TESTING OF MITOCHONDRIAL DNA AND MITOCHONDRIAL RESPIRATORY CHAIN COMPLEXES (REFERENCE – 2014.02.09)

Notice of Assessment

December 2014
1. GENERAL INFORMATION

1.1 Requester: Centre hospitalier universitaire Sainte-Justine

1.2 Applications for Review Submitted to MSSS: December 12, 2013, and January 14, 2014

1.3 Applications Received by INESSS: July 10, 2014

1.4 Notice Issued: October 31, 2014

Note:
This notice is based on the scientific and commercial information submitted by the requester and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2. TECHNOLOGY, COMPANY, AND LICENCE(S)

2.1 Name of the Technology
Five applications were received for addition to the Index. They involve four different techniques for diagnosing mitochondrial disorders:

- individual enzyme assays of mitochondrial respiratory chain complexes I to V in fibroblasts or muscle using spectrophotometry;
- detection of mitochondrial respiratory chain complexes I to V in fibroblasts or muscle using BN-PAGE (blue native polyacrylamide gel electrophoresis or blue native electrophoresis);
- mitochondrial DNA sequencing (Sanger method);
- MLPA (multiplex ligation-dependent probe amplification).

2.2 Brief Description of the Technologies, and Clinical and Technical Specifications

Biochemical Assays of Mitochondrial Respiratory Chain Complexes I to V

According to information provided by the requester, mitochondrial biochemical assays (enzyme activity using spectrophotometry and BN-PAGE) are performed using commonly affected tissue such as skeletal muscle, heart muscle, and the liver, or using cultured fibroblasts. Fresh or frozen tissue is treated to produce suitable fractions for BN-PAGE measurement of enzyme activity. First, the homogenate (H) analytical fraction is obtained, in which mitochondria are released by rupturing cell walls. Following centrifugation, a supernatant with intact mitochondria (S fraction) is obtained. The S1 fraction is used to assay complex I to IV activity. Assaying complex V activity (fresh tissue only) and extracting respiratory chain complexes for blue native electrophoresis require additional purification stages using isolated mitochondria. Specifically, complex V activity is assayed after an additional purification step to produce an M fraction. This step is required to reduce interference from enzymes present in the S1 fraction. A mitochondrial-enriched fraction must be used for fibroblast samples so the ratio of complex activity to enzyme contaminant activity is higher.

Individual Enzyme Assays Using Spectrophotometry

Respiratory chain complex enzyme activity is assayed by spectrophotometry using an automated analyzer. The analyzer has filters for measuring at each wavelength required and
a probe for collecting precise volumes of reagents and samples determined by a computer program. Each complex is assayed in an individual dish using a separate chemical reaction. Total activity for each complex is compared with residual activity when a complex-specific inhibitor is present, or with initial activity in the absence of a substrate. To properly assess respiratory chain complex enzyme activity, the results are expressed relative to citrate synthase (nuclear DNA-encoded enzyme), used as an enzyme marker of mitochondrial abundance [Spinazzi et al., 2011].

**Blue Native Electrophoresis (BN-PAGE) Detection of Complexes I to V**

This is a non-denaturing electrophoresis technique developed by Schägger and von Jagow [1991] to analyze membrane protein complexes such as the mitochondrial OXPHOS system composed of respiratory chain complexes. This technique maintains complexes in their biochemically active native state and allows them to be separated without dissociation so their structural organization can be studied. Serva Blue G (also known as Coomassie Blue G-250) dye is used in the extraction and electrophoresis running buffers to preserve the native state of protein complexes. Like sodium dodecyl sulphate (SDS) used in conventional electrophoresis, Serva Blue G provides the charged protein complexes required for migration through the electric field applied during gel electrophoresis. Following migration, the protein bands obtained are transferred from the polyacrylamide gel onto a nitrocellulose membrane and revealed using specific antibodies for the complexes (Western blot technique). Band intensity is measured by densitometry, and the results are expressed as a ratio relative to complex II. Complex II, which is entirely encoded by nuclear DNA (not affected by mtDNA mutations), is a good marker of mitochondrial abundance [DiMauro et al., 2013]. The size, assembly, and relative abundance of individual complexes can be analyzed with this technique [Leary, 2012].

**Mitochondrial DNA Sequencing (Sanger Method)**

The following description is the classic method described by Sanger. Details of the method used by the requester were not provided. The Sanger sequencing method [Sanger et al., 1977] involves the following steps:

1. A specific primer is selected that is complementary to a single strand of a DNA template amplified by either PCR or RT-PCR;
2. The single-strand DNA template is combined with the primer, DNA polymerase and the four bases (deoxyribonucleoside triphosphates, namely A, G, T, and C);
3. The mixture is then divided into reaction aliquots, each containing one of the four terminating bases labelled with a different fluorochrome (dideoxyribonucleotide triphosphates, namely A, G, T, and C, that lack the hydroxyl group required for synthesis) (ddNTP);
4. The reaction continues until the chance incorporation of a terminating base (ddNTP), which prevents incorporation of other bases and therefore stops fragment synthesis;
5. This results in four mixtures that contain fragments separated by size (by capillary or gel electrophoresis);

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1 OXPHOS: oxidative phosphorylation, a process associated with production of ATP by mitochondrial respiratory chain enzyme complexes.
6. A laser passes through the capillary and excites the fluorochrome on the terminal ddNTP of each fragment separated by length;
7. The original DNA sequence is revealed (e.g., by a chromatogram) and is normally analyzed using software.

**Multiplex Ligation-Dependent Probe Amplification (MLPA)**

The MLPA technique can detect variations in locus copy numbers [Schouten et al., 2002]. This targeted detection method has the advantage of being able to study several loci simultaneously. The principle consists of obtaining an amplified fragment of a different size for each locus so the fragments can be differentiated and quantified (Figure 1).

