Test Definition: CMITO

Combined Mitochondrial Full Genome and Nuclear Gene Panel, Varies

Overview

Useful For

Diagnosing 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

Identifying variants 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 utilizes next-generation sequencing to detect single nucleotide and copy number variants in 221 nuclear genes and amplification of the entire mitochondrial genome by long-range polymerase chain reaction: *AARS2, ABAT, ABCB7, ACACA, ACAD9, ACO2, AFG3L2, AGK, AIFM1, ALDH3A2, APOPT1* (*COA8*), *APTX, ATP5F1A, ATP5F1E, ATPAF2, AUH, BCS1L, BOLA3, C12orf65* (*MTRFR*), *CA5A, CARS2, CHAT, CHCHD10, CLPP, COA5, COA6, COA8* (*APOPT1*), *COASY, COQ2, COQ4, COQ6, COQ7, COQ8A, COQ8B, COQ9, COX10, COX14, COX15, COX20, COX4I1, COX4I2, COX6A1, COX6A2, COX6B1, COX7B, COX8A, CPT1C, CYC1, D2HGDH, DARS2, DGUOK, DLAT, DLD, DNA2, DNAJC19, DNM1L, EARS2, ELAC2, ETFA, ETFB, ETFDH, ETHE1, FARS2, FASTKD2, FBXL4, FDX2, FDXR, FH, FOXRED1, FXN, GAMT, GARS1, GCDH, GDAP1, GFER, GFM1, GFM2, GLYCTK, GPT2, GTPBP3, HARS2, HIBCH, HK1, HSPD1, IARS2, IBA57, IDH2, INF2, ISCU, L2HGDH, LARS2, LIAS, LRPPRC, LYRM4, LYRM7, MARS2, MFF, MGME1, MICU1, MPC1, MPV17, MRPL3, MRPL44, MRPS16, MRPS2, MRPS22, MRPS7, MSTO1, MTFMT, MTO1, MTPAP, MTRFR* (*C12orf65*), *NARS2, NBAS, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFA2, NDUFA4, NDUFA9, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NFU1, NR2F1, NUBPL, OGDH, OPA1, OPA3, OXCT1, PANK2, PARS2, PC, PCK2, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PET100, PNKD, PNPT1, POLG, POLG2, PTRH2, PUS1, QARS1, RARS1, RARS2, RMND1, RNASEH1, RRM2B, RTN4IP1, SACS, SARS2, SCO1, SCO2, SDHAF1, SERAC1, SFXN4, SLC19A3, SLC25A1, SLC25A12, SLC25A19, SLC25A22, SLC25A26, SLC25A3, SLC25A4, SLC25A42, SLC25A46, SLC52A2, SLC9A6, SOD1, SPG7, SUCLA2, SUCLG1, SUGCT, SURF1, TACO1, TAFAZZIN* (*TAZ*), *TARS2, TAZ* (*TAFAZZIN*), *TFAM, TIMM8A, TK2, TMEM126A, TMEM126B, TMEM70, TOP3A, TPK1, TRIT1, TRMT10C, TRMU, TRNT1, TSFM, TTC19, TUFM, TWNK, TYMP, UQCC2, UQCRB, UQCRC2, UQCRQ, VARS2, WDR45, XPNPEP3,* and *YARS2*.

See Targeted Genes and Methodology Details for Combined Mitochondrial Full Genome and Nuclear Gene Panel, Varies and Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, familial screening,

and genetic counseling for mitochondrial disease.

Testing Algorithm

If skin biopsy is received, fibroblast culture will be added at an additional charge. If viable cells are not obtained, the client will be notified.

For more information see:
-Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm
-Neuromuscular Myopathy Testing Algorithm

Special Instructions

- Molecular Genetics: Biochemical Disorders Patient Information
- Informed Consent for Genetic Testing
- Hereditary Peripheral Neuropathy Diagnostic Algorithm
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions
- Targeted Genes and Methodology Details for Combined Mitochondrial Full Genome and Nuclear Gene Panel, Varies

Method Name

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

Ordering Guidance

The diagnostic workup for a mitochondrial disorder may include testing to demonstrate elevations of the lactate-to-pyruvate ratio and an elevated growth differentiation factor 15 concentration. Consider LAPYP / Lactate Pyruvate Panel, Plasma and GDF15 / Growth Differentiation Factor 15, Plasma.

Customization of this panel and single gene analysis for any gene present on this panel are available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for the genes on this panel. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call

800-533-1710.

Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

Specimen Required

Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with whole blood or dried blood spot testing. For instructions for testing patients who have received a bone marrow transplant, call 800-533-1710

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA) or yellow top (ACD)
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) 4 days/Refrigerated 4 days

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: Cultured fibroblast
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
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.

