Overview

Useful For
Diagnosis of the subset of mitochondrial diseases that results from mutations in the mitochondrial genome

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

Identifying mutations 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 (LRPCR) followed by sequencing on the Illumina next-generation sequencing (NGS) platform to evaluate for mutations within the mitochondrial genome.

Highlights
Next-generation sequencing (NGS) is used to test for the presence of mutations within the mitochondrial genome (includes 13 protein coding genes, 22 tRNA genes, and 2 rRNA genes).

Large deletions within the mitochondrial genome are first detected by gel electrophoresis (as size-shifted PCR bands), and the locations of the deletions in the mtDNA are then determined from the Illumina NGS data.

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

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CULFB</td>
<td>Fibroblast Culture for Genetic Test</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Testing Algorithm
If skin biopsy is received, fibroblast culture will be added and charged separately.

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
Test Definition: MITOP
Mitochondrial Full Genome Analysis

- Neuromuscular Myopathy Testing Algorithm
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

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

NY State Available
Yes

Specimen

Specimen Type
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

Supplies: Fibroblast Biopsy Transport Media (T115)

Specimen Type: Skin biopsy

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. Tubes can be supplied upon request (Eagle’s minimum essential medium with 1% penicillin and streptomycin).

Specimen Volume: 4-mm punch

Specimen Stability Information: Refrigerated (preferred)/Ambient

Supplies: Muscle Biopsy Kit (T541)

Specimen Type: Muscle Tissue biopsy

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

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

Specimen Type: Blood spot

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 Mayo Clinic Laboratories for test suitability.

**Specimen Stability Information**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varies</td>
<td>Varies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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 mutations 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 mutations 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.

Mutations 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 mutations in nuclear genes, which are present in either 0, 1, or 2 copies, mitochondrial mutations 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 mutant mitochondria present with more severe disease than those with lower percentages of mutant alleles. The sensitivity for the detection of mutant 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. 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 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 mutation that is not identified by the methods performed. The absence of a mutation, therefore, does not eliminate the possibility of a mitochondrial disease due to mutation in the mitochondrial genome. Mutations 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 mutation 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 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 polymorphisms 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


Performance

Method Description

Next-generation sequencing (NGS) is used to test for the presence of mutations within the mitochondrial genome (includes 13 protein coding genes, 22 tRNA genes, and 2 rRNA 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 PCR bands), and the locations of the deletions in the mtDNA are then determined from the Illumina NGS data.


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

**LOINC® Information**

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MITOP</td>
<td>Mitochondrial Full Genome Analysis</td>
<td>In Process</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>55281</td>
<td>Result Summary</td>
<td>50397-9</td>
</tr>
<tr>
<td>55282</td>
<td>Result</td>
<td>82939-0</td>
</tr>
<tr>
<td>55283</td>
<td>Interpretation</td>
<td>69047-9</td>
</tr>
<tr>
<td>55284</td>
<td>Additional Information</td>
<td>48767-8</td>
</tr>
<tr>
<td>55285</td>
<td>Specimen</td>
<td>31208-2</td>
</tr>
<tr>
<td>55286</td>
<td>Source</td>
<td>31208-2</td>
</tr>
<tr>
<td>55287</td>
<td>Released By</td>
<td>18771-6</td>
</tr>
</tbody>
</table>