**Figure 1: MLPA concept**

![MLPA concept diagram](source)

There are two probes of different sizes for each locus. Each probe has two parts: one is complementary to the target sequence (specific hybridization), and the other is required for PCR amplification of all loci studied. Each fragment can thus be quantified and compared with a control for detection of locus copy numbers. This method is also used to detect deletions and duplications in one or more mitochondrial DNA regions [Kwon et al., 2011].

2.2 **Company or Developer:** Protocols provided by the requester.

2.3 **Licence(s):** Not applicable.

2.4 **Patent, If Any:** Not applicable.

2.5 **Approval Status (Health Canada, FDA)**

- Enzyme assays and BN-PAGE: These are in-house protocols. All reagents and solutions are prepared in the requesting centre’s biochemical genetics laboratory in accordance with strict detailed procedures described in the standard operating procedures (SOP) provided by the requester.
- Sanger sequencing and MLPA: in-house protocols.
2.6 Weighted Values

- Enzyme assays: 713.46
- BN-PAGE: 410.52
- Mitochondrial DNA sequencing: 1,524.43
- MLPA: 318.09
- MLPA + sequencing: 1,627.67

3. CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

These tests are indicated for patients who have a clinical diagnosis of a mitochondrial disorder, or in whom a mitochondrial disorder is strongly suspected.

3.2 Targeted Disease(s)

Mitochondria are organelles responsible for oxidative phosphorylation that produces energy in the form of adenosine triphosphate (ATP). This process is performed by the respiratory chain located in the mitochondrial inner membrane. Mitochondrial disorders are caused by a mitochondrial respiratory chain dysfunction that affects the oxidative phosphorylation process. This group of disorders is the most genetically heterogeneous, involving both the 16.6 kb mitochondrial genome and approximately 1,500 genes encoded in the nuclear genome [Tang et al., 2013; Wong, 2013].

The respiratory chain consists of five complexes (with 85 subunits) embedded in the mitochondrial inner membrane; complex I is the largest (more than 40 subunits). Only 13 subunits are encoded by mitochondrial DNA (mtDNA). Consequently, each of the five complexes is encoded partly by mtDNA and partly by nuclear DNA (nDNA), except complex II, which is entirely encoded by nDNA [DiMauro et al., 2013]. The respiratory chain performs a series of oxidation-reduction reactions in which electrons from the Krebs cycle and β-oxidation spirals are transferred from one complex to another while an electrochemical proton gradient is created, ultimately allowing complex V to create ATP [DiMauro et al., 2013].

The role of mitochondria in the energy production chain (production of ATP) explains the preferential involvement of organs and tissues that depend on it, such as skeletal and heart muscle and the central nervous system. Mitochondrial disorders are manifested by a broad spectrum of clinical phenotypes, ranging from early onset multi-systemic disease with rapid deterioration and death at a young age, to mild exercise intolerance presenting at an advanced age [Rodenburg et al., 2013; Haas et al., 2007]. Certain clinical characteristics suggest the presence of a mitochondrial disorder (failure to thrive, encephalopathy, cardiomyopathy, exercise intolerance, ophthalmological abnormalities, muscle weakness, etc.) while other signs and symptoms are specific to certain syndromes [Liang et al., 2014]. Although multisystem mitochondrial disorders involve both mitochondrial and nuclear DNA, some syndromes are associated specifically with mtDNA mutations (KSS, PS, MELAS, MERRF, NARP, MILS, LHON) and others with nDNA mutations (MNGIE, SANDO, Alpers syndrome, DOA2) [DiMauro et al., 2013].

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2 DOA: dominant optic atrophy; KSS: Kearns-Sayre syndrome; LHON: Leber hereditary optic neuropathy; MELAS: mitochondrial encephalomyopathy; MERRF: myoclonus epilepsy with ragged-red fibres; MILS: maternally inherited Leigh syndrome; MNGIE: mitochondrial encephalomyopathy.
More than 300 point mutations, deletions, and duplications in mtDNA have now been reported [Vafai and Mootha, 2012]. Mutations have been identified in the five respiratory chain complexes, but most have been in the genes of complex I, affecting 16 of its 45 subunits [DiMauro et al., 2013]. Consequently, complex I deficiency is the most common abnormality in patients with a mitochondrial disorder, and Leigh syndrome associated with this deficiency is the most frequently observed clinical presentation [Leshinsky-Silver et al., 2009]. Complex I deficiency accounts for 23% of all childhood cases [Hoefs et al., 2010]. Complex II, III, IV, and V deficiencies are rarer [Menezes et al., 2014]. In this regard, fewer nuclear genes have been associated with deficiencies of these complexes compared with complex I [Menezes et al., 2014].

**Epidemiology**

According to data collected in England over a 15-year period (1990 to 2004), the prevalence of mtDNA disorders in working-age adults (16 to 65 years) is 9.18 in 100,000 (95% CI: 7.8 to 10.8/100,000). In addition, the prevalence in asymptomatic first-degree relatives of patients with mtDNA point mutations, both adults and children, is 16.5 in 100,000 (95% CI: 14.8 to 18.3/100,000) [Schaefer et al., 2008]. No information was found on the prevalence of mitochondrial disorders in Quebec.

**Prognosis**

The prognosis is generally very poor for patients with a nuclear DNA-related complex I deficiency [Distelmaier et al., 2009]. More than half of patients die before the age of 2 years, and 79% die before the age of 10 years [Koene et al., 2012]. Although the difference is not significant, survival is higher in patients with an mtDNA-related complex I deficiency than in patients with a nuclear encoded complex I deficiency [Swalwell et al., 2011].

**Treatment**

Only supportive treatment is available to prolong and improve the quality of life of affected patients. This includes respiratory care, cataract and eyelid surgery (cases of ptosis), as well as antiepileptic drugs [Genge and Massie, 2014; DiMauro et al., 2013; Pfeffer and Chinnery, 2013].

3.3 **Number of Patients Targeted**

- Respiratory chain complex enzyme assays and BN-PAGE: expected provincial volume is 100 cases per year.
- Sequencing and MLPA: expected provincial volume is 15 cases per year (minimum volume of 50 tests for the next three years).

