

## Overview

### Useful For

Diagnosis of suspected von Hippel-Lindau (VHL) disease

Diagnosis of suspected VHL-related hereditary erythrocytosis

### Special Instructions

- [Informed Consent for Genetic Testing](#)
- [VHL Gene Testing Patient Information](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)

### Method Name

Polymerase Chain Reaction (PCR) Followed by DNA Sequence Analysis and Gene Dosage Analysis by Multiplex Ligation-Dependent Probe Amplification (MLPA)

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Shipping Instructions

Specimen preferred to arrive within 96 hours of draw.

### Specimen Required

**Patient Preparation:** A previous bone marrow transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

**Specimen Type:** Whole blood

**Container/Tube:**

**Preferred:** Lavender top (EDTA) or yellow top (ACD)

**Acceptable:** Any anticoagulant

**Specimen Volume:** 3 mL

**Collection Instructions:**

1. Invert several times to mix blood.
2. Send specimen in original tube.

### Forms

[1. New York Clients-Informed consent is required.](#) Document on the request form or electronic order that a copy

is on file. The following documents are available in Special Instructions:

[-Informed Consent for Genetic Testing](#) (T576)

[-Informed Consent for Genetic Testing-Spanish](#) (T826)

2. [VHL Gene Testing Patient Information](#) (T641) in Special Instructions

3. If not ordering electronically, complete, print, and send an [Oncology Test Request](#) (T729) with the specimen.

### Specimen Minimum Volume

1 mL

### Reject Due To

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

### Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Frozen		
	Refrigerated		

## Clinical and Interpretive

### Clinical Information

von Hippel-Lindau (VHL) disease is an autosomal dominant cancer predisposition syndrome with a prevalence of approximately 1 in 36,000 livebirths. It predisposes affected individuals to the development of mainly 5 different types of neoplasms: retinal angioma (approximately 5%-70% penetrance), cerebellar hemangioblastoma (CHB) (44%-72% penetrance), clear-cell renal cell carcinoma (cRCC) (approximately 25%-60% penetrance), spinal hemangioblastoma (SHB) (approximately 13%-50% penetrance), and pheochromocytoma (PC) (approximately 10%-20% penetrance). Angiomas in other organs, pancreatic cysts/adenomas/carcinomas, islet cell tumors, and endolymphatic sac tumors can also occur. VHL-related tumors typically present in the second to third decade of life, but sometimes earlier, particularly for retinal angiomas. For each tumor type, the incidence rates rise steadily, albeit at different slopes, throughout life.

VHL disease is caused by heterozygous germline loss-of-function sequence variants, small deletions or insertions (approximately 80% of cases), or large germline deletions (approximately 20% of cases) of the *VHL* gene. Approximately 20% of cases are due to new (de novo) pathogenic variants, which in some cases result in disease mosaicism. This presents a diagnostic challenge for individuals who present with clinical signs of VHL disease, but test negative genetically because the pathogenic variant is not present in all peripheral leukocytes.

*VHL* encodes the VHL protein, a tumor suppressor protein that is involved in ubiquitination and degradation of a variety of other proteins, most notably hypoxia-inducible factor (HIF). HIF induces expression of genes that promote cell survival and angiogenesis under conditions of hypoxia. It is believed that diminished HIF degradation due to inactive VHL protein causes the tumors in VHL disease. Tumors form when the remaining intact copy of *VHL* is somatically inactivated in target tissues (2-hit model). Sporadic cRCC, unrelated to VHL disease, also shows somatic deletions, sequence variants, or aberrant methylation in 80% to 100% of cases.

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Retinal angioma, CHB, and SHB cause morbidity and some mortality through pressure on adjacent structures and through retinal or subarachnoid hemorrhages. VHL-related cRCC and PC follow a similar clinical course as their sporadic counterparts, with substantial morbidity and mortality. Early detection of VHL-related tumors can reduce these adverse outcomes, and surveillance of affected individuals is, therefore, widely advocated. Genetic testing is the most accurate way to identify presymptomatic individuals, who can then be entered into a surveillance program.

