

## Overview

### Useful For

Confirming a clinical diagnosis of familial hypercholesterolemia or sitosterolemia

Cascade screening of at-risk family members and early diagnosis, treatment, and dietary modifications

Ascertaining carrier status of family members of individuals diagnosed with familial hypercholesterolemia for genetic counseling purposes

### Genetics Test Information

This test includes next-generation sequencing (NGS) and supplemental Sanger sequencing to evaluate for variants in the *ABCG5*, *ABCG8*, *APOB*, *LDLR*, *LDLRAP1*, and *PCSK9* genes. Additionally, NGS is used to test for the presence of large deletions and duplications in a subset of genes.

Identification of a pathogenic variant may assist with prognosis, clinical management, familial screening, and genetic counseling.

### Special Instructions

- [Informed Consent for Genetic Testing](#)
- [Familial/Autosomal Dominant Hypercholesterolemia Patient Information](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)

### Method Name

Sequence Capture and Targeted Next-Generation Sequencing Followed by Polymerase Chain Reaction (PCR) and Supplemental Sanger Sequencing or quantitative PCR

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

**Ordering Guidance**

[Targeted testing for familial variants \(also called site-specific or known mutations testing\) is available for the genes on this panel. See FMTT / Familial Mutation, Targeted Testing, Varies.](#)

**Shipping Instructions**

Specimen preferred to arrive within 96 hours of collection.

**Necessary Information**

1. [Familial/Autosomal Dominant Hypercholesterolemia Patient Information \(T637\)](#) is strongly recommended, but not required, to be filled out and sent with the specimen. This information aids in providing a more thorough interpretation of test results. Ordering providers are strongly encouraged to complete the form and send it with the specimen.

2. Include physician name and phone number with specimen.

**Specimen Required**

Submit only 1 of the following specimens:

**Specimen Type:** Whole blood

**Container/Tube:** Lavender top (EDTA)

**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:** Extracted DNA

**Container/Tube:** 2 mL screw top tube

**Specimen Volume:** 100 mcL (microliters)

**Collection Instructions:**

1. The preferred volume is 100 mcL at a concentration of 250 ng/mcL.

2. Include concentration and volume on tube.

**Specimen Stability Information:** Frozen (preferred)/Ambient/Refrigerated

### 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. [Familial/Autosomal Dominant Hypercholesterolemia Patient Information \(T637\)](#) is recommended. See Special Instructions.

3. If not ordering electronically, complete, print, and send a [Cardiovascular Test Request \(T724\)](#) with the specimen.

### Reject Due To

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

### Specimen Minimum Volume

Whole blood: 1 mL

### Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies (preferred)		

### Clinical & Interpretive

#### Clinical Information

Familial hypercholesterolemia (FH) is an inherited condition that results in elevated levels of low-density lipoprotein cholesterol (LDL-C). FH is associated with premature cardiovascular disease and myocardial infarction. Early diagnosis and treatment help to mitigate these risks.

The most common form of FH is autosomal dominant heterozygous familial hypercholesterolemia (heFH) caused by loss-of-function variants found in the *LDLR* gene. Recent studies suggest that the prevalence of heFH is as high as 1 in 200 to 250 and may be even higher in some founder populations such as those of French Canadian, Ashkenazi Jewish, Lebanese, and South African descent. In general, FH heterozygotic individuals have 2-fold elevations in plasma cholesterol and develop coronary atherosclerosis after the age of 30. Hundreds of variants have been identified in the

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*LDLR* gene. The majority of variants in the *LDLR* gene are small point variants (missense, nonsense) or small insertions or deletions. Most of these variants are detectable by sequencing of the *LDLR* gene. An additional 10% of variants in the *LDLR* gene are large intragenic rearrangements, such as large exon deletions and duplications. Absent or decreased LDL-receptor results in a reduced capacity to clear LDL from circulation.

A more severe form of familial hypercholesterolemia can also be caused by homozygous or compound heterozygous (biallelic) variants in the *LDLR* gene. This condition is referred to as homozygous familial hypercholesterolemia (hoFH). Recent studies suggest the prevalence of hoFH is as high as 1 in 250,000. Individuals with homozygous FH typically have severe hypercholesterolemia (generally >650 mg/dL) with the presence of cutaneous xanthomas prior to 4 years of age, childhood coronary heart disease, and oftentimes, death from myocardial infarction prior to 20 years of age.

