the infection within 2 years without any long-term health consequences. Both high-risk HPV genotypes (especially HPV-16 and 18), as well as persistent HPV infection (eg, an infection that is not cleared by the patient's immune system over time), are associated with an increased chance of progressing to high-grade CIN and invasive cancer.

Data suggest that certain HPV genotypes types (eg, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are considered high-risk (HR) for the development of cervical cancer and its precursor lesions. Furthermore, HPV types

16 and 18 have been regarded as the genotypes most closely associated with progression to cervical cancer. HPV-16 is the most carcinogenic, and is associated with approximately 60% of all cervical cancers, while HPV-18 accounts for approximately 10% to 15% of cervical cancers.(1-3)

In developed countries with cervical cancer screening programs, the Pap smear has been used since the mid-1950s as the primary tool to morphologically detect CIN, the precursor to cervical cancer. Pap smear screening has decreased death rates due to cervical cancer dramatically, since in many cases CIN can be treated and eliminated (eg, by local excision) before it progresses to invasive carcinoma. Although Pap smears and other liquid-based cytology methods have many advantages, they also have limitations: they require subjective interpretation by a highly trained cytopathologist and misinterpretation can occur, morphologic changes that resemble HIV-associated CIN can be caused by other conditions (eg, inflammation), and Pap smear does not sample every cell within the cervix/anogenital region potentially leading to falsely negative results. Perhaps most importantly, Pap smear does not differentiate between HPV genotypes that are high or low risk for progression to cervical cancer and it does not detect very early infections, which may lack a morphological phenotype.

Nucleic acid (DNA) testing by polymerase chain reaction (PCR) has become a standard, noninvasive method for determining the presence of a cervical HPV infection. Proper implementation of nucleic acid testing for HPV may:

1. Increase the sensitivity of cervical cancer screening programs by detecting high-risk lesions earlier in women 30 years and older with normal cytology

2. Reduce the need for unnecessary colposcopy and treatment in patients 21 and older with cytology results showing atypical squamous cells of undetermined significance (ASC-US)

Recently, data suggest that individual genotyping for HPV types 16 and 18 can assist in determining appropriate follow-up testing and triaging women at risk for progression to cervical cancer. Studies have shown that the absolute risk of CIN-2 or worse in HPV-16 and/or HPV-18 positive women is 11.4% (95% confidence interval [CI] 8.4%-14.8%) compared with 6.1% (95% CI, 4.9%-7.2%) of women positive for other HR-HPV genotypes, and 0.8% (95% CI, 0.3%-1.5%) in HR-HPV-negative women.(4) Based in part on these data, the American Society for Colposcopy and Cervical Pathology (ASCCP) now recommends that HPV 16/18 genotyping be performed on women who are positive for HR-HPV but negative by routine cytology. Women who are found to be positive for HPV-16 and/or -18 may be referred to colposcopy, while women who are negative for genotypes 16 and 18 may have repeat cytology and HR-HPV testing in 12 months.(1)

Reference Values

Negative for human papillomavirus (HPV) genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68

Interpretation

A positive result indicates the presence of human papillomavirus (HPV) DNA due to 1 or more of the following

genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

A negative result indicates the absence of HPV DNA of the targeted genotypes.

For patients with atypical squamous cells of undetermined significance (ASC-US) Pap smear result and who are positive for high-risk (HR) HPV, consider referral for colposcopy, if clinically indicated.

For women aged 30 years and older with a negative Pap smear result but who are positive for HPV-16 and/or HPV-18, consider referral for colposcopy, if clinically indicated.

For women aged 30 years and older with a negative Pap smear, positive-HR-HPV test result, but who are negative for HPV-16 and HPV-18, consider repeat testing by both cytology and a HR-HPV test in 12 months.

Cautions

The cobas human papillomavirus (HPV) test is FDA-approved for cervical and endocervical samples collected in PreservCyt (ThinPrep) media. Other sample types (eg, vaginal) are not considered FDA-approved sources; however, verification studies have been completed by Mayo Clinic Laboratories and Mayo Clinic in compliance with CLIA regulations.

The cobas HPV test detects DNA of the high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. This test does not detect DNA of HPV low-risk types (eg, 6, 11, 42, 43, 44) since these are not associated with cervical cancer and its precursor lesions.

Prevalence of HPV infection in a population may affect performance. Positive-predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.

Infection with HPV is not an indicator of cytologic high-grade squamous intraepithelial lesion (HSIL) or underlying high-grade cervical intraepithelial neoplasia (CIN), nor does it imply that CIN2-3 or cancer will develop. Most women infected with 1 or more high-risk (HR) HPV types do not develop CIN2-3 or cancer.

A negative-HR-HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.

Cervical specimens often show visibly detectable levels of whole blood as a pink or light brown coloration. These specimens are processed normally on the cobas 4800 System. If concentrations of whole blood exceed 1.5% (dark-red or brown coloration) in PreservCyt solution, there is a likelihood of obtaining a false-negative result.

The cobas HPV Test performance has not been validated with PreservCyt specimens that have been treated with glacial acetic acid for removal of red blood cells. Any such processing of PreservCyt specimens prior to HPV testing would invalidate the cobas HPV Test results.

The cobas HPV Test performance has not been validated with PreservCyt specimens that have been filled past the maximum fill line of the primary vial. ThinPrep vials that have had any additional PreservCyt fluid volume added or any dissimilar fluid volume added to the initial specimen should not be submitted for testing.

The presence of polymerase chain reaction (PCR) inhibitors may cause false-negative or invalid results.

