MAYO CLINIC LABORATORIES

Test Definition: HPVP

Human Papillomavirus (HPV) DNA Detection with Genotyping, High Risk Types by PCR with Papanicolaou Smear Reflex, ThinPrep, Varies

Overview

Useful For

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

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

This testing is intended for use in clinical monitoring and management of patients. It is not intended for use in medical-legal applications.

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.

Reflex Tests

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

Testing Algorithm

If the patient is 25 years of age or older and this test is positive for high-risk types, then ThinPrep Pap testing will be reflexed and performed at an additional charge. If ThinPrep Pap results are abnormal, a pathologist will review the case at an additional charge.

Method Name

HPVP: Real-Time Polymerase Chain Reaction (PCR)
TPRCY: Light Microscopy

NY State Available

Yes

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Specimen

Specimen Type
Varies

Necessary Information
1. 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.
2. 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 broom 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 the 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 and on bag.

Specimen Minimum Volume

17 mL

Reject Due To

Specimen containing SurePath preservative, CytoRich Red preservative fluid or glacial acetic acid Patient less than 25 years Sources other than cervix/cervical or not including cervix/cervical	Reject

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.

Human papillomavirus 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 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. Sexual transmission of 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 long-term health consequences. Both high-risk HPV genotypes (especially HPV-16 and 18) and 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 suggests that certain HPV genotypes (eg, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are 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.

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 false-negative results. Perhaps most importantly, a 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 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

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. Based in part on these data, the American Society for Colposcopy and Cervical Pathology 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.

Recently, the US Food and Drug Administration 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 has dropped from 30 to 25 years old.

Reference Values

Human papillomavirus (HPV) with Genotyping polymerase chain reaction: 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 Pap smear result and who are positive for high-risk-HPV, consider referral for colposcopy, if clinically indicated.

For women 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 US Food and Drug Administration approved for cervical and endocervical samples collected in PreservCyt (ThinPrep) media. Primary screening of vaginal sources by the cobas HPV test cannot be performed.f

The cobas HPV test detects DNA from high-risk genotypes 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 systems. If concentrations of whole blood exceed 10% (dark red or brown coloration) in PreservCyt solution, there is a possibility of obtaining an invalid or false-negative result.

Human beta-globin amplification and detection is included in the cobas HPV test to differentiate HPV negative specimens from those that do not exhibit HPV signal due to insufficient cell mass in the specimen. All HPV negative specimens must have a valid beta-globin signal within a pre-defined range to be identified as valid negatives.

Human papillomavirus 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-positive, immunocompromised, history of sexually transmitted infection).

The effects of other potential variables (eg, vaginal discharge, use of tampons, douching) and specimen collection variables have not been evaluated.

The cobas HPV test performance has been validated with PreservCyt specimens that have been treated with up to 5%

glacial acetic acid for removal of red blood cells. Addition of glacial acetic acid over 5% in 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.

Clinical Reference

1. Perkins RB, Guido RS, Castle PE, et al. 2019 ASCCP Risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors [published correction appears in J Low Genit Tract Dis. 2020 Oct;24(4):427]. J Low Genit Tract Dis. 2020;24(2):102-131. doi:10.1097/LGT.0000000000000525
2. Wright TC Jr, Stoler MH, Behrens CM, Apple R, Derion T, Wright TL. The ATHENA human papillomavirus study: design, methods, and baseline results. Am J Obstet Gynecol. 2012;206(1):46.e1-46.e11. doi:10.1016/j.ajog.2011.07.024
3. Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. Gynecol Oncol. 2015;136(2):189-197. doi:10.1016/j.ygyno.2014.11.076

Performance

Method Description

The cobas HPV (human papillomavirus) test is a qualitative real-time polymerase chain reaction (PCR) test that detects 14 high-risk HPV genotypes. The test uses primers to define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the Master Mix is designed to amplify HPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). An additional primer pair targets the human beta-globin gene (330 base pair amplicon) as an internal control to monitor the entire sample preparation and PCR amplification process. Fluorescent oligonucleotide probes bind to polymorphic regions within the sequence defined by these primers. The test utilizes a low titer positive and a negative control.(Package insert: cobas HPV: Qualitative nucleic acid test for the cobas 5800/6800/8800 Systems. Roche Diagnostics, Inc; Rev. 2.0, 09/2024)

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

Day(s) Performed

Monday through Saturday

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Report Available
3 to 14 days

Specimen Retention Time
If Pap test has not been performed: 14 days; If Pap test has been performed: 14 days after Cytology report has been issued

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

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
87626
G0476 (if appropriate)
88142 (if appropriate)

LOINC® Information

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

Result ID	Test Result Name	Result LOINC® Value
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	77379-6