3.4 **Medical Specialties and Other Professions Involved**

Medical biochemistry, molecular biology, molecular genetics, cardiology, neurology, ophthalmology, genetic counselling.

3.5 **Testing Procedure**

The following descriptions are based primarily on information provided by the requester.

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neurogastrointestinal encephalopathy; NARP: neuropathy ataxia and retinitis pigmentosa; PS: Pearson syndrome; SANDO: sensory ataxic neuropathy dysarthria and ophthalmoparesis.
1. **Muscle biopsy:** A 250 mg specimen is collected under general or local anesthesia. It is sent fresh (no more than 2 hours post collection) or on dry ice (allowing specimens to be sent from anywhere in Quebec) directly to the requesting centre’s biochemical genetics laboratory. Depending on the tests ordered by the prescribing physician (based on the algorithm in Appendix A), the sample is divided into aliquots and, if required, one part is sent to the molecular diagnostics lab for mitochondrial DNA sequencing. If applicable, the other aliquots are used for enzyme assays or BN-PAGE.

2. **Skin biopsy:** The samples collected are cultured in the biochemical genetics laboratory. When a skin biopsy is performed at another hospital, fibroblasts are cultured on-site. Once there are sufficient flasks of cells, they are sent to the requesting centre’s biochemical genetics lab for enzyme assay or BN-PAGE. If required, and based on the algorithm in the appendix, fibroblasts are sent to the molecular biology laboratory for extraction and mitochondrial DNA sequencing.

3. **Blood samples:** A tube of EDTA-treated blood is sent to the molecular biology laboratory for DNA extraction and analysis.

   The turnaround time for biochemical assays (enzyme assays and BN-PAGE) is approximately 8 weeks; for fibroblasts, cell culture alone takes approximately 4 weeks. Frequency is 2 to 4 weeks, and varies depending on volumes received. The turnaround time for molecular testing (mitochondrial DNA) is one month after the sample is received.

   Appendix A provides the integrated diagnostic algorithm for samples from patients with a suspected mitochondrial disorder, and the molecular diagnostic algorithm for mitochondrial DNA disorders submitted with the requester’s application for inclusion in the Index.

4. **TECHNOLOGY BACKGROUND**

   4.1 **Nature of the Diagnostic Technology**

   Spectrophotometric measurement of respiratory chain enzyme activity (complexes I to V) and BN-PAGE quantification of complexes are complementary techniques. Molecular analysis of mtDNA also supplements biochemical testing. It can identify two categories of mutations: large deletions and duplications (by MLPA), and point mutations (by Sanger sequencing).

   4.2 **Brief Description of the Current Technological Context**

   There are four main avenues of research into mitochondrial disorders: metabolic, histochemical, biochemical, and genetic. Metabolic assays provide elements that may support the diagnosis of a mitochondrial disorder. However, only biochemical and molecular testing can confirm the diagnosis. The patient’s family history and clinical presentation, imaging techniques, and muscle histochemical analyses are part of the whole picture to be considered when investigating and diagnosing this diverse group of disorders. The diagnostic strategy developed by Chaussenot et al. (Appendix B) clearly shows the complexity and multidisciplinary nature of diagnosing mitochondrial disorders [Chaussenot et al., 2011].
Metabolic Assays
The most common metabolic assays include screening for lactic acidosis through lactate assays, lactate-to-pyruvate ratio, ratio of ketone bodies 3-hydroxybutyrate to acetoacetate, chromatography of blood amino acids, serum creatine kinase, and fibroblast growth factor 21 (FGF-21) [DiMauro et al., 2013; Chaussenot et al., 2011].

Lactic acidosis is characteristic of all mitochondrial diseases, but it is not always present or necessarily severe; serum creatine kinase is moderately elevated in most mitochondrial disorders, and the FGF-21 assay is not widely used, although its diagnostic accuracy is better than that of conventional biomarkers, including lactate [DiMauro et al., 2013].

Muscle Histochemical Analysis
Despite its invasive nature, muscle biopsy remains the gold standard for diagnosis of mitochondrial disorders. Histochemical analysis of frozen muscle tissue sections by staining with the enzyme succinate dehydrogenase (SDH, respiratory chain complex II) and cytochrome c oxidase (COX, respiratory chain complex IV) provides valuable diagnostic clues to respiratory chain activity [DiMauro et al., 2013; Pfeffer and Chinnery, 2013].

Ragged red fibres (RRF) revealed by staining with modified Gomori trichrome are a histological feature of some mitochondrial myopathies. RRF indicate a proliferation and accumulation of mitochondria in the periphery of muscle fibres [Sarnat and Marin-Garcia, 2005].

Biochemical Assays
Measurement of respiratory chain complex enzyme activity in frozen tissue is currently performed at Nijmegen Centre for Mitochondrial Disorders (Netherlands), at a unit cost of C$1,400. The problem is that it is impossible to measure complex V using frozen tissue. Therefore, the tests performed are partial and cannot completely rule out a respiratory chain deficiency.

Genetic Testing
Mitochondrial DNA testing generally begins with screening for common point mutations and large deletions using real-time PCR, long-range PCR, and MLPA [Pfeffer et al., 2014; Tang et al., 2013; Kwon et al., 2011; Finsterer et al., 2009]. If results are negative, the entire mitochondrial genome is usually sequenced using the Sanger method [Tang et al., 2013]. Next-generation sequencing (NGS) is a recent technique that can identify common and rare point mutations, as well as deletions. It can also provide heteroplasmy measurement3 [Tang et al., 2013].

However, interpretation of the clinical significance of the mutations and variations detected by this technique is complex because of the highly polymorphic nature of mtDNA [Liang et al., 2014; Tang et al., 2013; Wong, 2013].

Baylor College of Medicine (Texas, USA) currently performs mtDNA testing at a unit cost of US$3,000.

