

Overview

Useful For

Diagnosis of the subset of mitochondrial diseases that results from variants in the mitochondrial genome

A second-tier test for patients in whom previous targeted gene variant analyses for specific mitochondrial disease-related genes were negative

Identifying variants within genes of the mitochondrial genome that are known to be associated with mitochondrial disease, allowing for predictive testing of at-risk family members

Genetics Test Information

[This test includes amplification of the entire mitochondrial genome by long-range polymerase chain reaction \(LR-PCR\) followed by sequencing on the next-generation sequencing \(NGS\) platform to evaluate for variants within the mitochondrial genome.](#)

Highlights

Next-generation sequencing (NGS) is used to test for the presence of variants, including: 13 protein coding genes, 22 transfer RNA genes, and 2 ribosomal RNA genes, within the mitochondrial genome

Large deletions within the mitochondrial genome and their locations are determined from the NGS data.

This assay is only useful for detecting mitochondrial genomic variants. Depletion of mitochondrial DNA levels or variants in mitochondrial genes encoded by the nuclear genome is not within the scope of this assay.

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
FIBR	Fibroblast Culture	Yes	No
CRYOB	Cryopreserve for Biochem Studies	No	No

Testing Algorithm

If skin biopsy is received, fibroblast culture and cryopreservation for biochemical studies will be added at an additional charge.

The following algorithms are available in Special Instructions:

• [-Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm](#)

• [-Neuromuscular Myopathy Testing Algorithm](#)

Special Instructions

- [Muscle Biopsy Specimen Preparation](#)
- [Molecular Genetics: Biochemical Disorders Patient Information](#)
- [Informed Consent for Genetic Testing](#)
- [Blood Spot Collection Card-Spanish Instructions](#)

- [Blood Spot Collection Card-Chinese Instructions](#)
- [Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm](#)
- [Neuromuscular Myopathy Testing Algorithm](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)
- [Blood Spot Collection Instructions](#)

Method Name

Long-Range Polymerase Chain Reaction (LR-PCR) followed by Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen**Specimen Type**

Varies

Advisory Information

If testing for variants in the mitochondrial genes encoded by the nuclear genome is requested, order MITON / Mitochondrial Nuclear Gene Panel, Next-Generation Sequencing (NGS), Varies

Shipping Instructions

Ambient blood is preferred to arrive within 96 hours of collection.

Necessary Information

Provide a reason for referral with each specimen. The laboratory will not reject testing if this information is not provided, but appropriate testing and interpretation may be compromised or delayed.

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.

Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Cultured fibroblasts

Container/Tube: T-75 or T-25 flask

Specimen Volume: 1 full T-75 or 2 full T-25 flasks

Specimen Stability Information: Ambient (preferred)/Refrigerated <24 hours

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

Specimen Type: Muscle tissue biopsy

Supplies: Muscle Biopsy Kit (T541)

Collection Instructions: Prepare and transport specimen per instructions in [Muscle Biopsy Specimen Preparation](#) Sheet in Special Instructions.

Specimen Volume: 10-80mg

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

Specimen Type: Snap frozen nerve tissue biopsy

Collection Instructions: Prepare snap frozen tissue biopsy per surgical procedure

Specimen Volume: 0.25-0.5 cm

Specimen Stability Information: Frozen

Specimen Type: Blood spot

Supplies: Card-Blood Spot Collection (Filter Paper) (T493)

Preferred: Collection card (Whatman Protein Saver 903 Paper)

Acceptable: Ahlstrom 226 filter paper or Supplemental Newborn Screening Card

Specimen Volume: 2 to 5 Blood Spots on collection card

Collection Instructions:

1. An alternative blood collection option for a patient older than 1 year of age is finger stick.

2. Let blood dry on the filter paper at ambient temperature in a horizontal position for 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. For collection instructions, see [Blood Spot Collection Instructions](#) in Special Instructions.
2. For collection instructions in Spanish, see [Blood Spot Collection Card-Spanish Instructions](#) (T777) in Special Instructions.
3. For collection instructions in Chinese, see [Blood Spot Collection Card-Chinese Instructions](#) (T800) in Special Instructions.

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 1 of the following forms with the specimen:

- [Neurology Specialty Testing Client Test Request](#) (T732)
- [Inborn Errors of Metabolism Test Request](#) (T798)

Specimen Minimum Volume

Blood: 1 mL
Muscle tissue biopsy: 20 mg
Nerve tissue biopsy: See Specimen Required.
Blood Spots: 5 punches-3 mm diameter

Reject Due To

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

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical and Interpretive

Clinical Information

The mitochondrion occupies a unique position in eukaryotic biology. First, it is the site of energy metabolism, without which aerobic metabolism and life as we know it would not be possible. Second, it is the sole subcellular organelle that is composed of proteins derived from 2 genomes, mitochondrial and nuclear. A group of hereditary disorders due to variants in either the mitochondrial genome or nuclear mitochondrial genes have been well characterized.