Research has suggested that certain combinations of VHL tumors cluster in VHL families, and this may be driven by the type of *VHL* gene variant present in the family. This observation has led to a phenotype-based classification of VHL syndrome. However, it should be noted that these patterns are not clear cut, and should not necessarily be used for diagnostic or therapeutic purposes.

VHL Type 1: Retinal angioma, central nervous system (CNS) hemangioblastoma, renal cell carcinoma, pancreatic cysts, and neuroendocrine tumors. Low risk for pheochromocytoma. Associated with pathogenic truncating or missense variants that are predicted to grossly disrupt the folding of VHL protein.

VHL Type 2: Pheochromocytoma, retinal angiomas, and CNS hemangioblastoma. High risk for pheochromocytoma. Associated with pathogenic missense variants.

VHL Type 2 is further subdivided:

-Type 2A: Pheochromocytoma, retinal angiomas, and CNS hemangioblastomas; low risk for renal cell carcinoma

-Type 2B: Pheochromocytoma, retinal angiomas, CNS hemangioblastomas, pancreatic cysts, and neuroendocrine tumor; high risk for renal cell carcinoma

-Type 2C: Risk for pheochromocytoma only

Additionally, pathogenic sequence variants distinct from those associated with VHL syndrome can cause hereditary erythrocytosis or polycythemia. Cases of VHL disease and erythrocytosis are largely mutually exclusive, and patients who present with erythrocytosis do not typically develop the neoplasms discussed above, although they are sometimes associated with varicose veins and vertebral hemangiomas. Erythrocytosis due to *VHL* is caused by germline homozygous or compound heterozygous pathogenic sequence variants, and is inherited in an autosomal recessive manner. These patients usually have a markedly high erythropoietin level in the presence of an elevated hematocrit. Erythrocytosis due to a germline homozygous missense variant at nucleotide c.598C->T, p.R200W in the *VHL* gene has been found endemically in the Chuvash region of Russia, leading individuals with this variant to be labeled as having Chuvash polycythemia (CP), although further studies have determined that this variant can be found in other ethnic groups as well. These patients are at an increased risk to develop cerebrovascular and embolic complications. Heterozygous carriers are typically unaffected.

## Reference Values

An interpretive report will be provided.

## Interpretation

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics recommendations as a guideline.(1) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and predictions made by these tools may change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

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## Cautions

Some individuals who have involvement of the von Hippel-Lindau (*VHL*) gene may have a pathogenic variant that is not identified by the methods performed (eg, promoter variants, deep intronic variants). The absence of a variant, therefore, does not eliminate the possibility of VHL disease. For predictive testing of asymptomatic individuals, it is important to first document the presence of a pathogenic gene variant in an affected family member.

Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

In some cases, DNA variants of undetermined significance may be identified. Rarely, sequence variants in primer- or probe-binding sites can result in false-negative test results (DNA sequencing) or either false-positive or false-negative results (multiplex ligation-dependent probe amplification [MLPA]; deletion screening), due to selective allelic drop-out. False-negative or false-positive results can occur in MLPA deletion screening assays due to poor DNA quality. If results obtained do not match the clinical findings, additional testing should be considered.

In addition to disease-related probes, the multiplex ligation-dependent probe amplification technique utilizes probes localized to other chromosomal regions as internal controls. In certain circumstances, these control probes may detect other diseases or conditions for which this test was not specifically intended. Results of the control probes are not normally reported. However, in cases where clinically relevant information is identified, the ordering physician will be informed of the result and provided with recommendations for any appropriate follow-up testing.

Unless reported or predicted to cause disease, alterations found deep in the intron or alterations that do not result in an amino acid substitution are not reported. These and common benign variants identified for this patient are available upon request.