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3 Heteroplasmy is a characteristic of mitochondrial genetic disorders in which normal and mutant copies of the mitochondrial genome may coexist within a tissue or organ. With homoplasmy, all mtDNA molecules are identical (normal or mutant). Clinical expression (phenotype) of a pathogenic mtDNA mutation is largely determined by the relative proportion of normal and mutant genome copies in tissues, also called the level of heteroplasmy [DiMauro and Davidzon, 2005].
4.3 Brief Description of the Advantages Cited for the New Technology

Spectrophotometry and BN-PAGE can both clarify the diagnosis. The requester provided a few examples that show the relevance of these two tests.

- A mild ambiguous deficiency is confirmed if both tests are abnormal. This can happen in adult patients with milder clinical effects.
- A coenzyme Q deficiency is seen only on the enzyme assay.
- A deficiency at the enzymes’ active site is seen only on the enzyme assay.
- In some cases, enzyme activity is normal but clinical analysis reveals a mitochondrial disorder. BN-PAGE can show an assembly defect in one of the five complexes and confirm the diagnosis.
- When a very limited amount of tissue is available, BN-PAGE analysis is performed because it requires less material than enzyme activity testing.

These tests evaluating respiratory chain function can reveal an isolated complex deficiency or a combined deficiency suggesting, for example, a mitochondrial deletion. This can then guide and target molecular diagnosis. Mitochondrial DNA sequencing and MLPA are therefore fully complementary to biochemical assays.

Fresh muscle tissue is preferable to frozen tissue because the activity of all respiratory chain complexes can be measured, including complex V for which testing on frozen tissue is less reliable (information provided by the requester) [Kirby et al., 2007]. According to the requester, being able to use fresh tissue argues in support of providing this testing locally.

4.4 Cost of Technology and Options: Information not available.

5. EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced

Spectrophotometry and BN-PAGE do not replace any tests for diagnosis of mitochondrial diseases currently in the MSSS Index. However, BN-PAGE was in the Index (code 50047), but was removed in 2011 (possibly performed only in a research laboratory at that time – information from the requester). These two complementary methods generally provide similar results, but one method or the other may be better for detecting certain deficiencies.\(^4\)

MLPA and mtDNA sequencing would replace mtDNA molecular testing currently sent outside Quebec; this test was previously performed in part by a research laboratory in Quebec. These techniques do not identify nuclear gene mutations, for which samples must also be sent outside the province (see point 8 in the requester’s algorithm, Appendix A).

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\(^4\) Information taken from a letter dated January 7, 2014, from Dr. Catherine Brunel-Guitton.
5.1.2 Diagnostic or Prognostic Value

Diagnostic confirmation is essential to:

- optimize disease management and patient care;
- provide supportive treatment to improve the quality of life of affected patients;
- determine the type and frequency of follow-up testing;
- predict the course of the disease in affected individuals;
- provide appropriate genetic counselling for families and a prenatal diagnosis.

None of the studies found dealt with the usefulness of this testing for the patient, in terms of mortality, morbidity, quality of life, etc.

Diagnosis of mitochondrial diseases is complex because it involves mitochondrial and nuclear genomes, level of heteroplasmy, and mitotic segregation (change in phenotype with age) [Liang et al., 2014; Dimauro and Davidzon, 2005]. Poor genotype-phenotype correlation may compromise the accuracy of clinical diagnosis [Liang et al., 2014]. A few studies have recently illustrated the diagnostic value of assaying respiratory chain complexes and detecting mtDNA and nDNA mutations (Table 1).
Table 1: Diagnostic value of respiratory chain complex assays and mtDNA and nDNA mutation testing

<table>
<thead>
<tr>
<th>STUDY</th>
<th>CASE/COHORT</th>
<th>DIAGNOSIS</th>
<th>METHOD</th>
<th>TISSUES</th>
<th>DIAGNOSTIC VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budde et al., 2000</td>
<td>N = 2</td>
<td>Leigh-like Syndrome</td>
<td>Enzyme activity assay Direct sequencing</td>
<td>Muscle</td>
<td>↓ CI and CIII activity nDNA mutation in \textit{NDUFS4} gene</td>
</tr>
<tr>
<td>Leshinsky-Silver et al., 2009</td>
<td>M, 28 months</td>
<td>Leigh Syndrome</td>
<td>CI to CV spectrophotometry CI to CIV BN-PAGE mtDNA and nDNA sequencing</td>
<td>Fresh muscle Fibroblasts</td>
<td>Partial ↓ in CI activity (muscle) Normal CI activity (fibroblasts) CIII assembly defect 2 mutations in \textit{NDUFS4} gene</td>
</tr>
<tr>
<td>Hoefs et al., 2010</td>
<td>N = 3 (children)</td>
<td>Various symptoms Low residual CI activity</td>
<td>Direct sequencing BN-PAGE 2D BN/SDS-PAGE in-gel activity Spectrophotometry</td>
<td>Fresh muscle Frozen muscle Fibroblasts</td>
<td>Severe ↓ in CI activity (&lt; 30% of the lowest control value) 5 mutations in \textit{NDUFS1} gene</td>
</tr>
<tr>
<td>Kwon et al., 2011</td>
<td>M, 45 years</td>
<td>CPEO, ptosis</td>
<td>MLPA Sanger Gap-PCR</td>
<td>Blood</td>
<td>Detection of deletion of 4,407 bp segment in mtDNA</td>
</tr>
<tr>
<td>Assouline et al., 2012</td>
<td>N = 7</td>
<td>Leigh Syndrome and CI deficiency</td>
<td>BN-PAGE</td>
<td>Fibroblasts</td>
<td>Mutations of gene \textit{NDUFS4} associated with an abnormal BN-PAGE profile</td>
</tr>
<tr>
<td>Pfeffer et al., 2014</td>
<td>N = 68 (adults)</td>
<td>CPEO with or without mtDNA deletions</td>
<td>Sanger MLPA Whole exome sequencing qRT-PCR, histochemistry, Western blot</td>
<td>Skeletal muscle</td>
<td>Exon deletions, single and compound heterozygous mutations of \textit{SPG7} gene</td>
</tr>
</tbody>
</table>

Abbreviations: BN-PAGE: blue native polyacrylamide gel electrophoresis; bp: base pair; CI: complex I; CIII: complex III; CIV: complex IV; CPEO: chronic progressive external ophthalmoplegia; CV: complex V; M: male; mtDNA: mitochondrial deoxyribonucleic acid; nDNA: nuclear deoxyribonucleic acid; qRT-PCR: quantitative reverse transcription polymerase chain reaction.