Another form of autosomal dominant hypercholesterolemia is called familial defective apolipoprotein B-100 (FDB). FDB is caused by loss-of-function variants in the *APOB* gene that reduce the binding affinity between the protein encoded by *APOB* (apolipoprotein B-100) and the protein encoded by *LDLR* (low-density lipoprotein receptor). Individuals with heterozygous *APOB* variants have elevated LDL-C, although the elevation is typically less than that observed in individuals with heterozygous *LDLR* variants; increased rates of coronary artery calcifications; and premature myocardial infarction. Approximately 1 in 1667 Northern European Caucasians carry the R3500Q (HGVS: c.10580G>A, p.Arg3527Gln) variant in the *APOB* gene, and approximately 1 in 800 East Asians carry the R3500W (HGVS: c.10579C>T, p.Arg3527Trp) variant in the *APOB* gene. Although other variants resulting in autosomal dominant hypercholesterolemia have been described in *APOB*; most appear within a hotspot (or frequently affected) region surrounding the p.Arg3527 residue. Homozygosity and compound heterozygosity for *APOB* variants can also occur; these individuals typically have LDL-C levels above 300 mg/dL. Individuals with homozygous FDB are sometimes misdiagnosed with heFH.

Autosomal dominant hypercholesterolemia can also be caused by gain-of-function variants in the *PCSK9* gene. Variants in this gene are rare, but when present, they result in increased PCSK9 protein levels, leading to increased degradation of low-density lipoprotein receptors. Recently, drugs targeting PCSK9 (called PCSK9 inhibitors) have been developed. These drugs inhibit the binding of PCSK9 to LDL-receptors, thus reducing degradation of LDL-receptors and increasing the amount of LDL-C cleared in certain individuals.

Loss-of-function variants in the *LDLRAP1* gene cause a rare form of familial hypercholesterolemia called autosomal recessive familial hypercholesterolemia. Once LDL-C binds to the LDL-receptor the *LDLRAP1* protein binds to the complex and internalization of the complex, which results in degradation of either the LDL particle or the entire complex occurs. Unlike autosomal dominant hypercholesterolemia caused by heterozygous variants in *LDLR*, *APOB*, and *PCSK9*, biallelic variants in *LDLRAP1* are required for elevated LDL-C levels. Individuals with homozygous or compound heterozygous *LDLRAP1* variants typically have LDL-C levels above 400 mg/dL, cutaneous and tendon xanthomas, and coronary artery disease. Heterozygosity for *LDLRAP1* variants does not result in elevated cholesterol levels, so the parents of children with biallelic *LDLRAP1* variants are typically normocholesterolemic.

Sitosterolemia, a rare autosomal recessive inherited lipid metabolism disease, is caused by biallelic variants in the *ABCG5* or *ABCG8* genes and has similar clinical manifestations to familial hypercholesterolemia. Sitosterolemia is characterized by increased intestinal absorption of plant sterols (15% to 60% compared to <5% in unaffected individuals). These individuals also typically have elevated total cholesterol and LDL cholesterol levels, although individuals with normal LDL-C levels have also been reported. Untreated individuals with sitosterolemia exhibit tendon and tuberous xanthomas in childhood, premature atherosclerosis, myocardial infarction, and coronary heart disease. At least one report of an individual with sitosterolemia being misdiagnosed with homozygous FH has been reported. The authors noted that the Dutch Lipid Clinic Network diagnostic (DLCN) criteria could not distinguish between homozygous FH and sitosterolemia in this individual.

Identification of the genetic cause of an individual's clinical features helps to determine the appropriate treatment for their clinical features. Treatment is aimed at lowering plasma LDL levels and plasma sterol levels. Common treatments included statins, LDL apheresis, dietary modifications, and more recently PCSK9 inhibitors. Screening of at-risk family members allows for effective primary prevention by instituting appropriate therapy and dietary modifications at an early stage.

**Table 1. Genes included in this panel**

Gene symbol	Protein	OMIM	Inheritance	Phenotype disorder
<i>ABCG5</i>	ATP-binding cassette, subfamily G, member 5	60545 9	AR	Sitosterolemia
<i>ABCG8</i>	ATP-binding cassette, subfamily G, member 8	60546 0	AR	Sitosterolemia
<i>APOB</i>	Apolipoprotein B	10773 0	AD  AR	Hypercholesterolemia, due to ligand-defective apo B  Hypobetalipoproteinemia
<i>LDLR</i>	Low density lipoprotein receptor	60694 5	AD	Hypercholesterolemia, familial
<i>LDLRAP1</i>	Low density lipoprotein receptor adaptor protein 1	60574 7	AR	Hypercholesterolemia, familial, autosomal recessive
<i>PCSK9</i>	Proprotein convertase, subtilisin/kexin-type, 9	60778 6	AD	Hypercholesterolemia, familial, 3

AR: autosomal recessive

**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 and Genomics (ACMG) 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.

