MAYO CLINIC LABORATORIES

Test Definition: DHPVR

Human Papillomavirus (HPV) Type 16 with Type 18, 31, 33, and 35 Reflex, Droplet Digital PCR, Blood

Overview

Useful For

Aid in the diagnosis of cancers caused by human papillomavirus (HPV) high-risk genotypes 16, 18, 31, 33, and 35

Pre-diagnostic screening (ie, prior to confirmation of an HPV-related cancer through traditional biopsy)

This test **should not be used** to screen asymptomatic patients.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
DHPVB	HPV-18/31/33/35, cfDNA, ddPCR, B	Yes	No

Testing Algorithm

If testing is positive for the human papillomavirus (HPV)-16 genotype, no further testing is performed. If negative for HPV-16, reflex testing for the less frequently encountered HPV-18, -31, -33 and -35 genotypes is performed at an additional charge.

Method Name

Droplet Digital Polymerase Chain Reaction (ddPCR)

NY State Available

No

Specimen

Specimen Type

Whole blood

Ordering Guidance

This test should be used as an initial pre-diagnostic screen to detect and quantify circulating cell free DNA from 5 high-risk human papillomavirus genotypes, including HPV-16, -18, -31, -33 & -35. This test is specific for HPV-16 which accounts for 90% of HPV associated head and neck squamous cell carcinomas. This test should be used as a pre-diagnostic screen without prior knowledge of HPV genotype. If the HPV genotype is known, specific tests for HPV-16 (DHPVA / Human Papillomavirus [HPV] Type 16, Droplet Digital PCR, Blood) or HPV-18, -31, -33 & -35 (DHPVB / Human Papillomavirus [HPV] Types 18, 31, 33, and 35, Droplet Digital PCR, Blood) should be ordered individually for monitoring

purposes, as appropriate.

For routine cervical cancer screening, order VHPV / Human Papillomavirus (HPV) Vaginal Detection with Genotyping for High-Risk Types by PCR.

Specimen Required

Supplies: Streck Tan Top Tube Kit (T715)
Container/Tube: Streck Cell-Free DNA blood collection kit
Specimen Volume: Two 10-mL Streck Cell-Free DNA blood collection tubes
Additional Information: Only blood collected in Streck Cell-Free DNA tubes will be accepted.

Specimen Minimum Volume

One 10 mL Streck Cell-Free DNA blood collection tube

Reject Due To

Specimen collected in tube other than Streck Cell-Free DNA collection tube	Reject

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)	7 days	
	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

Human papillomavirus (HPV) is a double-stranded DNA virus from the Papillomaviridae family. There are over 200 known types of HPV, which can be classified into low-risk types, associated with benign conditions such as genital warts, and high-risk types, which are linked to the development of cancers, including cervical, anal, oropharyngeal, vulvar, penile, and vaginal cancers.(1-3)

Human papillomavirus infects the basal epithelial cells of the skin or mucous membranes. The virus integrates into host epithelial cells, where it can exist as either an episomal (extrachromosomal) or integrated form in the host DNA.(4,5) Low-risk HPV types (eg, HPV-6 and -11) usually remain in episomal form, while high-risk types (eg, HPV-16 and -18) are more likely to integrate into the host genome, causing dysregulation of the cell cycle. The virus produces proteins (E6

and E7) that interfere with tumor suppressor genes, p53 and pRb, and may lead to uncontrolled cell proliferation and a higher risk of progression to malignancy.(5)

Most HPV infections are transient and cleared by the host immune system within 1 to 2 years. However, persistent infection with high-risk HPV types increases the likelihood of progression to premalignant lesions and ultimately invasive cancer.(5)

Human papillomavirus cell free DNA has been shown to be an important biomarker circulating in patients with HPV-related cancers, including those of the head and neck. Fragmented HPV is released into circulation by apoptotic tumor cells and can be detected by molecular techniques. This assay utilizes droplet digital polymerase chain reaction to detect, subtype, and quantify high-risk HPV cfDNA in plasma samples.

Reference Values

Not detected

Interpretation

A result of "Not Detected" indicates the absence of human papillomavirus (HPV)-16 cfDNA in the plasma (see Cautions below). If HPV-16 is not detected, presence of other genotypes 18/31/33/35 will be assessed.

A result of "Detected" indicates the presence of HPV-16 cfDNA in the plasma. If HPV-16 cfDNA is detected, a quantitative value will be reported in fragments/mL.

The quantification range of this assay is 100 to 309,600 fragments/mL.

A result of "<100 fragments/mL" indicates that HPV-16 cfDNA was detected in the plasma, but the assay could not accurately quantify the HPV-16 cfDNA present below this level.

A result of ">309,600 fragments/mL" indicates that HPV-16 cfDNA was detected in the plasma, but the assay could not accurately quantify the HPV-16 cfDNA present above this level.

Samples with an HPV signal below the limit of detection but above the limit of blank will be reported as "not detected" with an accompanying comment: "This specimen yielded signal above the limit of blank but below the assay's limit of detection, which may not be reproducible and should be interpreted in the context of the patient’s clinical presentation. If clinically indicated, recollect and submit a new sample for testing in 2-4 weeks."

