Overview

Useful For
Detection of high-risk (HR) genotypes associated with the development of cervical cancer
An aid in triaging women with abnormal Pap smear results
Individual genotyping of human papillomavirus (HPV)-16 and/or HPV-18, if present

Method Name
Real-Time Polymerase Chain Reaction (PCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Necessary Information
Specimen source, collection date, and patient identifiers are required.

Specimen Required

Supplies: HPV SurePath Transport Tube 13 mL (T710)

Specimen Type: Cervical (endocervical or ectocervical) or vaginal

Specimen Volume: 1.5 mL

Collection Instructions:
1. Aliquot a minimum of 1 mL SurePath specimen into SurePath HPV aliquot tube.
2. Bag specimens individually as they have a tendency to leak during transport.
3. Place labels on the vial and on the bag.

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

Specimen Minimum Volume
1 mL

Reject Due To

| Other                                      | SurePath enriched cell pellet |

Specimen Stability Information
Test Definition: SHPV  
HPV with Genotyping, PCR, Surepath

<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>14 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
<td>14 days</td>
<td></td>
</tr>
</tbody>
</table>

Clinical and Interpretive

Reference Values
Negative for 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/endocervical samples collected in PreservCyt (ThinPrep) media. Other sample types (eg, vaginal) collected in media, such as SurePath, are not considered FDA-approved sources; however, verification studies have been completed in compliance with CLIA-regulations by Mayo Clinic Laboratories.

Prolonged storage (>14 days) of clinical samples in SurePath media may impact the detection of high-risk (HR) HPV, especially if the amount of nucleic acid present in the sample is initially at a low concentration. Therefore, samples should be submitted for testing as soon as possible following collection.

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 low-risk HPV types (eg, 6, 11, 42, 43, 44), which are not associated with invasive cervical cancer and its precursor lesions. Low-risk HPV types are associated with noninvasive genital warts and laryngeal papillomatosis.

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 high-grade cervical intraepithelial neoplasia (CIN), nor does it indicate that a high-grade intraepithelial lesion (eg, HSIL or CIN2-3) or cancer will develop. Most women infected with 1 or more HR HPV types do not develop CIN2-3 or cancer.
A negative HR HPV result does not exclude the possibility of a patient developing a high-grade intraepithelial lesion (eg, HSIL or CIN2-3) or cancer in the future.

**Supportive Data**

To assess the accuracy of the Roche cobas human papillomavirus (HPV) test using cervical/endocervical and vaginal samples collected in SurePath media, a combination of spiking and comparison testing was performed. For spiking studies, 30 analyte-negative clinical samples (cervical/endocervical or vaginal matrix in SurePath media) were spiked with AcroMetrix HPV positive genotype controls (type 68 [n=10], type 16 [n=10], type 18 [n=10]) at 1 dilution above the limit of detection (LoD). The results are summarized in Table 1 below:

Table 1. Verification of accuracy for the Roche cobas HPV test using spiked cervical/endocervical samples in SurePath media.

<table>
<thead>
<tr>
<th>Source</th>
<th>Positives</th>
<th>Negatives</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV Genotype 68</td>
<td>10/10</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>HPV Genotype 16</td>
<td>10/10</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>HPV Genotype 18</td>
<td>10/10</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>

In addition to the spiking studies described above, clinical samples (n=26) collected in SurePath media and initially tested by the Roche cobas HPV assay at an outside laboratory were tested at Mayo Clinic Laboratories in a blinded fashion. The results are summarized in Table 2 below:

Table 2. Comparison of SurePath samples tested by Roche cobas HPV at an outside laboratory and the Virology Laboratory at Mayo Clinic Laboratories (MCL).

<table>
<thead>
<tr>
<th>Roche cobas 4800 - MCL</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>10</td>
<td>26</td>
</tr>
</tbody>
</table>

Agreement: 92.3% (74.7-99.0%)

**Reference Range:**

Cervical/endocervical samples (n=27) and vaginal samples (n=22) collected in SurePath media for routine Pap smear screening were tested by the Roche cobas HPV assay.

All 49 samples (100%) had negative Pap results and negative Roche cobas HPV 4800 results.

The reference range for the Roche cobas HPV test is negative.
Limit of Detection (Analytical Sensitivity):

To assess the analytical sensitivity of the Roche cobas HPV test, pools of cervical/endocervical/vaginal specimens in SurePath media were created. Pools were spiked at a high starting concentration using each of the 3 AcroMetrix HPV Genotype controls (cell lines infected with HPV genotypes 16, 18, or 68). Serial dilutions were made into analyte-negative sample containing cells to achieve dilution of the analyte to the point of extinction. At least 6 replicates of each dilution were tested, including the panel member that was 1 dilution below the predicted limit of detection (LoD). The LoD was established as the highest dilution in which 6 of 6 replicates were positive.