**Cautions****[Clinical Correlations:](#)**

Some individuals who have involvement of 1 or more of the genes on the panel may have a variation 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 familial hypercholesterolemia (FH) or a related disorder.

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.

If testing was performed because of a family history of FH or a related disorder, it is often useful to first test an affected family member. Identification of a pathogenic variant in an affected individual would allow for more informative testing of at-risk individuals.

**[Technical Limitations:](#)**

Next-generation sequencing may not detect all types of genetic variants. Additionally, rare alterations (ie, polymorphisms) may be present that could lead to false-negative or false-positive results. If the patient has had an allogeneic blood or marrow transplant or a recent (ie, <6 weeks from time of sample collection) heterologous blood transfusion, these results may be inaccurate due to the presence of donor DNA.

**[Reclassification of Variants Policy:](#)**

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At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that are detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

Contact the laboratory if additional information is required regarding the transcript or human genome assembly used for the analysis of this patient's results.

**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. Youngblom E, Pariani M, Knowles JW: Familial hypercholesterolemia. In: [Adam MP, Ardinger HH, Pagon RA, et al.](#) eds. *GeneReviews* [Internet]. University of Washington, Seattle; 2014. Updated December 2016. Accessed February 16, 2018. Available at [www.ncbi.nlm.nih.gov/books/NBK174884/](http://www.ncbi.nlm.nih.gov/books/NBK174884/)
3. Singh S, Bittner V: Familial hypercholesterolemia-epidemiology, diagnosis, and screening. *Curr Atheroscler Rep* 2015;17(2):482
4. Nordestgaard BG, Chapman MJ, Humphries SE, et al: Familial hypercholesterolemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J.* 2013;34:3478-3490
5. Vandrovcova J, Thomas ER, Atanur SS, et al: The use of next-generation sequencing in clinical diagnosis of familial hypercholesterolemia. *Genet Med.* 2013;15:12:948-957
6. Bouhaire VE, Goldberg AC: Familial hypercholesterolemia. *Cardiol Clin.* 2015;33(2):169-179
7. Andersen LH, Miserez AR, Ahmad Z, Andersen RL: Familial defective apolipoprotein B-100: A review. *J Clin Lipidol.* 2016;10(6):1297-1302
8. Priest JR, Knowles JW: Standards of evidence and mechanistic inference in autosomal recessive hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2016;36(8):1465-1466
9. Fellin R, Arca M, Zuliani G, et al: The history of autosomal recessive hypercholesterolemia (ARH). From clinical observations to gene identification. *Gene.* 2015;555(1):23-32
10. Brinton EA, Hopkins PN, Hegele RA, et al: The association between hypercholesterolemia and sitosterolemia, and report of a sitosterolemia kindred. *J Clin Lipidol.* 2018 Jan-Feb;12(1):152-161
11. Wang W, Jiang L, Chen PP, et al: A case of sitosterolemia misdiagnosed as familial hypercholesterolemia: A 4-year follow-up. *J Clin Lipidol.* 2018 Jan-Feb;12(1):236-239

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**Performance****Method Description**

Next-generation sequencing (NGS) is performed using an Illumina instrument with paired-end reads. The DNA is prepared for NGS using a custom Agilent SureSelect Target Enrichment System. Data is analyzed with a bioinformatics software pipeline for sequence variants and the presence of large intragenic deletions and duplications. Supplemental Sanger sequencing or quantitative polymerase chain reaction (qPCR) may be performed occasionally in regions where NGS is insufficient for data capture or not specific enough to correctly identify a variant. Sanger sequencing or qPCR may also be used for confirmatory testing.(Unpublished Mayo method)

Genes analyzed: *ABCG5*, *ABCG8*, *APOB*, *LDLR*, *LDLRAP1*, and *PCSK9*.

**PDF Report**

No

**Specimen Retention Time**

2 months

**Performing Laboratory Location**

Rochester

**Fees & Codes****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 US Food and Drug Administration.

**CPT Code Information**

81479

81406 x 2

81407

**LOINC® Information**



Test ID	Test Order Name	Order LOINC Value
FHRGP	Hypercholesterolemia Gene Panel	In Process

Result ID	Reporting Name	LOINC®
601697	Gene(s) Evaluated	48018-6
601708	Result Summary	50397-9
601709	Result Details	82939-0
601710	Interpretation	69047-9
601711	Additional Information	48767-8
601712	Method	85069-3
601713	Disclaimer	62364-5
601714	Reviewed By	18771-6