Test Definition: HPVP

HPVG PCR w/ Pap Reflex, ThinPrep

Overview

Useful For

Screening for infection with high-risk (HR) human papillomavirus associated with the development of cervical cancer

Individual genotyping of HPV-16 and/or HPV-18, if present

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

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.

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.

Testing Algorithm

When this test is ordered, human papillomavirus (HPV) with genotyping by polymerase chain reaction (PCR) will be performed. If the patient is > or =25 years old and HPV with genotyping by PCR is positive for other high-risk types and negative for types 16 and 18, then HPV cytology reflex test will be performed at an additional charge.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
TPRCY	HPV Cytology Reflex	No	No
TPSPC	Physician Interp Screen	No	No

Method Name

HPCG: Real-Time Polymerase Chain Reaction (PCR)

TPRCY: Light Microscopy

NY State Available

Yes

Specimen

Specimen Type

Varies

Necessary Information

An acceptable cytology request form must accompany specimen containers and include the following: Patient's name, medical record number, date of birth, sex, source (exact location and procedure used), date specimen was taken, name of ordering physician and pager number. Submit any pertinent history, clinical information, or date of last menstrual period.

Specimen Required

Original ThinPrep/PreservCyt collection vial is required for testing.

Only 1 aliquot may be removed from PreservCyt sample vial prior to performing the ThinPrep Pap Test, regardless of the volume of the aliquot (maximum aliquot volume=3 mL).

For optimal interpretation, Pap smears should be collected near the middle of the menstrual cycle. Avoid douching, lubricant use, and sexual intercourse for 24 hours prior to specimen collection.

Specimen source is required.

Submit only 1 of the following specimens:

Broom Collection Device:

Specimen Type: Cervical (endocervical or ectocervical)

Supplies: Thin Prep Media with Broom Kit (T056)

Container/Tube: ThinPrep/PreservCyt vial

Specimen Volume: 20 mL of solution in ThinPrep/PreservCyt vial

Collection Instructions:

1. Obtain adequate sampling from cervix using a broom-like collection device. If desired, use lukewarm water to warm and lubricate the speculum. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently and rotate the broom in a clockwise direction 5 times.

2. Rinse the broom as quickly as possible into the PreservCyt solution vial by pushing broom into bottom of vial 10 times, forcing the bristles apart.

3. As a final step, swirl broom vigorously to further release material. Discard the collection device.

4. Tighten cap on vial so that the torque line on the cap passes the torque line on the vial.

5. **Specimen vial must be labeled with a minimum of 2 unique identifiers** (patient's name and medical record number or date of birth).

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

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

Endocervical Brush/Spatula Collection Device:

Specimen Type: Ectocervix and endocervix

Supplies: Thin Prep Media with Spatula and Brush Kit (T434)

Container/Tube: ThinPrep/PreservCyt vial

Specimen Volume: 20 mL of solution in ThinPrep/PreservCyt vial

Collection Instructions:

1. Obtain an adequate sampling from the ectocervix using a plastic spatula. If desired, use lukewarm water to warm and lubricate the speculum. Select contoured end of plastic spatula and rotate it 360 degrees around the entire exocervix while maintaining tight contact with exocervical surface.

2. Rinse spatula as quickly as possible into the PreservCyt solution vial by swirling spatula vigorously in vial 10 times. Discard the spatula.

3. Next, obtain an adequate specimen from endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottommost fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction. **Do not over-rotate.**

4. Rinse the brush as quickly as possible in the PreservCyt solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall.

5. Swirl brush vigorously as final step to further release material. Discard the brush.

6. Tighten the cap so that the torque line on the cap passes the torque line on the vial.

7. **Specimen vial must be labeled with a minimum of 2 unique identifiers** (patient's name and medical record number or date of birth).

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

9. Place labels on the vial.

Forms

If not ordering electronically, complete, print, and send Gynecologic Cytopathology Request Form with the specimen.

Reject Due To

Specimen containing CytoRich red preservative fluid and/or glacial acetic acid	Reject
Patient <25 years old	
Sources other than cervix/cervical or not including cervix/cervical, SurePath preservative	

Specimen Minimum Volume

17 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 8000 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)

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% 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/Pap smear. 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/or 18 may have repeat cytology and HR-HPV testing in 12 months.(1)

Recently, the Food and Drug Administration (FDA) approved the use of the Roche cobas HPV test for primary screening of cervical and endocervical samples collected in ThinPrep/PreservCyt media. In addition, the age at which patients may be screened by the HPV test dropped from 30 to 25 years old.

Reference Values

Human papillomavirus (HPV) with Genotyping polymerase chain reaction (PCR): Negative for HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68

ThinPrep Pap Test: Satisfactory for evaluation. Negative for intraepithelial lesion or malignancy.

Interpretation

Human papillomavirus with genotyping polymerase chain reaction:

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 25 years and older who are positive for HPV-16 and/or HPV-18, but negative by Pap smear, consider referral for colposcopy, if clinically indicated.

Cytology:

Standard reporting, as defined by the Bethesda System (TBS) is utilized.

Cautions

When used as a primary screening assay, the cobas human papillomavirus (HPV) test is FDA-approved for cervical and endocervical samples collected in PreservCyt (ThinPrep) media. Primary screening of vaginal sources by the cobas HPV test cannot be performed.

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 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 possibility of obtaining an invalid or false-negative result.

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 infection).

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

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 manually filled past the maximum fill line of the primary vial. ThinPrep vials that have had any dissimilar fluid volume added to the initial specimen should not be submitted for testing.

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

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

Overall Agreement: 91.0% (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

Sensitivity=91.4%

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

Sensitivity=51.9%

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.

5. Solomon D, Schiffman M, Tarone R, ALTS Study group: Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst. 2001;93:293-299. doi: 10.1093/jnci/93.4.293.

6. Soloman D, Davey D, Kurman R, et al: The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002;287:2114-2119. doi: 10.1001/jama.287.16.2114.

7. Massad LS, Einstein MH, Huh WK, et al: 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013 April;17(5 Suppl 1):S1-S27. doi: 10.1001/jama.287.16.2114.

8. Sherman ME, Lorincz A, Scott DR, et al: Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Nat Cancer Inst. 2003 January;95(1):46-52. doi: 10.1093/jnci/95.1.46.

9. Huh WK, Ault KA, Chelmow D, et al: Use of primary high-risk human papillomavirus testing for cervical cancer screening: Interim clinical guidance. Gynecol Oncol. 2015 Feb;136(2):178-182. doi: 10.1016/j.ygyno.2014.12.022.

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.(Package insert: cobas HPV test. Roche Diagnostics; version 05641268001-20EN. 03/2021)

The ThinPrep Pap specimen is processed on a T2000 or T5000 processor, producing a slide that is stained with a Papanicolaou stain. The stained slides are examined microscopically.(Instruction manuals: ThinPrep 2000 System. Hologic; 2017; ThinPrep 5000 Processor. Hologic; 2016)

PDF Report

No

Specimen Retention Time

1 week if Pap test has not been performed, 14 days after Cytology report has been issued if Pap test has been performed.

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)

88142 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HPVP	HPVG PCR w/ Pap Reflex, ThinPrep	71432-9

Result ID	Reporting Name	LOINC®
SRCPV	Specimen Source	31208-2
36402	HPV High Risk type 16, PCR	77399-4
36403	HPV High Risk type 18, PCR	77400-0
36404	HPV other High Risk types, PCR	71431-1
37310	Interpretation	59464-8