The diagnosis of mitochondrial disease can be particularly challenging as the presentation can occur at any age, involving virtually any organ system, and with widely varying severities. This test utilizes massively parallel sequencing, also termed next-generation sequencing (NGS), to determine the exact sequence of the entire 16,569 base-pair mitochondrial genome. The utility of this test is to assist in the diagnosis of the subset of mitochondrial diseases that result from variants in the mitochondrial genome (mtDNA). This includes certain types of myopathies and neuro-ophthalmologic diseases, such as mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), mitochondrial myopathy (MM), neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP), Leigh syndrome, Leber hereditary optic neuropathy (LHON), and chronic progressive external ophthalmoplegia (CPEO). In addition to the detection of single base changes with these disorders, large deletions, such as those associated with Kearns-Sayre or Pearson syndromes, are also detected. Variants in mitochondrial proteins that are encoded by genes in the nucleus, such as the enzymes of fatty acid oxidation, are not detected using this test.

In contrast to variants in nuclear genes, which are present in either 0, 1, or 2 copies, mitochondrial variants can be present in any fraction of the total organelles, a phenomenon known as heteroplasmy. Typically, the severity of disease presentation is a function of the degree of heteroplasmy. Individuals with a higher fraction of altered mitochondria present with more severe disease than those with lower percentages of altered alleles. The sensitivity for the detection of altered alleles in a background of wild-type (or normal) mitochondrial sequences by NGS is approximately 10%.

Reference Values

An interpretive report will be provided.

Interpretation

All detected alterations are evaluated according to American College of Medical Genetics and Genomics recommendations.⁽¹⁾ Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. The degree of heteroplasmy of each single nucleotide or INDEL (insertion/deletion) variant, defined as the ratio (percentage) of variant sequence reads to the total number of reads, will also be reported. Large deletions will be reported as either homoplasmic or heteroplasmic, but the degree of heteroplasmy will not be estimated, due to possible preferential amplification of the smaller deletion product by long-range PCR.

Cautions

Clinical Correlations:

A small percentage of individuals who have mitochondrial genome involvement may have a variant that is not identified by the methods performed. The absence of a variant, therefore, does not eliminate the possibility of a mitochondrial disease due to variant in the mitochondrial genome. Variants in mitochondrial genes encoded by the nuclear genome will not be detected with this assay. For predictive testing of asymptomatic individuals, it is important to first document the presence of a gene variant in an affected family member.

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.

Technical Limitations:

In some cases, DNA variants of undetermined significance may be identified.

Rare alterations exist that could lead to false-negative or false-positive results. If results obtained do not match the clinical findings, additional testing should be considered.

Evaluation Tools:

Multiple in-silico evaluation tools were used to assist in the interpretation of these results. These tools are updated regularly; therefore, changes to these algorithms may result in different predictions for a given alteration. Additionally, the predictability of these tools for the determination of pathogenicity is currently unvalidated.

Unless reported or predicted to cause disease, alterations in protein coding genes that do not result in an amino acid substitution are not reported. The mitochondrial haplogroup classification of the patient will be reported, but the individual nucleotide changes that define the haplogroup will not be reported. These and common alterations identified for this patient are available upon request.

Reclassification of Variants-Policy:

At this time, it is not standard practice for the laboratory to systematically review likely deleterious alterations 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.

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. Munnich A, Rotig A, Cormier-Daire V, Rustin P: Clinical presentation of respiratory chain deficiency. In: Valle D, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA eds. *The Online Metabolic and Molecular Basis of Inherited Disease.* McGraw-Hill; 2019. Accessed September 28, 2020.
<https://ommbid.mhmedical.com/content.aspx?bookid=2709§ionid=225086827>
3. Wallace DC, Lott MT, Brown MD, Kerstann K: Mitochondria and neuro-ophthalmologic diseases. In: Valle D, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA et al, eds. *The Online Metabolic and Molecular Basis of Inherited Disease.* McGraw-Hill; 2019. Accessed September 28, 2020.
<https://ommbid.mhmedical.com/content.aspx?bookid=2709§ionid=225088522>
4. Wong LJ: Molecular genetics of mitochondrial disorders. *Dev Disabil Res Rev.* 2010 Jun;16(2):154-162

Performance

Method Description

Next-generation sequencing (NGS) is used to test for the presence of variants within the mitochondrial genome (includes 13 protein coding genes, 22 transfer RNA genes, and 2 ribosomal RNA genes) and to determine the mitochondrial haplogroup of the patient. Large deletions within the mitochondrial genome are first detected by gel electrophoresis (as size-shifted polymerase chain reaction bands), and the locations of the deletions in the mtDNA are then determined from the NGS data.

The haplogroup is computed using the software package HaploGrep.(Kloss-Brandstatter A, Pacher D, Schonherr S, et al: HaploGrep: a fast and reliable algorithm for automatic classification of mitochondrial DNA haplogroups. Hum Mutat 2011 Jan;32:25-32) and PhyloTree.(van Oven M, Kayser M: Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 2009;30[2]:E386-E394 Available at www.phylotree.org)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday; Varies

Analytic Time

8 weeks

Maximum Laboratory Time

10 weeks

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

81460-Whole Mitochondrial Genome

81465-Whole Mitochondrial Genome Large Deletion Analysis

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

88240-Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
MITOP	Mitochondrial Full Genome Analysis	In Process

Result ID	Test Result Name	Result LOINC Value
55281	Result Summary	50397-9
55282	Result	82939-0
55283	Interpretation	69047-9
55284	Additional Information	48767-8
55285	Specimen	31208-2
55286	Source	31208-2
55287	Released By	18771-6