## Supportive Data

Accuracy of this assay was assessed by sequencing 25 specimens from patients with clear-cell renal cell carcinoma (cRCC) of which 6 (24%) showed pathogenic variants. These results are in agreement with published estimates of pathogenic variant rates of 29% to 61% for von Hippel-Lindau (*VHL*) in cRCC. Additionally, 2 specimens with known variants were tested. Sequences were 100% concordant with published data. Both inter- and intraassay testing showed 100% consistency in sequencing. Fifteen normal specimens tested; all showed 100% normal sequences.

Multiplex ligation-dependent probe amplification (MLPA) analysis was tested using 5 specimens with known sequences. Three of the 5 had large deletions. All specimens showed 100% concordance with published results and with inter- and intra-assay testing. An additional study was conducted in which 50 normal specimens were tested for deletions of *VHL*. All specimens were normal.

## Clinical Reference

1. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-423
2. Online Mendelian inheritance in Man-OMIM. Available at [www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=193300](http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=193300)
3. Universal Mutation database-UMD-VHL mutations database page. Available at [www.umd.be:2020/](http://www.umd.be:2020/)
4. Maher ER, Kaelin WG Jr: von Hippel-Lindau disease (Reviews in Molecular Medicine). *Medicine* 1997;76:381-391
5. Pack SD, Zbar B, Pak E, et al: Constitutional von Hippel-Lindau (*VHL*) gene deletions detected in VHL families by fluorescence in situ hybridization. *Cancer Res* 1999;59:5560-5564

6. Richards FM: Molecular pathology of von Hippel-Lindau disease and the *VHL* tumor suppressor gene. *Expert Rev Mol Med* 2001;3:1-27
7. Hes FJ, Hoppener JW, Lips CJ: Clinical review 155: pheochromocytoma in von Hippel-Lindau disease. *J Clin Endocrinol Metab* 2003;88:969-974
8. Ong KR, Woodward ER, Killick P, et al: Genotype-phenotype correlations in von Hippel-Lindau disease. *Hum Mutat* 2007;28:143-149
9. Lonser RR, Glenn GM, McClellan W, et al: Von Hippel-Lindau disease. *Lancet* 2003;361:2059-2067
10. Stebbins CE, Kaelin WG, Pavletich NP: Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function. *Science* 1999;284:455-461
11. Frantzen C, Links TP, Giles RH: Von Hippel-Lindau Disease. In *GeneReviews*. Edited by RA Pagon, MP Adam, HH Ardinger, et al: University of Washington, Seattle. 1993-2015. 2000 May 17. Updated 2012 June 21. Available at [www.ncbi.nlm.nih.gov/books/NBK1463/](http://www.ncbi.nlm.nih.gov/books/NBK1463/)

## Performance

### Method Description

Bidirectional sequence analysis was performed to test for the presence of sequence variants in the 3 coding exons and intron/exon boundaries of the *VHL* gene (GenBank accession number NM\_000551; build GRCh37 [hg19]). Additionally, multiplex ligation dependent probe amplification (MLPA) was used to test for the presence of large genomic deletions and duplications in the *VHL* gene. (Unpublished Mayo method)

### PDF Report

No

### Day(s) Performed

Varies

### Report Available

14 to 20 days

### Specimen Retention Time

Whole Blood: 2 weeks (if available) Extracted DNA: 3 months

### 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 was developed and its performance characteristics 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**

81404-*VHL* (von Hippel-Lindau tumor suppressor) (eg, von Hippel-Lindau familial cancer syndrome), full gene sequence

81403-*VHL* duplication/deletion

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
VHLZ	VHL Gene, Full Gene Analysis	82533-1

Result ID	Test Result Name	Result LOINC Value
37453	Result Summary	50397-9
37454	Result	82939-0
37455	Interpretation	69047-9
37456	Additional Information	48767-8
37457	Specimen	31208-2
37458	Source	31208-2
37459	Released By	18771-6