Two case studies and one cohort study dealt with the importance of respiratory chain complex assays (enzyme activity and detection of complexes I to IV) for guiding molecular diagnosis. The study by Budde et al. dealing with two patients with Leigh-like syndrome showed that a mutation in gene \textit{NDUFS4} (which codes for a subunit of complex I) can cause a combined complex I and III deficiency, and it suggested that patients with this type of combined deficiency should be screened for mutations in this gene [Budde et al., 2000]. In another case of Leigh syndrome, a partial deficiency in complex I activity was noted in muscle tissue with a normal result in cultured fibroblasts. Analysis of a group of mutations associated with Leigh syndrome did not reveal any pathogenic mutations. However, BN-PAGE results revealed a complex I assembly defect, suggesting the possibility of detecting mutations in gene \textit{NDUFS4} (subunit of complex I). Two mutations in this gene were found. Consequently, the authors conclude that BN-PAGE is an important tool to guide molecular diagnosis [Leshinsky-Silver et al., 2009]. A study involving seven patients with Leigh syndrome and a complex I deficiency indicated that mutations in gene \textit{NDUFS4} were associated with an abnormal BN-PAGE profile, revealing a
complex I assembly defect [Assouline et al., 2012]. The authors conclude that BN-PAGE can identify patients who may have NDUFS4 gene mutations and target them more narrowly before DNA sequencing is performed.

A case study involving three patients showed that mutations in the NDUFS1 gene of respiratory chain complex I can explain very low residual activity in complex I (< 30% of the lowest control value) expressed in cultured fibroblasts from these patients [Hoefs et al., 2010]. A study of a case of CPEO, a type of mitochondrial myopathy with progressive bilateral ptosis, showed that MLPA detected deletion of a 4,407 bp segment in mtDNA; the deletion was than confirmed by gap-PCR and Sanger sequencing [Kwon et al., 2011]. A cohort study of 68 patients with CPEO was conducted recently to understand the genetic basis of the disorder; it showed that SPG7 gene mutations are an important cause of CPEO associated with multiple mtDNA deletions [Pfeffer et al., 2014].

Additionally, appropriate genetic counselling and prenatal diagnosis have been suggested to inform affected families about the risks of a recurrence of mitochondrial disorders. For example, ten couples who had previously given birth to a child with a mitochondrial disorder were given a prenatal diagnosis based on measurement of respiratory chain complex enzyme activity in choriocytes (12 tests) and amniocytes (16 tests) collected during 21 pregnancies [Faivre et al., 2000]. In 7 of the 21 pregnancies, the test was performed twice, at the end of the first and second trimesters. Twelve healthy newborns with normal results remained healthy after follow-up that continued beyond the age of onset of the disease in the proband, seven pregnancies were terminated after an enzyme deficiency was found and five of these were confirmed by tissue analysis, and two fetuses that appeared normal after prenatal diagnosis turned out to be affected at birth. The authors conclude that an enzyme activity deficiency indicates recurrence, but a normal result at ten weeks of gestation is not necessarily conclusive. They also propose guidelines that state, among other things, that complex I activity cannot be accurately measured in fetal cells.

In a more recent case study, an assembly defect of respiratory chain complex I as revealed by BN-PAGE guided molecular testing and confirmed the diagnosis. Based on these results, prenatal diagnosis was performed for the subsequent pregnancy [Leshinsky-Silver et al., 2009].
### 5.2 Clinical Validity

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PRESENCE</th>
<th>ABSENCE</th>
<th>NOT APPLICABLE</th>
</tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>X</td>
<td></td>
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<tr>
<td>Specificity</td>
<td>X</td>
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<td></td>
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<tr>
<td>Positive predictive value (PPV)</td>
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<td>X</td>
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<tr>
<td>Negative predictive value (NPV)</td>
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<td>X</td>
<td></td>
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<tr>
<td>Likelihood ratio (LR)</td>
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<td>X</td>
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<tr>
<td>ROC Curve</td>
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<td>X</td>
<td></td>
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<tr>
<td>Accuracy</td>
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</tbody>
</table>

The results of a cohort study of 57 patients with well-characterized mtDNA mutations associated with respiratory chain complex activity deficiencies indicate that testing of respiratory chain enzyme activity is not sensitive enough (although it is specific) to diagnose mitochondrial myopathy in patients with transfer RNA (tRNA) gene mutations [Wibrand et al., 2010]. Decreased enzyme activity of one or more respiratory chain complexes was found in 10% of patients with the 3243A>G mutation. However, 83% of patients had other point mutations and 62% of patients had mutations with deletions. Enzyme analysis was compared with muscle fibre histochemistry, lactate concentration, and VO$_2$ max. Abnormal muscle histochemistry seems to be a better indicator of mitochondrial myopathy than enzyme activity (overall sensitivity of 77% and 39%, respectively). The sensitivity of lactate concentration and VO$_2$ max is 50% and 27%, respectively. However, the specificity of the four tests is quite high (93% to 100%).