Cautions

This assay only detects genetic fragments of the following human papillomavirus (HPV) genotypes: 16, 18, 31, 33 and 35.

This assay will not detect other HPV genotypes.

This assay will not specify an anatomic location or source of detectable levels of HPV.

A result of "Not Detected" does not necessarily eliminate the possibility of HPV infection, or a tumor caused by HPV.

Serial measurement of HPV in platelet poor plasma may be recommended based on clinical presentation.

This assay should only be used for patients with a clinical history and/or symptoms consistent with a confirmed or suspected HPV-related tumor.

Results must be interpreted in the context of the patient's clinical picture.

Variants within the HPV E2, E6 and E7 gene regions targeted by this assay may affect primer and/or probe binding, resulting in the under quantitation of viral fragments or failure to detect the presence of viral fragments. The assay attempts to mitigate this risk by inclusion of multiple gene target regions.

Supportive Data

Analytical Sensitivity/Detection Capability:
The 95% limit of detection of this assay is 17.54 fragments/mL of human papillomavirus (HPV)-16 cfDNA in platelet poor plasma. This was determined following a serial dilution series of plasma spiked with a known concentration of HPV-16 DNA with subsequent probit analysis.

Accuracy:
Forty-eight contrived samples yielded 100% overall, positive and negative agreement. Additionally, 8 plasma samples from patients confirmed to have an oropharyngeal tumor caused by HPV-16 were positive by this assay.

Precision:
Intra- and inter-assay reproducibility experiments yielded 100% qualitative precision. Replicates at three levels within the analytical measuring range produced quantitative values with standard deviations of 0.01- 0.07 log[10] fragments/mL.

Analytical Specificity:
A panel of 38 bacterial, fungal, parasitic and viral nucleic acid extracts from related organisms or organisms commonly found in plasma were tested by this assay with no cross-reactivity detected.

Inclusivity (*in silico* analysis) was performed using the NCBI GenBank database. Homology of assay primers and probes was determined using 20 representative strains from HPV-16 sublineages. Homology of oligos ranged from 89.5% to 100% across all strains analyzed.

Reference Interval:
Platelet poor plasma from 20 healthy donors was tested by this assay. No samples yielded detectable HPV-16 cfDNA above the defined limit of detection.

Clinical Reference

1. HPV and Cancer. National Cancer Institute. Updated May 9, 2025. Accessed October 14,2025. Available at www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer
2. Jamal Z, Anjum F. Oropharyngeal Squamous Cell Carcinoma. In: StatPearls [Internet]. Updated April 27, 2023. Accessed October 14, 2025. Available at www.ncbi.nlm.nih.gov/books/NBK563268/

3. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55-F70. doi:10.1016/j.vaccine.2012.06.083
4. Gribb JP, Wheelock JH, Park ES. Human papilloma virus (HPV) and the current state of oropharyngeal cancer prevention and treatment. Dela J Public Health. 2023;9(1):26-28. Published 2023 Apr 22. doi:10.32481/djph.2023.04.008
5. Munger K, Phelps WC, Bubb V, Howley PM, Schlegel R. The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J Virol. 1989;63(10):4417-4421
6. Fernholz EC, Routman DM, Van Abel KM, et al. Detection, quantitation, and genotyping of human papillomavirus circulating tumor DNA by droplet digital PCR. J Clin Microbiol. 2025;63(9):e0058525. doi:10.1128/jcm.00585-25

Performance

Method Description

This laboratory-developed test utilizes whole blood samples collected in Streck Cell-Free DNA blood collection tubes. Cell-free DNA (cfDNA) is isolated from double-centrifuged plasma using the EZ2 Connect extraction system (Qiagen). Nucleic acid eluate is combined with polymerase chain reaction (PCR) reaction mix and partitioned into approximately 20,000 droplets by oil emulsion on the Automated Droplet Generator (Bio-Rad). Following PCR on the VeritiPro Thermal Cycler (Applied Biosystems), droplets are analyzed for presence or absence of amplified viral target sequences using the QX600 Droplet Reader (Bio-Rad).

The PCR reaction mix contains four primer and probe sets directed against the E2, E6 and E7 gene regions of the human papillomavirus (HPV) type 16 genome producing amplicons ranging from 81 – 95 base pairs. The reaction mix also contains a primer-probe set directed against the human reference gene (ribonuclease P protein subunit 30 [RPP300]), which serves as an internal process control. The HPV-16 and RPP30 hydrolysis probes are coupled with unique fluorescent dyes that can be differentiated on the QX600. Each droplet is individually tallied and evaluated for fluorescence. Results are analyzed using dedicated software and Poisson statistics to provide absolute quantification of target and reference concentrations. Calculated results are reported as detected or not detected for HPV-16 cfDNA. A concentration is also reported for samples with detectable levels (fragments of HPV-16 cfDNA per milliliter of plasma).(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Varies

Report Available

5 to 10 days

Specimen Retention Time

7 days

Performing Laboratory Location

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 was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

0661U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
DHPVR	High Risk HPV ddPCR, Reflex	112586-3

Result ID	Test Result Name	Result LOINC® Value
623293	HPV-16 ddPCR Result w/ Reflex	61372-9
623294	HPV-16 ddPCR Quant w/ Reflex	112587-1