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

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPRCY</td>
<td>HPV Cytology Reflex</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>TPSPC</td>
<td>Physician Interp Screen</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Testing Algorithm
When this test is ordered, HPV with genotyping PCR will be performed. If the results include the criteria below, the HPV Cytology Reflex test will be ordered by reflex and performed at an additional charge:

- HPV with genotyping PCR is positive for other high-risk types and negative for types 16 and 18
- Patient is > or =25 years of age

Method Name
Real-Time Polymerase Chain Reaction (PCR) with ThinPrep Pap Cytology Screening by Light Microscopy

NY State Available
Yes

Specimen

Specimen Type
Varies

Specimen Required
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 (LMP).

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: (T056)**

**Specimen Type:** Cervical (endocervical or ectocervical)

**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: (T434)**

**Specimen Type:** Ectocervix and endocervix

**Container/Tube:** ThinPrep/PreservCyt vial

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

**Collection Instructions:**

1. Obtain an adequate sampling from 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 and on the bag.

Forms

Arizona:

If not ordering electronically, complete, print, and send Gynecologic Cytopathology Request Form http://mayoweb.mayo.edu/sp-forms/mc4600-mc4699/mcs4689.pdf

Specimen Minimum Volume

17 mL

Reject Due To

| Other | Specimen containing CytoRich red preservative fluid and/or glacial acetic acid, patient <25 years old, sources other than cervix/cervical or not including cervix/cervical, SurePath preservative |

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varies</td>
<td>Ambient (preferred)</td>
<td>42 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
<td>42 days</td>
<td></td>
</tr>
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</table>

Clinical and Interpretive

Clinical Information

Persistent infection with human papillomavirus (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 >99% of cervical cancers worldwide. 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 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 (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)

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 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 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 and 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% 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/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

HPV with Genotyping 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

HPV with Genotyping PCR:

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**

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 in compliance with CLIA-regulations by Mayo Clinic Laboratories. 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.

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 (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.

The Cobas HPV 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.

The Cobas HPV test is **not** intended for women who have undergone hysterectomy.

The Cobas HPV 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.

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 additional PreservCyt fluid volume added or any dissimilar fluid volume added to the initial specimen should not be submitted for testing.

The presence of PCR 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)

<table>
<thead>
<tr>
<th></th>
<th>Digene hc2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Roche Cobas 4800</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>353</td>
</tr>
<tr>
<td>Negative</td>
<td>42(b)</td>
</tr>
<tr>
<td>Total</td>
<td>395</td>
</tr>
</tbody>
</table>

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).
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 ≥CIN2.

Finally, the results of the Roche Cobas HPV-16/18 genotype test were compared to a tissue diagnosis of ≥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).

<table>
<thead>
<tr>
<th>Tissue Diagnosis &gt; or =CIN2*</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Cobas 4800 16/18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>42(a)</td>
<td>36(c)</td>
<td>78</td>
</tr>
<tr>
<td>Negative</td>
<td>39(b)</td>
<td>233</td>
<td>272</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>269</td>
<td>350</td>
</tr>
</tbody>
</table>

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**


**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. Indianapolis, IN, version 05641268001-01EN)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Friday; Varies

**Analytic Time**

3-8 days
Maximum Laboratory Time
6-14 days

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 and 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 Regional Manager. For assistance, contact Customer Service.

Test Classification
This test has been cleared or approved by the U.S. 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

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<tr>
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<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<tbody>
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<td>HPVG PCR w/ Pap Reflex, ThinPrep</td>
<td>71432-9</td>
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<table>
<thead>
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<td>HPV other High Risk types, PCR</td>
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<td>Interpretation</td>
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