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 1 of the following forms with the specimen:

-Neurology Specialty Testing Client Test Request (T732)
-Biochemical Genetics Test Request (T798)

Specimen Minimum Volume
Whole blood: 1 mL; Skin biopsy or cultured fibroblasts: See Specimen Required

Reject Due To
All specimens will be evaluated 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. 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 221 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 mitochondrial DNA (mtDNA) maintenance (ie, mtDNA 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%.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(1-2) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:
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.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

Genes may be added or removed based on updated clinical relevance. For detailed information regarding gene-specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(1) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

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;17(5):405-424
2. McCormick EM, Lott MT, Dulik MC, et al. Specifications of the ACMG/AMP standards and guidelines for mitochondrial DNA variant interpretation. Hum Mutat. 2020;41(12):2028-2057
3. 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 March 8, 2024. Available at https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225086827
4. Wallace DC, Lott MT, Brown MD, Kerstann K. Mitochondria and neuro-ophthalmologic diseases. 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 March 8, 2024. Available at https://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225088522
5. Wong LJ. Molecular genetics of mitochondrial disorders. Dev Disabil Res Rev. 2010;16(2):154-162

6. Barca E, Long Y, Cooley V, et al. Mitochondrial disease in North America: An analysis of the NAMDC Registry. Neurol Genet. 2020;6(2):e402

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated to be over 99% for single nucleotide variants, over 94% for deletions-insertions (delins) less than 40 base pairs (bp), and over 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. See Targeted Genes and Methodology Details for Combined Mitochondrial Full Genome and Nuclear Gene Panel, Varies for details regarding the targeted genes analyzed for each test and specific gene regions not routinely covered.(Unpublished Mayo method)

Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

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 mitochondrial DNA are then determined from the NGS data.

The haplogroup is computed using the software package HaploGrep and PhyloTree.(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;32[1]:25-32; 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)

Genes analyzed: *AARS2, ABAT, ABCB7, ACACA, ACAD9, ACO2, AFG3L2, AGK, AIFM1, ALDH3A2, APOPT1* (*COA8), APTX, ATP5F1A, ATP5F1E, ATPAF2, AUH, BCS1L, BOLA3, C12orf65* (*MTRFR*), *CA5A, CARS2, CHAT, CHCHD10, CLPP, COA5, COA6, COA8* (*APOPT1*), *COASY, COQ2, COQ4, COQ6, COQ7, COQ8A, COQ8B, COQ9, COX10, COX14, COX15, COX20, COX4I1, COX4I2, COX6A1, COX6A2, COX6B1, COX7B, COX8A, CPT1C, CYC1, D2HGDH, DARS2, DGUOK, DLAT, DLD, DNA2, DNAJC19, DNM1L, EARS2, ELAC2, ETFA, ETFB, ETFDH, ETHE1, FARS2, FASTKD2, FBXL4, FDX2, FDXR, FH, FOXRED1, FXN, GAMT, GARS1, GCDH, GDAP1, GFER, GFM1, GFM2, GLYCTK, GPT2, GTPBP3, HARS2, HIBCH, HK1, HSPD1, IARS2, IBA57, IDH2, INF2, ISCU, L2HGDH, LARS2, LIAS, LRPPRC, LYRM4, LYRM7, MARS2, MFF, MGME1, MICU1, MPC1, MPV17, MRPL3, MRPL44, MRPS16, MRPS2, MRPS22, MRPS7, MSTO1, MTFMT, MTO1, MTPAP, MTRFR* (*C12orf65*), *NARS2, NBAS, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFA2, NDUFA4, NDUFA9, NDUFAF1, NDUFAF2, NDUFAF3,*

NDUFAF4, NDUFAF5, NDUFAF6, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NFU1, NR2F1, NUBPL, OGDH, OPA1, OPA3, OXCT1, PANK2, PARS2, PC, PCK2, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PET100, PNKD, PNPT1, POLG, POLG2, PTRH2, PUS1, QARS1, RARS1, RARS2, RMND1, RNASEH1, RRM2B, RTN4IP1, SACS, SARS2, SCO1, SCO2, SDHAF1, SERAC1, SFXN4, SLC19A3, SLC25A1, SLC25A12, SLC25A19, SLC25A22, SLC25A26, SLC25A3, SLC25A4, SLC25A42, SLC25A46, SLC52A2, SLC9A6, SOD1, SPG7, SUCLA2, SUCLG1, SUGCT, SURF1, TACO1, TAFAZZIN (*TAZ), TARS2, TAZ (TAFAZZIN*), *TFAM, TIMM8A, TK2, TMEM126A, TMEM126B, TMEM70, TOP3A, TPK1, TRIT1, TRMT10C, TRMU, TRNT1, TSFM, TTC19, TUFM, TWNK, TYMP, UQCC2, UQCRB, UQCRC2, UQCRQ, VARS2, WDR45, XPNPEP3, YARS2* and mitochondrial genome

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; Cultured fibroblasts: 1 month

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

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
81440
81465

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value

CMITO	Combined mtDNA+Nuclear Gene Panel	86206-0

Result ID	Test Result Name	Result LOINC® Value
617104	Test Description	62364-5
617105	Specimen	31208-2
617106	Source	31208-2
617107	Result Summary	50397-9
617108	Result	82939-0
617109	Interpretation	69047-9
618173	Additional Results	82939-0
617110	Resources	99622-3
617111	Additional Information	48767-8
617112	Method	85069-3
617113	Genes Analyzed	48018-6
617115	Released By	18771-6
617114	Disclaimer	62364-5