MAYO CLINIC LABORATORIES

Test Definition: MITOP

Mitochondrial Full Genome Analysis, Next-Generation Sequencing (NGS), Varies

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

Reflex Tests

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

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.

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.

The following algorithms are available:
-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

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.

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

NY State Available
Yes

Specimen

Specimen Type
Varies

Ordering Guidance
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
Molecular Genetics: Biochemical Disorders Patient Information (T527) is available to provide information useful for accurate test interpretation. At minimum, provide a reason for testing with each specimen. Although testing may proceed without this information, **ordering providers are strongly encouraged** to complete the form and send it with the specimen.

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 whole blood specimen in original tube. **Do not aliquot.**
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
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: 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: Muscle tissue biopsy
Supplies: Muscle Biopsy Kit (T541)
Collection Instructions: Prepare and transport specimen per instructions in Muscle Biopsy Specimen Preparation Sheet.
Specimen Volume: 10-80 mg
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: PerkinElmer 226 (formerly Ahlstrom 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 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 spot, 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)

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. Molecular Genetics: Biochemical Disorders Patient Information (T527)
3. If not ordering electronically, complete, print, and send a Neurology Specialty Testing Client Test Request (T732) with the specimen.

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 & 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 .This includes certain types of myopathies and neuro-ophthalmologic

diseases, such as MELAS (mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes), MERRF (myoclonic epilepsy with ragged red fibers), mitochondrial myopathy, neurogenic muscle weakness, ataxia, retinitis pigmentosa, Leigh syndrome, Leber hereditary optic neuropathy, and chronic progressive external ophthalmoplegia . 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 delin (deletion/insertion) 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 polymerase chain reaction.

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 variants (ie, polymorphisms) 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 healthcare 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. Available at https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=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. Available at https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225088522
4. Wong LJ: Molecular genetics of mitochondrial disorders. Dev Disabil Res Rev. 2010 Jun;16(2):154-162. doi: 10.1002/ddrr.104

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 mitochondrial DNA 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(1):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) Performed
Monday

Report Available
28 to 42 days

Specimen Retention Time
Whole Blood: 2 weeks (if available); Extracted DNA: 3 months

Performing Laboratory Location
Rochester

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
81460-Whole Mitochondrial Genome
81465-Whole Mitochondrial Genome Large Deletion Analysis
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)
81479 (if appropriate for government payers)

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