Test Definition: SHPV

HPV with Genotyping, PCR, Surepath

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

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

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.

Reject Due To

SurePath enriched cell pellet	Reject

Specimen Minimum Volume

1 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)	14 days	
	Refrigerated	14 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, 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 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).

Recently, 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)

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.

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.

Source	Positive	Negative	Agreement
HPV Genotype 68	10/10	0	100%
HPV Genotype 16	10/10	0	100%
HPV Genotype 18	10/10	0	100%

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 (MCL) 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 MCL.

		Roche cobas 4800 - outside laboratory		
Roche cobas 4800 - MCL		**Positive**	**Negative**	**Total**
	Positive	14	14	14
	Negative	2	10	12
	Total	16	10	26

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.

LOD (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 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.

	Type 68- 5000 cells/mL	Type 16- 5000 cells/mL	Type 18- 5000 cells/mL
Day	**Crossing point**	**Crossing point**	**Crossing point**
0	31.6	31.8	36.7
7	32.5	31.4	36.6
14	34.2	31.9	39.2
AVERAGE	32.3	31.7	37.5
% CV	4.03	0.83	3.93

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.

	Patient genotype "Other HR HPV"	Patient Genotype HPV-16	Patient Genotype HPV-18
Day	**Crossing point**	**Crossing point**	**Crossing point**
0	37.2	27.5	29.6
7	36.4	28.7	30.4
14	37.4	28.9	29.8
AVERAGE	37.0	28.4	29.9
% CV	1.43	2.66	1.39

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. Gilbert L, Oates E, Ratnam S: Stability of cervical specimens in SurePath medium for HPV testing with the Roche cobas 4800. J Clin Microbiol. 2013 Oct;51(10):3412-3414. doi: 10.1128/JCM.01391-13

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.(Instruction manual and package insert: cobas HPV test. Roche Diagnostics. version 05641268001-20EN. 03/2021)

PDF Report

No

Specimen Retention Time

1 week

Performing Laboratory Location

Rochester

Fees & Codes

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 US Food and Drug Administration.

CPT Code Information

87624

G0476 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
SHPV	HPV with Genotyping, PCR, Surepath	77378-8

Result ID	Reporting Name	LOINC®
SS018	Specimen Source	31208-2
36003	HPV High Risk type 16, PCR	61372-9
36004	HPV High Risk type 18, PCR	61373-7
36005	HPV other High Risk types, PCR	77375-4