Overview

Useful For

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

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

Identification of variants 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 (NGS) and Sanger sequencing to evaluate for variants in the nuclear genes listed on the panel and amplification of the entire mitochondrial genome by long-range polymerase chain reaction (LR-PCR) followed by sequencing on the NGS platform to evaluate for variants within the mitochondrial genome.

This test includes next-generation sequencing (NGS) and supplemental Sanger sequencing to evaluate for variants 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, DLAT, DLD, DNA2, DNAJC19, DNM1L, EARS2, ELAC2, ETFA, ETFB, ETFDH, ETHE1, FARS2, FASTKD2, FBXL4, FH, FOXRED1, FXN, 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, NDUFV1, NDUFV2, NFU1, NUBPL, OGDH, OPA1, OPA3, OXCT1, PANK2, PC, PCK2, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PNKD, PNPT1, POLG, POLG2, PUS1, RARS2, RMND1, 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, TPK1, TRAP1, TRMU, TSFM, TTC19, TUFM, TWNK (C10orf2), TYMP, UQCRB, UQCRC2, UQCRQ, VARS2, XPNPEP3,* and *YARS2*.

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

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
- 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

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.

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: 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: 10-80 mg

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. 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: 20 mg

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. 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 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, 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 variants 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 alterations, 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 Kearns-Sayre or Pearson syndromes, are also detected. 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%.

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 (insertion/deletion) 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 variant that is not identified by the methods performed. The absence of a variant, 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 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.

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

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 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 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 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

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&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. 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

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing is performed to test for the presence of a variant 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, DLAT, DLD, DNA2, DNAJC19, DNM1L, EARS2, ELAC2, ETFA, ETFB, ETFDH, ETHE1, FARS2, FASTKD2, FBXL4, FH, FOXRED1, FXN, 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, NDUFV1, NDUFV2, NFU1, NUBPL, OGDH, OPA1, OPA3, OXCT1, PANK2, PC, PCK2, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PNKD, PNPT1, POLG, POLG2, PUS1, RARS2, RMND1, 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, TPK1, TRAP1, TRMU, TSFM, TTC19, TUFM, TWNK (C10orf2), TYMP, UQCRB, UQCRC2, UQCRQ, VARS2, XPNPEP3,* 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 NGS 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 NGS when appropriate.(Unpublished Mayo method)

NGS is also 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, SchAnherr 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. Human Mutation. 2009;30(2):E386-E394 Available at www.phylotree.org)

PDF Report

No

Day(s) and Time(s) Test Performed

Performed weekly; Varies

Analytic Time

8 weeks

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

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

88240-Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
MITOT	Combined Mitochondrial Analysis	In Process

Result ID	Test Result Name	Result LOINC Value
48346	Result Summary	50397-9

Result ID	Test Result Name	Result LOINC Value
48347	Result-mtDNA Genome Analysis	53034-5
35857	Result-Mitochondrial Nuclear Genes	40995-3
48348	Interpretation	69047-9
48349	Additional Information	48767-8
48350	Specimen	31208-2
48351	Source	31208-2
48352	Released By	18771-6