Mitochondrial Diagnostic Criteria
This information represented below is provided by The Foundation for Mitochondrial Medicine.

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The criteria below represent the careful investigations that need to be performed and assessed for mitochondrial disease diagnosis.

Nijmegen Clinical Criteria for Mitochondrial Disease: Clinical scoring for diagnostic assessment is according to (*Wolf NI, Smeitink JA. Mitochondrial disorders: a proposal for consensus diagnostic criteria in infants and children. Neurology. 2002 Nov 12;59(9):1402-5.)*

CLINICAL CRITERIA	SCORE
Neuromuscular manifestations (Maximum of 2 points)	
a. Progressive external ophthalmoplegia (2 points)	
b. Ptosis (1 point)	
c. Exercise intolerance (1 point)	
d. Muscle weakness (1 point)	
e. Rhabdomyolysis (1 point)	
f. Abnormal electromyogram (1 point)	
Central nervous system and other organ involvement (Maximum of 2 points)	
g. Isolated central nervous system involvement (1 point)	
h. Any other isolated organ system (1 point)	
i. Two or more organ systems (2 points)	
Metabolic and imaging studies (Maximum of 4 points)	
j. Elevated blood lactate on 3 occasions (2 points)	
k. Elevated cerebrospinal fluid lactate (2 points)	
l. Elevated blood alanine (2 points)	
m. Elevated cerebrospinal fluid alanine (2 points)	
n. Elevated urine tricarboxylic acid (Kreb) cycle intermediates (2 points)	
o. Elevated urine ethylmalonic, 3-methylglutcaonic, or dicarboxylic acids (1 point)	
p. Abnormal ^{31}P-MRS (magnetic resonance spectroscopy) in muscle with reduced Phosphocreatine/P_i ratio (2 points)	
q. Abnormal T2 signal in basal ganglia on brain MRI (2 points)	
r. Decreased resting metabolic rate or abnormal exercise studies (cycle ergometry protocol) (2 points)	
Tissue morphology (Maximum of 4 points)	
s. Ragged red fibers on muscle biopsy (2 points if present, 4 points if >2%)	
t. Diffuse reduction in cytochrome c oxidase histochemical reaction or scattered COX deficient fibers(4 points)	
u. Strongly succinate dehydrogenase positive vessels by histochemistry (1 point)	
	TOTAL SCORE

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Scoring for evaluation of Clinical Criteria:

Definite: 8-12 points

Probable: 5-7 points

Possible: 2-4 points

Unlikely: 1 point

The Nijmegen Biochemical Criteria are modified for incorporation of contemporary technologies. Genetic criteria for diagnosis are added.

BIOCHEMICAL CRITERIA	SCORE
1. Abnormal high resolution respirometry in muscle or fibroblasts (measurements <5% reference level) (Live (fresh tissue) assessment of Complex V function, coupling and protein leak across the mitochondrial membrane:)	
2. Abnormal OXPHOS subunit immunohistochemistry or immunofluorescece in skeletal muscle tissue sections (Qualitative assessment of OXPHOS enzyme assembly within tissue sections. Defects in OXPHOS enzyme assembly are readily recognized by this testing.)	
3. Abnormal OXPHOS enzymology (single or multiple enzyme defects) (activity measurements <5% reference level) (Testing must be performed on mitochondria isolated from fresh (not frozen) tissue to minimize risk of artifacts caused by freezing skeletal muscle prior to mitochondrial isolation).	
4. Abnormal quantitative Western Blot of Selected OXPHOS subunits from Complexes I-V (levels <5% reference level for subunit) (Western blot can detect defects that are not evident by other techniques. (*2*))	
5. Abnormal muscle CoQ10 level (<50% of control mean) (Allows assessment for primary defects in Coenzyme Q10 synthesis. (*3*))	
6. Supercomplex evaluation: Optimal OXPHOS function requires aggregation of individual OXPHOS enzymes into supercomplexes which allows efficient and rapid transport of electrons. (*4-8*) Supercomplexes allow efficient formation of an electrochemical (proton) gradient created by Complexes I, III, and IV that is then used by Complex V to synthesize ATP. Supercomplex formation is impaired in a variety of OXPHOS diseases. (*9-23*)	
a. Abnormal supercomplex formation (Score 0.5 if present only in Blue Native OR OXPHOS Clear Native Immunoblot. Score 1 if present in BOTH tests.)	
b. Abnormal monomeric OXPHOS enzyme complex formation (Score 0.5 if present only in Blue Native OR OXPHOS Clear Native Immunoblot. Score 1 if present in BOTH tests.)	

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7. Abnormal in-gel OXPHOS enzyme activity. (Qualitative in-gel assessment of OXPHOS enzyme activity in intact enzymes. This is particularly important in assessment of the ATPase activity of Complex V and Complex V assembly. (*11-15*))	
TOTAL SCORE	

Scoring for evaluation of Biochemical Criteria

Unlikely: Criteria Score 0

Possible: Criteria – Score is ≥1 and < 2

Probable: Criteria- Score = 2

Highly Probable: Score >2

GENETIC CRITERIA	SCORE
1. mtDNA depletion (mtDNA copy number <5% reference interval)	
2. Identification of confirmed pathogenic mtDNA or nuclear DNA mutation	
3. Identification of provisional pathogenic mtDNA or nuclear DNA mutation (i.e. mutation requires additional data supporting pathogenicity)	
4. No mutation identified	

Scoring for evaluation of Genetic Criteria

Definite: Criteria 1 or 2 are abnormal

Probable: Criteria for 3 is abnormal

Undetermined: Criteria 4 is present

Note: Criteria D: The failure to find a mutation does not exclude mitochondrial disease due to the large number of genes associated with mitochondrial disease and the large number of undiscovered genetic associations.