## Biomarker Testing

1. Was a biomarker analysis done?

Yes

No

If YES, please answer questions below:

1. Source:

Blood (serum/plasma)

Buccal swab

CSF

Fibroblasts

Imaging

MRS

Location:

Brain

Liver

Muscle

Other, specify:

Ultrasound

Location:

Brain

Liver

Muscle

Other, specify:

Other, specify:

Location:

Brain

Liver

Muscle

Other, specify:

Induced pluripotent stem cells (iPS)

Kidney biopsy

Leukocytes

Liver biopsy

Lymphoblasts (EBV)

Lymphocytes

Muscle biopsy

Organoid(s):

Brain

Kidney

Liver

Other, specify:

Red blood cells

Whole Blood

Cultured Cells

Saliva

Urine

Other, specify:

1. Clinical assessments:

Cardiac evaluation:

Echocardiogram

EKG

Holter Monitor

MRI

Other, specify:

Ergometry

Head circumference

Hearing

Height

Indirect calorimetry

Vision

Weight

Other, specify:

1. Which biomarker(s) were assessed from the specimen's (serum/plasma) sample?

Acylcarnitines

Amino acids

Ammonia

Carnitine levels

CBC

Cell-free mitochondrial DNA (cf-mtDNA)

CPK

Creatine

Cystatin C

Electrolytes

Endocrine testing:

A1C

Cortisol

Free fatty acids

Glucose

Insulin

Ketone bodies

Thyroid

Other, specify:

Fibroblast growth factor 21 (FGF21)

Growth differentiation factor 15 (GDF15)

Hepatic panel:

Albumin

Alk Phos

ALT

AST

GGT

INR

PT

PTT

Other, specify:

Lactate

Lactate/pyruvate ratio

LDH

Lipid panel

Metabolomics:

Targeted

Untargeted

Vitamin levels:

B12

Folate

Niacin

Pyridoxine

Riboflavin

Thiamine

Other, specify:

Pyruvate

Purines and pyrimidines

Other, specify:

1. Which biomarker(s) were assessed from the urine sample?

Acylglycines

Amino acids

Metabolomics:

Targeted

Untargeted

mtDNA heteroplasmy

Organic acids

Purines and pyrimidines

Urinalysis (UA)

Other, specify:

1. Which biomarker(s) were assessed from the cerebrospinal fluid (CSF) sample?

5-methyltetrahydrofolate

Amino acids

Cell count

GABA

Glucose (with simultaneous blood glucose)

Lactate

Metabolomics:

Targeted

Untargeted

Neurotransmitters

Protein

Pyruvate

Other, specify:

1. Which biomarker(s) were assessed from the specimen's fibroblasts sample?

ATP synthesis

Blue native gel electrophoresis (OXPHOS)

Coenzyme Q10

High resolution respirometry

Lactate/pyruvate ratio

OXPHOS enzymology

Pyruvate dehydrogenase enzymology

Seahorse live cell metabolic analysis

Other, specify:

1. Which biomarker(s) were assessed from the specimen's leukocytes sample?

Coenzyme Q10

Intracellular free glutathione (fGSH), oxidized disulfide (GSSG), fGSH/GSSG ratio

mtDNA copy number

mtDNA deletion/duplication

Pyruvate dehydrogenase enzymology

Thymidine phosphorylase enzymology

Other, specify:

1. Which biomarker(s) were assessed the specimen's lymphoblast sample?

ATP synthesis

High resolution respirometry

Seahorse live cell metabolic analysis

Other, specify:

1. Which biomarker(s) were assessed from the specimen's muscle biochemistry?

ATP synthesis

Blue native gel electrophoresis

Coenzyme Q10

Glutathione content

High resolution respirometry

mtDNA copy number

mtDNA deletion/duplication

OXPHOS enzymology

Pyruvate dehydrogenase enzymology

Seahorse live cell metabolic analysis

Other, specify:

1. Which biomarker(s) were assessed from the specimen's muscle histology?

Combined SDH + COX

Cytochrome C Oxidase (COX) (Complex IV)

Gomori trichrome

Nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR)

Succinate dehydrogenase (SDH)

Other, specify:

1. Which biomarker(s) were assessed from the specimen's genetics?

| **Biomarker** | **Specimen Type** |
| --- | --- |
| Exome sequencing (NGS) (nDNA) | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |
| Whole genome sequencing | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |
| Gene sequencing panel | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |
| RNA analysis | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |
| Mitochondrial gene expression profiling | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |
| Mitochondrial haplotype | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |
| mtDNA copy number | Leukocytes  Liver  Muscle  Other, specify: |
| mtDNA deletion/duplication | Leukocytes  Liver  Muscle  Other, specify: |
| mtDNA sequencing | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |
| Other, specify: | Buccal swab  Cultured cells  Leukocytes  Muscle  Urine  Whole blood  Other, specify: |

1. Specify other biomarker(s) assessed from specimen sources not listed above:

Recorder Signature: Date:

## General Instructions

This case report form contains data elements that assess biomarkers with potential utility to confirm or evaluate mitochondrial disease. Data collected may be used to substantiate or provide additional evidence of a mitochondrial disease diagnosis, indicate severity of disease, determine standard of care, and/or monitor medication/therapy.

Important note: None of the data elements included on this CRF Module are classified as Core (i.e., strongly recommended for all mitochondrial disease clinical studies to collect). All data elements are classified as Supplemental and should only be collected if the research team considers them appropriate for their study.

Please see the Data Dictionary for element classifications.

## Specific Instructions

Please see the Data Dictionary for definitions for each of the data elements included in this CRF Module.

* For each biomarker, the source should be specified. If not listed, use “other” and designate source.
* Source type – Kidney and liver biopsy samples may be taken from organs in vivo or from whole organs ex vivo.
* Vitamin level type – For assessment of vitamin B12 or folate deficiency, evaluation of plasma methylmalonic acid (MMA) and total homocysteine is recommended.
* Date/time should be recorded to the level of granularity known (e.g., year, year and month, complete date plus hours and minutes, etc.) and in an unambiguous format acceptable to the study database like DD-MMM-YYYY. When date/time data are prepared for aggregation or sharing, they should be converted to the format specified by [ISO 8601](https://www.iso.org/iso-8601-date-and-time-format.html);  YYYY-MM-DD T:hh:mm:ss.

## References

Please see Guidance Document.