MAYO CLINIC LABORATORIES

Test Definition: CTPCO

ThinPrep with Human Papillomavirus (HPV) Co-Test-Screen with p16/Ki67 Dual Stain Reflex, Varies

Overview

Useful For

Screening for cervical carcinoma or intraepithelial lesions and the presence or absence of high-risk human papillomavirus (HR-HPV) when screening women aged 30 to 65 years for possible cervical neoplasia

Aiding in triaging women with abnormal Papanicolaou (Pap) smear results

Aiding in triaging women with positive HR-HPV but negative Pap smear results

Aiding in triaging women aged 30 to 65 years with NILM (negative for intraepithelial lesion or malignancy) and 12 other HR-HPV positive test results using the cobas 4800 HPV Test in adjunctive cervical cytology and HR-HPV screening, to determine the need for referral to colposcopy

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
TPSPC	Physician Interp Screen	No	No
CINPC	CINtec IHC Multiplex, PC	No	No

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
HPV	HPV with Genotyping, PCR, ThinPrep	Yes	Yes

Testing Algorithm

When this test is ordered, a ThinPrep Papanicolaou (Pap) cytology screen and human papillomavirus (HPV) high-risk DNA detection with genotyping by polymerase chain reaction test will be performed.

If the ThinPrep Pap results are abnormal, a pathologist will review the case at an additional charge.

If the cytology screen results are negative for intraepithelial lesion or malignancy and HPV test results are positive, p16/Ki67 dual stain immunocytochemistry will be performed at an additional charge.

Special Instructions

- Gyn-Cytology Patient Information

Method Name

Light Microscopy/Real-Time Polymerase Chain Reaction (PCR)/Immunocytochemistry as needed

NY State Available
Yes

Specimen

Specimen Type
Varies

Ordering Guidance
1. Mayo Clinic Laboratories' clients need prior laboratory approval to order cytology testing.
2. This test is not approved for patients outside the ages of 30 to 65 years. If this test is ordered and the patient is outside the ages of 30 to 65 years, it will be canceled and automatically reordered by the laboratory as one of the following:
-STPCO / ThinPrep with Human Papillomavirus (HPV) Co-Test-Screen, Varies if the patient is older than 65 years.
-STHPV / ThinPrep Screen with Human Papillomavirus (HPV) Reflex, Varies if the patient is younger than 30 years.
3. This test is not approved on vaginal specimens. Testing ordered on vaginal specimens will be canceled and automatically reordered by the laboratory as one of the following:
-STPCO if the patient is older than 30 years
-STHPV If the patient is younger than 30 years

Necessary Information
1. An acceptable cytology request must accompany specimen containers and include the following:
Patient's name
Medical record number
Date of birth
Sex
Specimen source (exact location and procedure used)
Date specimen was collected
Name and pager number of ordering healthcare professional
2. Submit any pertinent history or clinical information.

Specimen Required
Patient Preparation: For optimal interpretation, Papanicolaou (Pap) smears should be collected near the middle of the menstrual cycle. No douching, lubricant use, or sexual intercourse for 24 hours before specimen collection.

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: 4 mL).

Submit only 1 of the following specimens:

Specimen Type: Cervical
Supplies: Thin Prep Media with Broom Kit (T056)
Container/Tube: ThinPrep/PreservCyt vial
Specimen Volume: 16 mL
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 tend to leak during transport.
7. Place labels on the vial and on the bag.

Specimen Type: Ectocervix and endocervix
Supplies: Thin Prep Media with Spatula and Brush Kit (T434)
Container/Tube: ThinPrep/PreservCyt vial
Specimen Volume: 16 mL
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 ectocervix while maintaining tight contact with ectocervical 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 one-quarter or one-half turn in 1 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 tend to leak during transport.
9. Place labels on the vial and bag.

Forms

Gyn-Cytology Patient Information (T601)

Specimen Minimum Volume

See Specimen Required

Reject Due To

SurePath vial	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

The majority (>99%) of cervical epithelial neoplasms are the result of human papillomavirus (HPV) infection. High-risk (HR) HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) can result in both low-grade squamous intraepithelial lesions and high-grade squamous intraepithelial lesions, as well as invasive carcinomas.(1,2) Patients with both a negative cytology and negative HPV have been shown to be at extremely low risk for cervical neoplasia.(1,2)

For women 30 years and older who have received a negative Pap test and concurrent negative HPV result, the American Cancer Society and American College of Obstetricians and Gynecologists recommendations for cervical screening state that physicians may lengthen the screening interval to 3 years when using the combined test. Patients deemed to be high risk by the clinician should be screened more frequently.

