MAYO CLINIC LABORATORIES

Test Definition: HCHLG

Hypercholesterolemia Gene Panel, Varies

Overview

Useful For

Providing a genetic evaluation for patients with a personal or family history suggestive of familial hypercholesterolemia (FH), sitosterolemia, or other monogenic forms of inherited hypercholesterolemia

Establishing a diagnosis of FH, sitosterolemia, or other monogenic forms of inherited hypercholesterolemia

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 12 genes associated with familial hypercholesterolemia (FH), sitosterolemia, and other monogenic forms of inherited hypercholesterolemia: *ABCG5, ABCG8, APOB, APOE, CETP, CYP27A1, LDLR, LDLRAP1, LIPA, LPL, LRP6,* and *PCSK9*. See Targeted Genes and Methodology Details for Hypercholesterolemia Gene Panel and Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for FH, sitosterolemia, and other monogenic forms of inherited hypercholesterolemia.

This test also reports homozygous status for the *APOE* E2 allele, a risk allele for type III hyperlipoproteinemia. This test does NOT report other, non-cardiovascular *APOE* disease associations, including Alzheimer disease.

Prior Authorization is available for this assay.

Testing Algorithm

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

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Hereditary Dyslipidemia Patient Information
- Targeted Genes and Methodology Details for Hypercholesterolemia Gene Panel
- Hypercholesterolemia Gene Panel (HCHLG) Prior Authorization Ordering Instructions

Method Name

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

Sanger Sequencing.

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Upon request and after initial testing is complete, WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies may be added to this test. To obtain more information about this option or add WESPR testing, call 800-533-1710.

Customization of this panel and single gene analysis for any gene present on this panel are available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies. To modify this panel via CGPH, please use the Cardiovascular/Connective Tissue/Dyslipidemia/Cerebrovascular/Primary Ciliary Dyskinesia disease state for step 1 on the Custom Gene Ordering Tool.

Targeted testing for familial variants (also called site-specific or known mutations testing) is available for the genes on this panel. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Necessary Information

Prior Authorization is available, **but not required**, for this test. If proceeding with the prior authorization process, submit the required form with the specimen.

Specimen Required

Patient Preparation: A previous hematopoietic stem cell transplant from an allogenic donor will interfere with testing. For instructions for testing patients who have received a hematopoietic stem cell transplant, call 800-533-1710.

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Green top (Sodium heparin)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:

1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Saliva
Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.
Supplies:
DNA Saliva Kit High Yield (T1007)
Saliva Swab Collection Kit (T786)
Container/Tube:
Preferred: High-yield DNA saliva kit
Acceptable: Saliva swab
Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786
Collection Instructions: Collect and send specimen per kit instructions.
Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days
Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Specimen Type: Cultured fibroblasts
Source: Skin
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Tissue biopsy
Supplies: Hank's Solution (T132)
Container/Tube: Sterile container with sterile Hank's balanced salt solution, Ringer's solution, or normal saline
Specimen Volume: 0.5 to 3 cm(3) or larger
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for

specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are 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 filter paper or blood spot collection card
Specimen Volume: 2 to 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 a Dried Blood Spot Sample.
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. Blood spot specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from blood spots, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.
2. Due to lower concentration of DNA yielded from blood spot, it is possible that additional specimen may be required to complete testing.
3. For collection instructions, see Blood Spot Collection Instructions
4. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
5. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

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:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
2. Hereditary Dyslipidemia Patient Information
3. Hypercholesterolemia Gene Panel (HCHLG) Prior Authorization Ordering Instructions
4. If not ordering electronically, complete, print, and send a Cardiovascular Test Request Form (T724) 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
Hypercholesterolemia, characterized by elevated cholesterol levels in the blood, can be an inherited (genetic) condition or can be acquired due to either lifestyle factors such as diet or exercise or an underlying medical condition. The genetic influence on cholesterol levels can be complex, with monogenic (single gene) or polygenic (many genes) etiologies. This gene panel assesses for only monogenic causes of hypercholesterolemia.

Autosomal dominant familial hypercholesterolemia (FH) is the most common inherited hypercholesterolemia condition and is characterized by elevated levels of low-density lipoprotein cholesterol (LDL-C), leading to increased risk of premature cardiovascular disease and myocardial infarction. Affected individuals may also exhibit xanthomas that worsen with age. The majority of cases of FH are due to loss-of-function variants in the *LDLR* gene, but FH can also be caused by loss-of-function variants in the *APOB* gene or gain-of-function variants in the *PCSK9* gene.(1,2) The most common form of FH is autosomal dominant heterozygous familial hypercholesterolemia (heFH) caused by single, heterozygous variants in *LDLR*, *APOB*, or *PCSK9*. A more severe form of FH, homozygous FH (hoFH), results when an individual inherits two variants in one of the three associated genes, either in the homozygous or compound heterozygous state.(1,2) Recent studies suggest that the prevalence of heFH is as high as 1:200 to 1:250, and the prevalence of hoFH is between 1:160,000 to 1:250,000.(1,2)