Two retrospective cohort studies based on a database review revealed the phenotypic variability of mutations frequently reported in the literature. The first cohort study consisting of 129 patients [Nesbitt et al., 2013] revealed that, although the 3243A>G mutation in mtDNA gene MTTL1 is found in most cases of MELAS$^5$ syndrome, only 10% of patients in this cohort exhibited a classical MELAS phenotype. A large proportion of patients (30%) presented MIDD$^6$ syndrome, 28% presented a range of clinical characteristics not related to a classical syndrome associated with the mutation, and 9% were asymptomatic. The second study consisting of 42 patients revealed that most patients with the 8344A>G mutation (mtDNA) did not present classical MERRF$^7$ syndrome [Mancuso et al., 2013]. Although this mutation is reported as the main cause of this syndrome [Shoffner et al., 1990], it also presents with a large variety of phenotypes.

A case study of a 53-year-old presenting with the classical NARP$^8$ phenotype, primarily associated with the m.8993T>C/G mutation in subunit 6 of the ATP synthase (complex V), revealed a new point mutation [Duno et al., 2013]. Analysis for three common mtDNA mutations, m.3243A>G (associated with MELAS syndrome), m.8344A>G (associated with MERRF syndrome), and c.8993T>G/C (associated with NARP syndrome), showed normal sequences but revealed heteroplasmic presence of the m.8989G>C mutation in the blood.

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5 MELAS: mitochondrial encephalomyopathy.
6 MIDD: maternally inherited deafness and diabetes.
7 MERRF: myoclonus epilepsy with ragged-red fibres.
8 NARP: neuropathy, ataxia, retinitis, pigmentosa.
From a muscle standpoint, decreased complex V activity corroborates the pathogenic effect of the m.8989G>C mutation.

A few studies show the prevalence of detection of mtDNA and nDNA mutations associated with various mitochondrial phenotypes (Table 2).

**Table 2: Prevalence of mutations associated with mitochondrial disorders**

<table>
<thead>
<tr>
<th>STUDY</th>
<th>TECHNIQUE</th>
<th>PHENOTYPE</th>
<th>NUMBER OF CASES</th>
<th>NUMBER OF INDIVIDUALS WITH MUTATIONS (%)</th>
<th>TYPE OF MUTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swalwell et al., 2011</td>
<td>dHPLC, NGS, RFLP</td>
<td>Leigh, MELAS, mitochondrial encephalomyopathy</td>
<td>109</td>
<td>30 (27.5) 44 (40.4)</td>
<td>18 mtDNA missense AR point and nDNA deletions</td>
</tr>
<tr>
<td>Janer et al., 2012</td>
<td>Genome sequencing (confirmed by Sanger)</td>
<td>Encephalopathy</td>
<td>1</td>
<td>1 (100)</td>
<td>1 homozygous missense</td>
</tr>
<tr>
<td>Nesbitt et al., 2013</td>
<td>Direct sequencing</td>
<td>Leigh syndrome</td>
<td>4</td>
<td>3 (75)</td>
<td>3 missense</td>
</tr>
<tr>
<td>Ma et al., 2014</td>
<td>PCR</td>
<td>Complex I deficiency, heterogeneous phenotypes</td>
<td>62</td>
<td>32 (51.6)</td>
<td>24 missense in 15 mitochondrial genes</td>
</tr>
</tbody>
</table>

Abbreviations: AR: autosomal recessive; dHPLC: denaturing high pressure liquid chromatography; MELAS: mitochondrial encephalomyopathy; mtDNA: mitochondrial deoxyribonucleic acid; nDNA: nuclear deoxyribonucleic acid; NGS: next generation sequencing; PCR: polymerase chain reaction; RFLP: restriction fragment-length polymorphism.

A complex I deficiency is the most frequently observed abnormality in patients with a mitochondrial disorder, and Leigh syndrome associated with this deficiency is the most common clinical presentation [Leshinsky-Silver et al., 2009]. The Swalwell study [2111] dealing with clinical and genetic data from 109 patients diagnosed with a mitochondrial disorder with complex I deficiency shows the association between the different phenotypes and mutations in different mtDNA genes (Table 3). Pathogenic mtDNA mutations were identified in 30 of 109 patients (28%), 15 of whom had Leigh syndrome. The authors report that these mutations were previously described in other studies and were considered pathogenic. In half the cases, the percentage of mutant load or heteroplasmy was greater than or equal to 90. Generally, the higher the levels of heteroplasmy, the more severe the disease [Steffann et al., 2014].

By analyzing maternal DNA from 19 of the 29 probands, Swalwell et al. [2011] also showed that the mutant load was much lower in tissue from the mothers than in their affected child. In eight cases, no mutation was found in the mother, which could indicate a de novo mutation in the child, or that the mutation was not detected. It should be noted that the maternal tissue analyzed was usually blood.
<table>
<thead>
<tr>
<th>GENE (NUMBER OF PATIENTS)</th>
<th>MUTATION (NUMBER OF PATIENTS)</th>
<th>% MUTANT LOAD</th>
<th>TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELAS (n = 7)</td>
<td>MTND1 (3)</td>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>m.3697G&gt;A (1)</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m.3946G&gt;A (1)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m.3949T&gt;C (1)</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MTTL1 (4)</td>
<td>de = 60 to ≈ 70</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>m.3243A&gt;G (3)</td>
<td>&gt; 98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m.3271C&gt;T (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS (n = 14)</td>
<td>MTND3 (2)</td>
<td>91 and 97</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>MTND5 (6)</td>
<td>65</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≈ 60</td>
<td>Various*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>from 26 to 44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MTND6 (6)</td>
<td>&gt; 98</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95 and 97</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ME (n = 4)</td>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>MTND3 (2)</td>
<td>= 90 and ≈ 100</td>
<td>Muscle/blood</td>
</tr>
<tr>
<td></td>
<td>MTND6 (1)</td>
<td>&gt; 98</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>MTTL1 (1)</td>
<td>&gt; 98</td>
<td>Muscle</td>
</tr>
<tr>
<td>MM (n = 2)</td>
<td>MTTL1 (2)</td>
<td>81</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>LIMD (n = 3)</td>
<td>MTND3 (2)</td>
<td>&gt; 98</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 98</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>MTTW (1)</td>
<td>&gt; 90</td>
<td>Muscle</td>
</tr>
</tbody>
</table>

Source: adapted from Swalwell et al., 2011.