The presence of HR-HPV types in cervical specimens identifies a subgroup of patients with a greater likelihood of having a high-grade squamous intraepithelial lesion. Current guidelines for follow-up of a cytology-negative/HPV-positive patient recommend repeat HPV testing in 12 months.(2)

Persistent infection with HPV is the principal cause of cervical cancer and its precursor, cervical intraepithelial neoplasia (CIN).(1-3) The presence of HPV has been implicated in more than 99% of cervical cancers worldwide. 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 that can infect the human anogenital mucosa. However, data suggest that 14 of these types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are considered high risk 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.(4-6)

Although persistent infection with HR-HPV is necessary for the development of cervical cancer and its precursor lesions, only a very small percentage of infections progress to these disease states. 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. An infection with any HPV type can produce CIN, although this also usually resolves once the HPV infection has been cleared.

In developed countries with cervical cancer screening programs, the Papanicolaou (Pap) smear has been used since the mid-1950s as the primary tool to detect early precursors to cervical cancer. Although it has decreased the death rates due to cervical cancer dramatically in those countries, the Pap smear and subsequent liquid-based cytology methods require subjective interpretation by highly trained cytopathologists, and misinterpretation can occur. Cytological abnormalities are primarily due to infection with HPV; however, various inflammatory conditions or sampling variations can result in false-positive cytology results. Triage of an abnormal cytology result may involve repeat testing, colposcopy, and biopsy. A histologically confirmed high-grade lesion must be surgically removed or ablated in order to prevent the development of invasive cervical cancer.

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 aged 30 years and older with normal cytology
2. Reduce the need for unnecessary colposcopy and treatment in patients aged 21 years and older with cytology results showing atypical squamous cells of undetermined significance

Recent 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 women who are HPV-16 and HPV-18 positive 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 women who are HR-HPV negative.(7) 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. Women who are found to be positive for HPV-16 or HPV-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.(4)

The IMPACT trial evaluated the performance of the CINtec *PLUS* Cytology test as a triage test to stratify women aged 25 to 65 years who are cobas 4800 HPV positive in primary HPV cervical cancer screening for referral to colposcopy, and the performance of the CINtec *PLUS* Cytology test as a triage test to stratify women aged 30 to 65 years who are cobas 4800 HPV positive with NILM (negative for intraepithelial lesion or malignancy) Pap cytology in adjunctive cervical cytology and HPV screening for referral to colposcopy.(8)

It is the responsibility of the physicians and other healthcare professionals or the US guidelines to provide guidance as to whether women with positive CINtec *PLUS* Cytology results should be referred to colposcopy, and what type of follow-up should be recommended for women who test negative for the CINtec *PLUS.*

Reference Values

ThinPrep PAPANICOLAOU
Satisfactory for evaluation. Negative for intraepithelial lesion or malignancy.

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

CINtec PLUS Cytology, Immunocytochemical dual stain for p16/Ki-67 has been performed.
Result: Negative

Interpretation

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

Human papillomavirus (HPV):
A positive result indicates the presence of 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.

CINtec *PLUS* Cytology:
If no cervical epithelial cells show simultaneous brown cytoplasmic immunostaining and red nuclear immunostaining, the CINtec *PLUS* Cytology test result is considered negative.

For patients with a Papanicolaou (Pap) smear result of atypical squamous cells of undetermined significance and who are positive for high-risk HPV (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 or HPV-18, consider referral for colposcopy if clinically indicated.

For women aged 30 to 65 years with a negative Pap smear, positive HR-HPV test result, but negative HPV-16 and HPV-18 and CINtec Plus positive, consider referral for colposcopy if clinically indicated

For women aged 30 to 65 years with a negative Pap smear, positive HR-HPV test result, but negative HPV-16 and HPV-18 and CINtec Plus negative, consider repeat testing by both cytology and a HR-HPV test in 12 months.

Cautions

For women aged 30 to 65 years old with NILM (negative for intraepithelial lesion or malignancy) and HPV16/18 positive test results using the cobas 4800 HPV Test in adjunctive cervical cytology and HR HPV screening, the CINtec PLUS Cytology test results should be used in conjunction with the healthcare professional's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

The Papanicolaou (Pap) test is a screening test for cervical cancer with inherent false-negative results. A negative human papillomavirus (HPV) test or Pap smear result does not preclude the presence of carcinoma or intraepithelial lesion. The false-negative rates of the Pap test range from 15% to 30%.

