Test Definition: 2OHGP

2-Hydroxyglutaric Aciduria Gene Panel, Varies

Overview

Useful For

Follow up for abnormal biochemical results suggestive of 2-hydroxyglutaric aciduria

Establishing a molecular diagnosis for patients with 2-hydroxyglutaric aciduria

Identifying variants within genes known to be associated with 2-hydroxyglutaric aciduria, allowing for predictive testing of at-risk family members

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in 4 genes associated with 2-hydroxyglutaric aciduria: *D2HGDH*, *IDH2*, *L2HGDH*, *SLC25A1*. See Targeted Genes and Methodology Details for 2-Hydroxyglutaric Aciduria Gene Panel in Special Instructions and Method Description for additional details.

Identification of a pathogenic variant may assist with diagnosis, prognosis, clinical management, familial screening, and genetic counseling for 2-hydroxyglutaric aciduria.

Additional first tier testing may be considered/recommended. For more information see Ordering Guidance.

Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture testing will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Special Instructions

- Molecular Genetics: Biochemical Disorders Patient Information
- Informed Consent for Genetic Testing
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions
- Targeted Genes and Methodology Details for 2-Hydroxyglutaric Aciduria Gene Panel

Method Name

Sequence Capture and Targeted Next-Generation Sequencing followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type
Varies

Ordering Guidance
The recommended first-tier test for 2-hydroxyglutaric aciduria is urine organic acids; order OAU / Organic Acids Screen, Random, Urine.

Customization of this panel and single gene analysis for any gene present on this panel is available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies.

Shipping Instructions
Specimen preferred to arrive within 96 hours of collection.

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.

Submit only 1 of the following specimens:

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. **Do not aliquot**.
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 14 days

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
Specimen Volume: 4-mm punch
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblast

Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred)/Refrigerated (<24 hours)
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Blood spot
Supplies: Card-Blood Spot Collection (Filter Paper) (T493)
Container/Tube:
Preferred: Collection card (Whatman Protein Saver 903 Paper)
Acceptable: PerkinElmer 226 (formerly Ahlstrom 226) filter paper, or blood spot collection card
Specimen Volume: 5 Blood spots
Collection Instructions:
1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect Dried Blood Spot Samples.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry.
Specimen Stability Information: Ambient (preferred)/Refrigerated
Additional Information:
1. Due to lower concentration of DNA yielded from blood spots, it is possible that additional specimen may be required to complete testing.
2. For collection instructions, see Blood Spot Collection Instructions.
3. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777).
4. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800).

Specimen Type: Saliva
Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.
Supplies: Saliva Swab Collection Kit (T786)
Specimen Volume: 1 swab
Collection Instructions: Collect and send specimen per kit instructions.
Specimen Stability Information: Ambient 30 days
Additional Information: Due to lower concentration of DNA yielded from saliva, it is possible that additional specimen may be required to complete testing.

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. Molecular Genetics: Biochemical Disorders Patient Information (T527) in Special Instructions
3. If not ordering electronically, complete, print, and send a Biochemical Genetics Test Request (T798) with the specimen.

Specimen Minimum Volume
See Specimen Required

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

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information
The 2-hydroxyglutaric aciduria disorders are a group of cerebral organic acidurias that present biochemically with an elevation of 2-hydroxyglutaric acid (2-HG) in the urine. There are two enantiomers or forms of 2-hydroxyglutaric acid, the D-form and the L-form. Depending on the genetic defect, individuals may have an elevation of one or both forms of 2-HG. Routine organic acid analysis (OAU / Organic Acids Screen, Random, Urine), while able to detect 2-HG, is unable to distinguish between the two enantiomers; however, they can be separated with more specialized biochemical testing.

L-2-hydroxyglutaric aciduria (L-2-HGA) is caused by defects in *L2HGDH* and is characterized by progressive cerebellar ataxia and intellectual disability, seizures, and macrocephaly beginning in infancy or early childhood. Symptoms worsen over time leading to severe disability by early adulthood. Magnetic resonance imaging findings include subcortical leukoencephalopathy, generalized cerebellar and cerebral atrophy, and atrophy of the corpus callosum.

