Test Definition: MITOT
Combined Mitochondrial Analysis

Overview

Useful For
Diagnosis of mitochondrial disease that results from mutations in either nuclear-encoded genes or the mitochondrial genome

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

Identification of mutations known to be associated with mitochondrial disease, allowing for predictive testing of at-risk family members

Genetics Test Information
This test includes next-generation sequencing and Sanger sequencing to evaluate for mutations in the nuclear genes listed on the panel and 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.

See Targeted Genes Interrogated by Mitochondrial Nuclear Gene Panel in Special Instructions for details regarding the targeted gene regions identified by this test.

Reflex Tests

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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
- Targeted Genes Interrogated by Mitochondrial Nuclear Gene Panel
- Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm
- Neuromuscular Myopathy Testing Algorithm
- Informed Consent for Genetic Testing (Spanish)

Method Name
Custom 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

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

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
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(Eagle's minimum essential medium with 1% penicillin and streptomycin [T115]).

**Specimen Volume:** 4-mm punch

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

**Supplies:** Muscle Biopsy Kit (T541)

**Specimen Type:** Tissue biopsy

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

**Additional Information:** Muscle Biopsy Shipping Kits (T541) are available.

**Specimen Volume:** 10-80 mg

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

**Additional Information:** To ensure minimum volume and concentration of DNA is met, the preferred volume of blood must be submitted. Testing may be canceled if DNA requirements are inadequate.

**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
Tissue Biopsy: 200 mg

**Reject Due To**

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

**Specimen Stability Information**

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Clinical and Interpretive

Clinical Information

The mitochondrion occupies a unique position in eukaryotic biology. It is the site of energy metabolism, and 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, involve virtually any organ system, and be associated with widely varying severities. Due to the considerable overlap in the clinical phenotypes of various mitochondrial disorders, it is often difficult to distinguish these specific inherited disorders without genetic testing. This test utilizes massively parallel sequencing, also termed next-generation sequencing (NGS), to analyze 176 nuclear-encoded genes implicated in mitochondrial disease and 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 mitochondrial diseases that result from mutations in both nuclear encoded genes and in the mitochondrial genome. Those diseases involving nuclear genes include disorders of mitochondrial protein synthesis, coenzyme Q10 biosynthesis, respiratory chain complexes, and mtDNA maintenance (ie, mitochondrial DNA depletion disorders). Disorders of the mitochondrial genome include those caused by point mutations, such as mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), mitochondrial myopathy (MM), neurogenic muscle weakness, ataxia, and 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 Kears-Sayre or Pearson syndromes, are also detected. 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%.

See Targeted Genes Interrogated by Mitochondrial Nuclear Gene Panel in Special Instructions for details regarding the targeted nuclear genes identified by this test.

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. For mitochondrial DNA (mtDNA) alterations, 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 mtDNA 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 involvement of one of more of the genes on the panel or of the
mitochondrial genome 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. 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.

Depletion of mitochondrial DNA levels is not within the scope of this assay.

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 DNA 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:

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

Clinical Reference

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

The Metabolic and Molecular Bases of Inherited Disease. Available at Scriver's The Online Metabolic and Molecular
Retrieved 2013

Scriver's The Online Metabolic and Molecular Basis of Inherited Disease (OMBBID). Edited by D Valle, A Beaudet, B
Performance

Method Description

Next-generation sequencing and/or Sanger sequencing is performed to test for the presence of a mutation in the following genes: AARS2, AASS, ABAT, ABCB7, ACACA, ACAD9, ACO2, AFG3L2, AGK, AIFM1, ALDH3A2, AMPD1, APOPT1, APTX, ATP5A1, ATP5E, ATP5G3, ATPAF2, AUH, BCS1L, BOLA3, C12orf65, CA5A, CHAT, CLPP, COA5, COA6, COQ2, COQ4, COQ6, COQ8A (ADCK3), COQ8B (ADCK4), COQ9, COX10, COX14, COX15, COX20, COX4I2, COX6B1, COX7B, CYC1, D2HGDH, DARS2, DGUOK, DLT, DLD, DNA2, DNAJC19, DNM1L, EARS2, ELAC2, ETF2, ETFB, ETFDH, ETIHE1, FARS2, FASTKD2, FBXL4, FH, FOXRED1, FNX, GAMT, GARS, GCDH, GFER, GFM1, HARS2, HIBCH, IARS2, IBA57, IDH2, ISCU, L2HGDH, LARS2, LIAS, LRPPRC, LYRM4, LYRM7, MARS2, MGME1, MICU1, MPC1, MPV17, MRPL3, MRPL44, MRPS16, MRPS22, MTFMT, MTO1, MTPAP, NDUFA1, NDUFA2, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUVF1, NDUVF2, NFU1, NUBPL, OGDH, OPA1, OPA3, OXCT1, PANK2, PC, PCK2, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PNKD, PNPT1, POLG, POLG2, PUS1, RARS2, RMRD1, RRM2B, SACS, SARS2, SCO1, SCO2, SDHAF1, SERAC1, SFXN4, SLC19A3, SLC25A1, SLC25A3, SLC25A4, SLC25A12, SLC25A19, SLC52A2, SUCLA2, SUCLG1, SUGCT, SURF1, TACO1, TARS2, TAZ, TIMM8A, TIMM44, TK2, TMEM126A, TMEM70, TP1, TRAP1, TRMU, TSFM, TTC19, TUFM, TWIN (C10orf2), TYMP, UQCRB, UQCRCC2, UQCRQ, VARS2, XPNEP3, and YARS2.

There are regions of the genes COX10, COX20, NDUFV2, and TSFM that cannot be effectively sequenced as a result of technical limitations of the assay. Regions of homology, high GC-rich content, and repetitive sequences may not provide accurate sequence. Therefore, all reported alterations detected by next-generation sequencing are confirmed by an independent reference method. However, this does not rule out the possibility of a false-negative result in these regions.

Sanger sequencing is used to confirm alterations detected by next-generation sequencing when appropriate. (Unpublished Mayo method)

Next-generation sequencing (NGS) is also 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

Performed weekly; Varies

Analytic Time

8 weeks
Test Definition: MITOT
Combined Mitochondrial Analysis

Maximum Laboratory Time
10 weeks

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

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
81440-Nuclear Encoded Mitochondrial Genes
81465 - Whole Mitochondrial Genome Large Deletion Analysis

LOINC® Information

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