

## Mitochondrial Genome: Sequencing

**Test Code:** JD

**Turnaround time:** 8 weeks

**CPT Codes:** 81460 x1

### Condition Description

Mitochondrial disorders are a frequent cause of genetic disease [1]. They comprise a clinically heterogeneous group of diseases caused by mutations of either nuclear or mitochondrial DNA (mtDNA) which may result in decreased cellular energy production due to a dysfunctional mitochondrial respiratory chain. This sequencing assay is available to detect mutations in the mtDNA genome. Mutations in nuclear genes with mitochondrial function will not be detected by this analysis.

Clinical presentation of mtDNA disorders is variable. Most involve multiple organ systems and frequently present with neurologic and myopathic symptoms, which may be intermittent, but disorders may be confined to one organ such as the eye in Leber hereditary optic neuropathy (LHON). Age of onset also varies though symptoms may frequently develop in childhood.

Common clinical features of mtDNA disorders include external ophthalmoplegia, ptosis, cardiomyopathy, diabetes mellitus, sensorineural deafness, optic atrophy, pigmentary retinopathy, myopathy and exercise intolerance [3]. The central nervous system findings are often seizures, dementia, migraine, stroke-like episodes, ataxia, spasticity and encephalopathy. However, due to a significant clinical variability, some individuals do not fit into a specific clinical diagnosis. Heteroplasmy, which is the uneven distribution of mtDNA molecules during cell division, may result in variable penetrance and severity of symptoms, depending on the level of mutant mitochondria [4].

Some discrete clinical syndromes are well established, for which targeted testing is available:

Kearns-Sayre syndrome (KSS)

Pearson syndrome

Chronic progressive external ophthalmoplegia (CPEO)

Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)

Myoclonic epilepsy with ragged-red fibers (MERRF)

Retinitis Pigmentosa and Ataxia (NARP)

Leber hereditary optic neuropathy (LHON)

Leigh syndrome (LS)

Sequence analysis of the entire mtDNA genome is available to test for mitochondrial mutations. This test is intended for patients with a diagnosis of a mitochondrial disorder. If applicable, testing for common mutations associated with specific mitochondrial disorders should be performed first. Low levels of heteroplasmy may not be detected.

#### References:

1. Taylor and Turnbull. Mitochondrial DNA Mutations in Human Disease. *Nat Rev Genet* 2005, 6:389-402
2. Vu et al. Mitochondrial diseases. *Neurol Clin N Am* 2002, 20:809-839.
3. Scaglia et al. Clinical Spectrum, Morbidity, and Mortality in 113 Pediatric Patients With Mitochondrial Disease. *Pediatrics* 2004, 114(4):925-931.
4. Holt et al. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990, 46:428-33.
5. Majamaa et al. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. *Am J Hum Genet* 1998, 63:447-454.
6. Schaefer et al. The epidemiology of mitochondrial disorders - past, present and future. *Biochim Biophys Acta* 2004, 1659:115-120.
7. Chinnery and Turnbull. Epidemiology and Treatment of Mitochondrial Disorders. *Am J Med Genet C* 2001, 106:94-101.
8. Wilson et al. A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. *Science* 2004, 306:1190-1194.
9. Trifunovic et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 2004, 429:417-423.

### Indications

This test is indicated for:

- Individual with a clinical diagnosis of a mitochondrial DNA disorder

### Methodology

**Methodology:** PCR was used to amplify the mitochondrial genome. Direct sequencing of the amplified region was performed using next generation short base pair read sequencing. Sequences are compared to revised Cambridge reference sequence (rCRS) NC\_012920.1. Sequence analysis is limited to m.577\_m.16023 region of the mitochondrial genome and excludes the highly variable control regions (m.1\_m.576 and m.16024\_m.16569).

**Heteroplasmy:** - Heteroplasmy is defined as the co-presence of a reference allele and an alternate allele (variant) at a given position in the mitochondrial genome. For any given variant, the level of heteroplasmy is calculated as a percentage of the total NGS reads (at that particular position) that have the variant. In general, heteroplasmic variants detected at <10% of alleles, are not reported. Known pathogenic or likely pathogenic variants detected at <10% heteroplasmy may be reported with recommendations for additional studies, as appropriate.

### Detection

Detection is related to the specific condition suspected (refer to the test descriptions for the recognized conditions listed above). Since in general, sequence analysis does not detect low level mutant heteroplasmy. Common mutations must be ruled out before the whole genome sequence is analyzed.

## Specimen Requirements

*Submit only 1 of the following specimen types*

### Type: DNA, Isolated

#### Specimen Requirements:

Microtainer

8µg

Isolation using the Perkin Elmer™ Chemagen™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

#### Specimen Collection and Shipping:

Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

### Type: Whole Blood (EDTA)

#### Specimen Requirements:

EDTA (Purple Top)

Infants and Young Children ( 2 years of age to 10 years old: 3-5 ml

Older Children & Adults: 5-10 ml

Autopsy: 2-3 ml unclotted cord or cardiac blood

#### Specimen Collection and Shipping:

Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.

### Type: Saliva

#### Specimen Requirements:

Oragene™ Saliva Collection Kit

Oragene™ Saliva Collection Kit used according to manufacturer instructions. Please contact EGL for a Saliva Collection Kit for patients that cannot provide a blood sample.

#### Specimen Collection and Shipping:

Please do not refrigerate or freeze saliva sample. Please store and ship at room temperature.

## Related Tests

- PCR amplification and restriction enzyme fragment analysis is available to test for Leigh syndrome (QD), Myoclonic epilepsy with ragged-red fibers (QH), and Leber hereditary optic neuropathy (QC).
- Testing for MELAS (Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) (QA) is available using sequence analysis of the MTTL1 gene and an allele-specific extension assay.
- Testing for Retinitis Pigmentosa and Ataxia (NARP) (QK) is available by PCR amplification with restriction enzyme fragment analysis and an allele-specific extension assay.
- Urine organic acid (OA) with lactic acid and pyruvic acid, and plasma acylcarnitine analysis (AR) may be considered for evaluation of specific mitochondrial disorders.
- Known mutation analysis (KM) is available to family members if mutation is identified in the proband by sequencing.