D-2-hydroxylglutaric aciduria (D-2-HGA) is characterized by elevated levels of D-2-hydroxyglutaric acid and typically manifests with developmental delay, seizures, and hypotonia, though can vary widely from asymptomatic to severe. There are 2 types of D-2-HGA depending on the genetic cause. D-2-HGA can either be autosomal recessive, resulting from variants in *D2HGDH* causing reduced enzymatic activity (Type I) or autosomal dominant gain-of-function variants in *IDH2* causing overproduction of D-2-HG (Type II).

D,L-2-hydroxylglutaric aciduria is the most severe of the 3 and caused by defects in *SLC25A1*, which encodes the mitochondrial citrate carrier. It is characterized by neonatal-onset encephalopathy with severe muscular weakness, intractable seizures, respiratory distress, and lack of psychomotor development resulting in early death. Because of the genetic heterogeneity of the 2-hydroxyglutaric acidurias and the specialized biochemical testing needed to distinguish among the conditions, this genetic panel, which incorporates *D2HGDH*, *L2HGDH*, *IDH2*, and *SLC25A1*, is an efficient way to diagnose these conditions.

Reference Values
An interpretive report will be provided.

Interpretation
All detected alterations are evaluated according to American College of Medical Genetics and Genomics (ACMG) recommendations.(1) Variants are classified based on known, predicted, or possible pathogenicity and reported with

interpretive comments detailing their potential or known significance.

Cautions

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

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of at least one reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller indels.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

Genes may be added or removed based on updated clinical relevance. Refer to the Targeted Genes and Methodology Details for 2-Hydroxyglutaric Aciduria Gene Panel for the most up to date list of genes included in this test. For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing

patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline. Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

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 periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgement.

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 May;17(5):405-424
2. Nota B, Struys EA, Pop A, et al: Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. Am J Hum Genet. 2013;92:627-631. doi: 10.1016/j.ajhg.2013.03.009
3. Kranendijk M, Struys EA, van Schaftingen E, et al: IDH2 mutations in patients with D-2-hydroxyglutaric aciduria. Science. 2010 Oct 15;330(6002):336. doi: 10.1126/science.1192632
4. Kranendijk M, Struys EA, Salomons GS, Van der Knaap MS, Jakobs C: Progress in understanding 2-hydroxyglutaric acidurias. J Inherit Metab Dis. 2012;35(4):571-587. doi: 10.1007/s10545-012-9462-5
5. Pop A, Struys EA, Jansen EEW, et al: D-2-hydroxyglutaric aciduria Type I: functional analysis of D2HGDH missense variants. Hum Mutat. 2019; 40(7):975-982. doi: 10.1002/humu.23751

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing is performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known pathogenic variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for insertions/deletions (indels) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction (PCR)-based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed. See Targeted Genes and Methodology Details for 2-Hydroxyglutaric Aciduria Gene Panel in Special Instructions for details regarding the targeted genes analyzed.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. See Targeted Genes and Methodology Details for 2-Hydroxyglutaric Aciduria Gene Panel in Special Instructions for details regarding the specific gene regions not routinely covered.(Unpublished Mayo method)

Genes analyzed: *D2HGDH, IDH2, L2HGDH, SLC25A1*

PDF Report
Supplemental

Day(s) Performed
Varies

Report Available
28 to 42 days

Specimen Retention Time
Whole Blood: 2 weeks (if available); Extracted DNA: 3 months; Blood spots/Saliva: 1 month

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
81479
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
2OHGP	2-OH Glutaric Aciduria Gene Panel	105341-2

Result ID	Test Result Name	Result LOINC® Value

608764	Test Description	62364-5
608765	Specimen	31208-2
608766	Source	31208-2
608767	Result Summary	50397-9
608768	Result	82939-0
608769	Interpretation	69047-9
608770	Resources	99622-3
608771	Additional Information	48767-8
608772	Method	85069-3
608773	Genes Analyzed	48018-6
608774	Disclaimer	62364-5
608775	Released By	18771-6