Abbreviations: LIMD: lethal infantile mitochondrial disease; LS: Leigh syndrome; ME: mitochondrial encephalomyopathy; MELAS: mitochondrial encephalomyopathy lactic acidosis, stroke-like episodes; MM: mitochondrial myopathy; n: number of patients.

* For each of 4 patients, the % of mutant load was 31 (muscle), 44 (fibroblasts), 30 (blood) and 26 (muscle).

† For each of 3 patients, the % of mutant load was 95 (muscle); 97 (fibroblasts), 97 (muscle).
5.3 Analytical (or Technical) Validity

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PRESENCE</th>
<th>ABSENCE</th>
<th>NOT APPLICABLE</th>
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<tbody>
<tr>
<td>Repeatability</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproducibility</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analytical sensitivity</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analytical specificity</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix effect</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Concordance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation between test and comparator</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

**Internal Validation of Biochemical Assays**

In a letter regarding the application for inclusion sent to MSSS (attached), the requester states that internal validation of the mitochondrial tests (enzyme assays and BN-PAGE) has been completed. For example, the results of four patient cases were submitted with the application. Based on information received from the requester, the validation process involved testing 25 positive cases (with respiratory chain complex deficiency). These cases were also sent to the referral centre in Netherlands, and the deficiencies found were confirmed.

**Internal Quality Control**

Each enzyme activity assay is performed on two aliquots prepared from a tissue biopsy or a single fibroblast culture. Each aliquot is assayed on two different days using freshly prepared reagents. The average of both results for each complex is calculated and sent to the requesting physician if the values for each measurement do not differ from the mean by more than 10%. If they do, a third measurement is performed on a third aliquot with recently prepared reagents and the average of the closest two values is reported.

Reliability of results is ensured by parallel analysis of normal fibroblast and muscle controls. Reference values have been established for each of the five complexes.

For genetic testing of mitochondrial DNA, tissue samples from patients who received a positive test result from an external diagnostic laboratory (Europe, USA) will be analyzed as an internal control.
External Quality Control

According to the requester, there is no external quality control program at this time for enzyme assays and the BN-PAGE technique. The possibility of exchanging a few samples once a year with another reference laboratory that performs similar tests will be reviewed. For analysis of mutations through sequencing and MLPA of mitochondrial DNA, a partnership with the College of American Pathologists9 (CAP) is planned to ensure the quality of genetic testing.

Published Results of Analytical Validation

Biochemical Assays: a study was conducted to measure the effect of various conditions on the analytical performance of enzyme activity assays for each complex of the mitochondrial respiratory chain [Spinazzi et al., 2011]. Enzyme assay protocols were optimized for each complex and a new method was developed for complex III, generally considered the most difficult to measure [Chretien et al., 2004]. The tissue homogenization protocol, choice of homogenization buffer (composition, concentration, and pH), choice of reagents and spectrophotometric parameters were assessed. The results of the analytical performance of enzyme activity assays on muscle homogenates were presented for each complex except complex V (Table 4).

The authors conclude that homogenization protocol and analytical conditions have a significant effect on the results of spectrophotometric assays of respiratory chain complex enzyme activity used to diagnose mitochondrial disorders.

The results of enzyme activity of complexes I to IV in cultured fibroblasts (10 patients) were compared with those from another laboratory [Kramer et al., 2011]. Interpretation of the results concorded for 91% of the samples with known deficiencies of certain respiratory chain complexes. Mean intra-assay CV for 20 replicates for each complex was 5.9%. Interassay variation was evaluated using two samples analyzed three times each. Mean CV was 13% for complex I, 18% for complex II + III (combined activity), and 13% for complex IV.

9 CAP offers a quality control program in which samples are sent on a regular and random basis to verify that the techniques used and the resulting analysis meet specific quality standards.
Table 4: Analytical performance of spectrophotometric assays of respiratory chain enzyme activity in muscle homogenates

<table>
<thead>
<tr>
<th></th>
<th>CI</th>
<th>CII</th>
<th>CIII</th>
<th>CIV</th>
<th>CI + CIII</th>
<th>CII + CIII</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle protein (µg)</td>
<td>30</td>
<td>24</td>
<td>6</td>
<td>1.5</td>
<td>30</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Intraday CV (n = 10)</td>
<td>3%</td>
<td>5.2%</td>
<td>5.2%</td>
<td>3%</td>
<td>1.7%</td>
<td>2.8%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Interday CV (n = 10)</td>
<td>4.1%</td>
<td>4%</td>
<td>7.4%</td>
<td>3.7%</td>
<td>6.4%</td>
<td>6.7%</td>
<td>5.4%</td>
</tr>
<tr>
<td>% inhibition (n = 3)</td>
<td>98%</td>
<td>97% (mal)</td>
<td>67%</td>
<td>100%</td>
<td>79%</td>
<td>99% (mal)</td>
<td>-</td>
</tr>
<tr>
<td>Linearity index (n = 5)</td>
<td>0.89 ± 0.02</td>
<td>0.95 ± 0.05</td>
<td>0.82 ± 0.03</td>
<td>0.77 ± 0.08</td>
<td>0.85 ± 0.012</td>
<td>1.01 ± 0.09</td>
<td>1.01 ± 0.07</td>
</tr>
</tbody>
</table>

Source: Spinazzi et al., 2011.

Abbreviations: Aa: antimycin; CI: complex I; CII: complex II; CIII: complex III; CIV: complex IV; CS: citrate synthase; CV: coefficient of variation; mal: malonate; n: number of samples; TTFA: 2-thienoyltrifluoroacetone 500 µM.