HPV-negative cancers of the cervix do occur in rare circumstances. Also, no cancer screening test is 100% sensitive. Use of this device for primary cervical cancer screening should be undertaken after carefully considering the performance characteristics put forth in the cobas HPV Test label, as well as recommendations of professional guidelines.

The use of this test has not been evaluated for the management of women with prior ablative or excisional therapy, hysterectomy, who are pregnant or who have other risk factors (eg, HIV+, immunocompromised, history of sexually transmitted infections).

The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc, and specimen collection variables have not been evaluated.

Supportive Data

Accuracy:

To assess the accuracy of the Roche cobas human papillomavirus (HPV) test, prospectively collected

cervical/endocervical samples (n=753) in ThinPrep media were tested by both the Digene hc2 (Qiagen) and Roche cobas HPV tests.

Table 1. Comparison of the Roche cobas 4800 HPV assay and the Digene hc2 using prospectively collected endocervical/cervical samples in ThinPrep media (n=753)

		Digene hc2		
		Positive	**Negative**	**Total**
Roche cobas 4800	**Positive**	353	26(a)	379
	Negative	42(b)	332	374
	Total	395	358	753

(88.7-92.8%)

a. When tested by a third FDA-approved high-risk (HR)-HPV assay, 4 of these samples resulted positive and 22 resulted negative.

b. When tested by a third FDA-approved HR-HPV assay, 13 of these samples resulted positive and 29 resulted negative.

In addition to comparing the accuracy data above, the Roche cobas HPV assay was also compared to the results of colposcopy (tissue biopsy) (n=350), with a clinical endpoint of cervical intraepithelial neoplasia (CIN)2 or worse being considered positive. The results are summarized below in Table 2.

Table 2. Comparison of the Roche cobas 4800 HPV test to cervical biopsy among 350 samples demonstrating atypical squamous cells of undetermined significance (ASC-US) or worse by cytology (Pap smear).

		Tissue diagnosis > or =CIN2		
		Positive	**Negative**	**Total**
Roche cobas 4800	**Positive**	74	185	259
	Negative	7	84	91
	Total	81	269	350

Specificity=31.2%

In comparison, the current Digene hc2 assay demonstrated a sensitivity of 97.5% (79/81) and specificity of 27.1% (73/269) compared to a colposcopy endpoint of > or =CIN2.

Finally, the results of the Roche cobas HPV-16/18 genotype test were compared to a tissue diagnosis of > or =CIN2.

Table 3. Comparison of the Roche cobas 4800 HPV 16/18 genotype test to cervical biopsy among 350 samples determined to be ASC-US by cytology (Pap smear).

		Tissue diagnosis > or =CIN2*		
		Positive	**Negative**	**Total**
Roche cobas 4800 16/18	**Positive**	42(a)	36(c)	78
	Negative	39(b)	233	272
	Total	81	269	350

Specificity=86.6%

a. 41 of these specimens were also positive by GenProbe APTIMA for HPV mRNA (not genotyped)

b. 32 specimens were Roche positive for HPV types other than 16/18. 33 were also positive by GenProbe APTIMA for HPV, not otherwise specified (NOS).

c. 31 were positive by GenProbe APTIMA for HPV, NOS.

Reference Range:

From the 30 years of age and over cytology (Pap) and HPV DNA cotesting population, cervical/endocervical (n=30) and vaginal (n=28) samples collected in ThinPrep media for routine HPV screening were tested.

58 out of 58 (100%) cervical/endocervical and vaginal samples tested had negative Pap results, negative Roche cobas HPV 4800 results, and negative Digene hc2 results.

The reference range for the Roche cobas HPV test is negative.

Clinical Reference

1. Saslow D, Solomon D, Lawson HW, et al: American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. J Low Genit Tract Dis. 2012;16(3):175-204. doi: 10.1097/LGT.0b013e31824ca9d5.

2. Walboomers JM, Jacobs MV, Manos MM, et al: Human papillomavirus is a necessary cause of invasive cervical cancer

worldwide. J Pathol. 1999;189:12-19. doi: 10.1002/(SICI)1096-9896(199909)189:1

3. de Sanjose S, Quint WG, Alemany L, et al: Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11:1048-1056. doi: 10.1016/S1470-2045(10)70230-8.

4. Wright TC Jr, Stoler MH, Sharma A, et al: Evaluation of HPV-16 and HPV-18 genotyping for the triage of women with high-risk HPV positive, cytology-negative results. Am J Clin Pathol. 2011 Oct;136(4):578-586. doi: 10.1309/AJCPTUS5EXAS6DKZ.

Performance

Method Description

The cobas human papillomavirus (HPV) test targets and detects nucleic acid from the L1 region of the HPV genome using real-time polymerase chain reaction (PCR) technology. The cobas HPV test is used for the in vitro qualitative detection of 14 high-risk HPV types commonly associated with cervical cancer. The assay is able to specifically assess for the presence or absence of HPV genotypes 16 and 18, while concurrently detecting the remaining 12 high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The cobas HPV test is used in conjunction with the cobas 4800 System. The cobas 4800 System comprises the cobas x 480 instrument and cobas z 480 analyzer that fully automates the cobas HPV from sample extraction through amplification, detection, and data reduction.(Instruction manual and package insert: cobas HPV test. Roche Diagnostics; version 05641268001-20EN. 03/2021)

PDF Report

No

Specimen Retention Time

1 week

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information

87624

G0476 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HPV	HPV with Genotyping, PCR, ThinPrep	77378-8

Result ID	Reporting Name	LOINC®
SS017	Specimen Source	31208-2
35924	HPV High Risk type 16, PCR	61372-9
35925	HPV High Risk type 18, PCR	61373-7
35926	HPV other High Risk types, PCR	77375-4