The LoD of the Roche cobas HPV genotype 16, 18, and "Other" high-risk HPV infected cells in SurePath media was determined to be 50 cells/mL, 1250 cells/mL, and 250 cells/mL, respectively.

Analytical Specificity:

A full specificity panel has been tested by the manufacturer that included bacteria, fungi and viruses, including those commonly found in the female urogenital tract. Also, several HPV types classified as low or undetermined risk were tested with the cobas HPV test to assess analytical specificity. Results indicated that none of these organisms interfered with detection of HPV 31, HPV16, and HPV18 or produced false-positive results from specimens negative for high-risk HPV.

Specimen Stability:

The stability of SurePath samples (endocervical/cervical and vaginal) at ambient (18-24 degrees C) was assessed using spiking studies and clinical samples. These results are summarized in Tables 3 and 4 below:

Table 3. Negative SurePath samples spiked with AcroMetrix positive genotype controls (68, 16 or 18). Samples were held at ambient temperature for 14 days.

<table>
<thead>
<tr>
<th>Type 68- Type 16- Type 18-</th>
<th>5000 cells/mL</th>
<th>5000 cells/mL</th>
<th>5000 cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Crossing Point</td>
<td>Crossing Point</td>
<td>Crossing Point</td>
</tr>
<tr>
<td>0</td>
<td>31.6</td>
<td>31.8</td>
<td>36.7</td>
</tr>
<tr>
<td>7</td>
<td>32.5</td>
<td>31.4</td>
<td>36.6</td>
</tr>
<tr>
<td>14</td>
<td>34.2</td>
<td>31.9</td>
<td>39.2</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>32.3</td>
<td>31.7</td>
<td>37.5</td>
</tr>
<tr>
<td>% CV</td>
<td>4.03</td>
<td>0.83</td>
<td>3.93</td>
</tr>
</tbody>
</table>

In addition to the spiking studies described above, clinical samples collected in SurePath media at an outside laboratory were held at ambient temperature over a period of 14 days and tested by the Roche cobas HPV assay.

Table 4. Positive SurePath pooled patient material collected at an outside laboratory were held at ambient temperature and tested over 14 days.
Test Definition: SHPV
HPV with Genotyping, PCR, Surepath

<table>
<thead>
<tr>
<th>Day</th>
<th>Patient Genotype</th>
<th>Crossing Point</th>
<th>Patient Genotype</th>
<th>Crossing Point</th>
<th>Patient Genotype</th>
<th>Crossing Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&quot;Other HR HPV&quot;</td>
<td>37.2</td>
<td>HPV-16</td>
<td>27.5</td>
<td>HPV-18</td>
<td>29.6</td>
</tr>
<tr>
<td>7</td>
<td>HPV-18</td>
<td>36.4</td>
<td></td>
<td>28.7</td>
<td></td>
<td>30.4</td>
</tr>
<tr>
<td>14</td>
<td>HPV-16</td>
<td>37.4</td>
<td></td>
<td>28.9</td>
<td>HPV-18</td>
<td>29.8</td>
</tr>
<tr>
<td></td>
<td>AVERAGE</td>
<td>37.0</td>
<td></td>
<td>28.4</td>
<td></td>
<td>29.9</td>
</tr>
<tr>
<td>% CV</td>
<td>1.43</td>
<td>2.66</td>
<td></td>
<td>1.39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Clinical Reference**


5. Procedure manual and package insert: cobas HPV test. Roche Diagnostics. Indianapolis, IN, version 05641268001-01EN


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

**PDF Report**

No
Day(s) and Time(s) Test Performed
Monday through Friday; Varies

Analytic Time
3 days

Maximum Laboratory Time
6 days

Specimen Retention Time
1 week

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 modified from the manufacturer’s instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
87624

G0476 (if appropriate)

LOINC® Information

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<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<tbody>
<tr>
<td>SHPV</td>
<td>HPV with Genotyping, PCR, Surepath</td>
<td>77378-8</td>
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<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS018</td>
<td>Specimen Source</td>
<td>31208-2</td>
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<tr>
<td>36003</td>
<td>HPV High Risk type 16, PCR</td>
<td>61372-9</td>
</tr>
<tr>
<td>36004</td>
<td>HPV High Risk type 18, PCR</td>
<td>61373-7</td>
</tr>
<tr>
<td>36005</td>
<td>HPV other High Risk types, PCR</td>
<td>77375-4</td>
</tr>
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</table>