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.

The cobas HPV test is not recommended for evaluation of suspected sexual abuse.

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 HPV (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.

Performance of the CINtec *PLUS* Cytology test was not established for women older than 65 years.

Clinical Reference

1. Lorincz AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. Arch Pathol Lab Med. 2003;127(8):959-968
2. Wright TC Jr, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. N Engl J Med. 2003;348(6):489-490
3. Nayar R, Wilbur DC, eds. The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes. 3rd ed. Springer International Publishing; 2015
4. 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
5. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12-19
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7. 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+, cytology-negative results. Am J Clin Pathol 2011;136(4):578-586
8. Package insert: CINtec *PLUS* Cytology. Roche Diagnostics; version 1018621US Rev D, 06/2024
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10. Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst. 2003;95(1):46-52
11. Clarke MA, Cheung LC, Castle PE, et al. Five-year risk of cervical precancer following p16/Ki-67 dual-stain triage of HPV-positive women. JAMA Oncol. 2019;5(2):181-186. doi:10.1001/jamaoncol.2018.4270
12. Wentzensen N, Clarke MA, Bremer R, et al. Clinical evaluation of human papillomavirus screening with p16/Ki-67 dual stain triage in a large organized cervical cancer screening program [published correction appears in JAMA Intern Med. 2019 Jul 1;179(7):1007. doi:10.1001/jamainternmed.2019.2636.]. JAMA Intern Med. 2019;179(7):881-888. doi:10.1001/jamainternmed.2019.0306

13. Wright TC Jr, Stoler MH, Ranger-Moore J, et al. Clinical validation of p16/Ki-67 dual-stained cytology triage of HPV-positive women: Results from the IMPACT trial. Int J Cancer. 2022;150(3):461-471. doi:10.1002/ijc.33812

Performance

Method Description

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 Operator's Manual. Hologic; MAN-02585-001 Rev. 006, 02/2017; ThinPrep 5000 Processor Operator's Manual. Hologic; MAN-02203-001 Rev. 002, 2016)

The cobas HPV test is a qualitative real-time 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 6800/8800 Systems. Roche Diagnostics; Rev. 2.0, 03/2021)

The CINtec *PLUS* Cytology test is a qualitative immunocytochemical assay intended for the simultaneous detection of the p16INK4a and Ki-67 proteins in cervical specimens collected by a clinician using an endocervical brush/spatula or broom collection device and placed in the ThinPrep Pap Test PreservCyt Solution. The CINtec *PLUS* Cytology test includes a ready-to-use cocktail of primary antibodies that contains a mouse monoclonal antibody directed against human p16INK4a (p16) protein (clone E6H4), and a recombinant rabbit monoclonal antibody directed against human Ki-67 protein (clone 274-11AC3V1) for use on the BenchMark ULTRA instrument with 3,3'-diaminobenzidine tetrahydrochloride and Fast Red detection systems.(Package insert: CINtec *PLUS* Cytology. Roche Diagnostics; version 1018621US Rev D, 06/2024)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

5 to 15 days

Specimen Retention Time

14 days after report issued

Performing Laboratory Location

Test Definition: CTPCO

ThinPrep with Human Papillomavirus (HPV) Co-Test-Screen with p16/Ki67 Dual Stain Reflex, Varies

Mayo Clinic Laboratories - Rochester Main Campus

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

88142
G0123 (Government payers)
G0124 (Government payers, if appropriate)
88141 -TPSPC (if appropriate)
88344 -CINTC/CINPC (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CTPCO	ThinPrep HPV CoTest w CINtec+Reflex	47527-7

Result ID	Test Result Name	Result LOINC® Value
616074	Interpretation	69965-2
616075	Participated in the Interpretation	No LOINC Needed
616076	Report electronically signed by	19139-5
616077	Addendum	35265-8
616078	Gross Description	22634-0
CY092	Pap Test Source	22633-2
CY093	Clinical History	22636-5
CY094	Menstrual Status (LMP, PM, Pregnant)	8678-5
CY095	Hormone Therapy/Contraceptives	8659-5
616079	Disclaimer	62364-5
616080	Case Number	80398-1