MITOCHONDRIAL GENOME SEQUENCING AND DEPLETIONS/ INTEGRITY

London Health Sciences Centre | St. Joseph's Health Care London

Pathology and Laboratory Medicine

Orderable - MD Mitochondrial Panel

Turnaround Time: 4-6 weeks **STAT: 4 weeks**

Laboratory:
Molecular Diagnostics Lab

Requisition:
MOLECULAR DIAGNOSTICS REQUISITION

Method of Analysis:
Mitochondrial regions of interest and all coding exons and 20 bp of flanking intronic sequence of nuclear encoded genes are enriched using an LHSC custom targeted hybridization protocol (Roche Nimblegen), followed by high throughput sequencing (Illumina). Sequence variants and copy number changes are assessed and interpreted using clinically validated algorithms and commercial software (SoftGenetics: Nextgene, Geneticist Assistant, Mutation Surveyor; and Alamut Visual). Nuclear encoded genes have 300x or higher mean read depth coverage, with a minimum 100x coverage

Alternate Name(s):

mtDNA sequencing
LHON
MELAS
MERF

Specimen:

Whole blood-2 x 4 mL Lavender EDTA top Vacutainer tube

Collection Information:

Sample may be transported at room temperature. Alternative tissues may be accepted after consult with laboratory.

Reference Ranges:

See report

Interpretive Comments:

The human mitochondrial DNA (mtDNA) encodes 37 genes coding for two rRNAs, 22 tRNAs and 13 polypeptides within its 16 569 bp.. The mtDNA-encoded polypeptides are all subunits of enzyme complexes of the oxidative phosphorylation system. Disease phenotypes resulting from mitochondrial mutations may appear as distinct syndromes, such as Kearns-Sayre syndrome (KSS), Leber's Hereditary Optic Neuropathy (LHON), mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes (MELAS), chronic progressive external ophthalmoplegia (CPEO), myoclonic epilepsy with ragged-red fibers (MERRF), neurogenic weakness with ataxia and retinitis pigmentosa (NARP) or Leigh syndrome (LS). More frequently, the clinical presentation is much more heterogeneous. Some common symptoms include ptosis, external ophthalmoplegia, proximal myopathy, exercise intolerance, cardiomyopathy, sensorineural deafness, migraine, stroke-like episodes, pigmentary retinopathy,

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diabetes mellitus, encephalopathy, seizures, ataxia, and spasticity. This panel has been augmented with a selected series of 19 nuclear genes known to be associated the mitochondrial depletion disorders. About 80-95% of patients with mitochondrial disorders do not harbor a pathogenic mutation in the mitochondrial genome. A large proportion of these cases may have defects in nuclear-encoded genes that are involved in the biosynthesis of the mitochondrial genome or in the maintenance of mtDNA integrity. The Mitochondrial Genome Sequencing and Depletion/Integrity panel is appropriate for patients suspected of having one of the various forms of mtDNA depletion syndrome and/or mtDNA multiple deletions.

Comments:

Mitochondrial encoded: NC_012920.1: MT-TY, MT-TW, MT-TV, MT-TT, MT-TS2, MT-TS1, MT-TR, MT-TQ, MT-TP, MT-TN, MT-TM, MT-TL2, MT-TL1, MT-TK, MT-TI, MT-TH, MT-TG, MT-TF, MT-TE, MT-TD, MT-TA, MT-RNR2, MT-RNR1, MT-ND6, MT-ND5, MT-ND4L, MT-ND4, MT-ND3, MT-ND2, MT-ND1, MT-CYB, MT-CO3, MT-CO2, MT-CO1, MT-TC, MT-ATP8, MT-ATP6
Nuclear encoded: APTX(NM_0011952248.1), DGUOK(NM_080916.2), DNA2(NM_001080449.2), FBXL4(NM_001278716.1), GFER(NM_005262.2), MGME1(NM_052865.3), MPV17(NM_002437.4), OPA1(NM_130837.2), OPA3(isoformA & B: NM_001017989.2 & NM_025136.3), POLG(NM_001126131.1), POLG2(NM_007215.3), RRM2B(NM_001172477.1 & NM_015713.4), SLC25A4(NM_001151.3), SPG7(isoform1 & 2:NM_003119.2 & NM_199367.2 (x10)), SUCLA2(NM_003850.2), SUCLG1(NM_003849.3), TK2(NM_004614.4), TWNK(C10orf2)(NM_021830.4), TYMP(NM_001257989.1)

Storage and Shipment:

Samples may be stored and shipped at room temperature

at a single nucleotide resolution. Mitochondrial genes have >1000x mean read depth coverage, with a minimum 500x coverage at a single nucleotide resolution. Test is validated for heteroplasmy detection sensitivity of 2-5%. Mitochondrial CNVs are routinely detectible to 15% heteroplasmy levels. LR-PCR is used to confirm mtDNA CNVs, and may be used to assess mitochondrial CNVs to 2-5% heteroplasmy levels. All variants interpreted as either ACMG category 1, 2, or 3 (pathogenic, likely pathogenic, VUS; PMID: 25741868) are confirmed using Sanger sequencing, MLPA, or other assays. ACMG category 4 and 5 variants (likely benign, benign) are not reported, but are available upon request. Analysis includes copy number assessment for the mitochondrial DNA deletion syndrome (Kearns-Sayre syndrome). This assay has been validated at a level of sensitivity equivalent to the Sanger sequencing and standard copy number analysis (>99%;

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PMID: 27376475, 28818680).

Test Schedule:
As required,
Monday to Friday 0800-1600 hours