Autosomal recessive FH, due to biallelic (homozygous or compound heterozygous) variants in the *LDLRAP1* gene is a rare form of inherited hypercholesterolemia and is typically characterized by LDL-C levels above 400 mg/dL as well as cutaneous and tendon xanthomas. While *LDLRAP1*-associated hypercholesterolemia is rare, emerging evidence suggests heterozygous carriers of disease-causing *LDLRAP1* variants may also present with hypercholesterolemia.(3,4,5,6)

Sitosterolemia is a rare, autosomal recessive inherited lipid metabolism disease caused by biallelic variants in the *ABCG5*

or *ABCG8* genes. The condition is characterized by increased intestinal absorption of plant sterols and has similar clinical manifestations to familial hypercholesterolemia, including elevated LDL-C, xanthomas, increased risk of premature cardiovascular disease, and increased risk of myocardial infarction. The prevalence of sitosterolemia has not been well established.(7)

Other, more rare genetic conditions that may present with elevated cholesterol levels include autosomal recessive lysosomal acid lipase deficiency due to variants in the *LIPA* gene; autosomal dominant hyperalphalipoproteinemia due to variants in the *CETP* gene; autosomal recessive lipoprotein lipase deficiency due to variants in the *LPL* gene; and atypical autosomal dominant hypercholesterolemia due to variants in the *APOE* gene.(8) In addition, emerging evidence suggests that the *LRP6* gene may be associated with autosomal dominant coronary artery disease, a condition in which hypercholesterolemia is a feature.(9,10)

Cerebrotendinous xanthomatosis is a rare, autosomal dominant condition caused by disease-causing variants in the *CYP27A1* gene. Individuals with cerebrotendinous xanthomatosis do not typically have elevated plasma cholesterol levels but do have clinical manifestations that overlap with hypercholesterolemia conditions, including xanthomas and increased risk for premature cardiovascular disease and myocardial infarction.(11)

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(12) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:
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 a 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 delins.

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.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic mutations 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. For the most up to date list of genes included in this test and 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 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 professionals 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.(12) 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 judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. Incidental findings may include, but are not limited to, results related to the sex chromosomes. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

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6. Junna N, Ruotsalainen S, Ripatti P, FinnGen, Ripatti S, Widen E. Novel Finnish-enriched variants causing severe hypercholesterolemia and their clinical impact on coronary artery disease: Atherosclerosis. 2023:117327
7. Tzavella E, Hatzimichael E, Kostara C, Bairaktari E, Elisaf M, Tsimihodimos V: Sitosterolemia: A multifaceted metabolic disorder with important clinical consequences. J Clin Lipidol. 2017;11(4):1095-1100. doi:10.1016/j.jacl.2017.04.116
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10. Singh R, Smith E, Fathzadeh M, et al. Rare nonconservative LRP6 mutations are associated with metabolic syndrome. Hum Mutat. 2013;34(9):1221-1225. doi:10.1002/humu.22360
11. Nie S, Chen G, Cao X, Zhang Y. Cerebrotendinous xanthomatosis: a comprehensive review of pathogenesis, clinical manifestations, diagnosis, and management. Orphanet J Rare Dis. 2014;9:179. doi:10.1186/s13023-014-0179-4
12. 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(5):405-424

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are 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 disease-causing 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 deletions-insertions (delins) 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 -based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

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 Hypercholesterolemia Gene Panel or details regarding the targeted genes analyzed for each test and specific gene regions not routinely covered.(Unpublished Mayo method)

Genes analyzed: *ABCG5, ABCG8, APOB, APOE, CETP, CYP27A1, LDLR, LDLRAP1, LIPA, LPL, LRP6*, and *PCSK9*

PDF Report
No

Day(s) Performed
Varies

Report Available
21 -to 35 days

Specimen Retention Time
Whole blood: 28 days (if available); Saliva: 30 days (if available); Extracted DNA: 3 months; Blood spots: 1 year

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
81406 x2
81407
81479

81479 (if appropriate for government payers)

Prior Auhtorization

Insurance preauthorization is available for this testing; forms are available.

Patient financial assistance may be available to those who qualify. Patients who receive a bill from Mayo Clinic Laboratories will receive information on eligibility and how to apply.

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
HCHLG	Hypercholesterolemia Gene Panel	107543-1

Result ID	Test Result Name	Result LOINC® Value
617268	Test Description	62364-5
617269	Specimen	31208-2
617270	Source	31208-2
617271	Result Summary	50397-9
617272	Result	82939-0
617273	Interpretation	69047-9
617274	Additional Results	82939-0
617275	Resources	99622-3
617276	Additional Information	48767-8
617277	Method	85069-3
617278	Genes Analyzed	48018-6
617279	Disclaimer	62364-5
617280	Released By	18771-6