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10 Definitions: Intraday CV: repeated measurements performed the same day; Interday CV: repeated measurements performed on different days; % inhibition: degree of inhibition by an appropriate enzyme inhibitor, specificity of enzyme activity; linearity index: linearity of the enzyme reaction, ratio of the enzymatic rate observed during the first 60 seconds and the activity measures between 120 and 180 seconds, which can estimate the decay of the enzymatic rate calculated two minutes after the initiation of the reaction; values less than 1 indicate a reduced rate of reaction over time.
A comparative study of five European diagnostic laboratories dealt with sample preparation methods and respiratory chain complex enzyme activity assays [Rodenburg et al., 2013]. Three control muscle samples (biopsies and homogenates) were analyzed in duplicate to assess enzyme assay reproducibility and the effect of sample preparation method. The average interlaboratory CV for complex I to IV assays was 16% for biopsies and 11% for homogenates. For complex V, the results were 28% for biopsies and 18% for homogenates. Fibroblasts from 16 patients and control cases were analyzed in 4 labs (64 measurements) to determine diagnostic accuracy. Most samples were correctly identified as a control or a sample with enzyme deficiency. One incorrect result was obtained in 3 cases, which corresponds to an error rate of 5%. According to the authors, sample preparation method is one of the main causes of variation, as previously reported in the study by Spinazzi et al. described above.

*Molecular Assays:* screening for mtDNA mutations and large deletions in blood samples is usually the first step in assessing a patient with a suspected mitochondrial disorder ([Wong et al., 2010], requester’s algorithm in Appendix A). mtDNA sequencing is used to screen for common point mutations and mutations associated with the so-called classic syndromes first. Additionally, identification of a biochemical or histochemical deficiency (isolated or generalized) in respiratory chain complexes can suggest the presence of mtDNA mutations or deletions, and the need to sequence certain genes [Wong et al., 2010].

A recent study involved 16 patients with symptoms of a mitochondrial disorder and negative test results for common mtDNA point mutations; its aim was to compare MLPA with three other molecular diagnostic methods (Southern blot, PCR, long-PCR) [Tonska et al., 2012]. The results showed that MLPA was not able to confirm all mtDNA deletions identified by PCR methods, especially when the level of heteroplasmy was low (less than 40%). Of the 22 samples analyzed (14 blood, 7 muscle, 1 liver), there were 12 positive results from PCR and long-PCR, 4 positive results (concordant) from MLPA, and 3 positive results from Southern blot. Nevertheless, the authors conclude that MLPA should be used given its low cost and ease of processing, but they recommend that it be used as a screening method and that results confirmed by PCR. For the requester, a positive MLPA result serves as diagnostic confirmation. Sequencing follows in cases where the result is negative (Appendix A, Algorithm 2).

### 5.4 Recommendations from Other Organizations

The Clinical Molecular Genetics Society (CMGS) has guidelines for molecular diagnosis of mitochondrial disorders [CMGS, 2008], which stress the importance of the type of tissue used to establish the diagnosis and its effect on the likelihood of detecting a mutation for certain categories of clinical presentations. Preferred tissues for these categories are presented in Table 5.
Table 5: Preferred tissue for diagnosis based on clinical presentation

<table>
<thead>
<tr>
<th>CLINICAL PRESENTATION</th>
<th>BLOOD</th>
<th>MUSCLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHON</td>
<td>***</td>
<td>Not necessary</td>
</tr>
<tr>
<td>Pearson syndrome</td>
<td>***</td>
<td>Not necessary</td>
</tr>
<tr>
<td>KSS/CPEO</td>
<td>*</td>
<td>****</td>
</tr>
<tr>
<td>Deletion</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>MELAS/MIDD</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>MERRF</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Leigh syndrome/NARP</td>
<td>***</td>
<td>Not necessary</td>
</tr>
<tr>
<td>Deafness (1555A&gt;G)</td>
<td>***</td>
<td>Not necessary</td>
</tr>
</tbody>
</table>

Adapted from Clinical Molecular Genetics Society (CMGS), 2008.

CMGS recommendations are arranged by clinical presentation groups, and the molecular testing techniques suggested are discussed based on clinical features. Mitochondrial genome sequencing is indicated for patients in whom investigations in affected tissue suggest a mitochondrial respiratory chain defect but there is no evidence of mtDNA rearrangements or common point mutations. Screening of nDNA-encoded genes is recommended for cases of isolated complex I, II, and IV deficiencies.

The European Federation of Neurological Sciences (EFNS) recommends a sequential procedure for genetic diagnosis of mitochondrial disorders that includes the test methods suggested [Finsterer et al., 2009].

1. Family and individual history, and clinical investigations by specialists in neurology, ophthalmology, otology, endocrinology, cardiology, gastroenterology, nephrology, hematology or dermatology.

2. The clinician must determine whether the phenotype observed corresponds to a mitochondrial syndrome (syndromic phenotype), and whether it occurred sporadically or followed Mendelian or maternal inheritance.

3. Genetic testing (Appendix C):
   - Syndromic phenotype due to mtDNA point mutations (MELAS, MERRF, NARP, LHON): DNA-microarray hybridization, real-time PCR or single-gene-sequencing.
   - Syndromic phenotype due to mtDNA deletion (mtPEO, KSS, Pearson syndrome): RFLP (restriction fragment length polymorphism) or Southern blot.
     - deletion with low heteroplasmcy rate: long-range PCR
     - single and multiple deletions absent: mtDNA sequencing
     - multiple deletions: sequencing of particular genes (POLG, TYMP, etc.)
• Non-syndromic phenotype: biochemical investigation of the most affected tissues (muscle, liver, brain, skin).
  ▪ autosomal transmission of multiple biochemical defects: Southern blot or sequencing
  ▪ maternal transmission: sequencing
  ▪ presence or absence of depletion: sequencing of genes involved in mitochondrial protein synthesis (see details in the figure in Appendix C).

6. ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources: Not assessed.

6.2 Economic Consequences of Introducing Test Into Quebec’s Health Care and Social Services System

Measurement of enzyme activity and mtDNA analysis are currently performed outside Quebec at a higher cost than that of the weighted values calculated by the requester. Therefore, there would be a cost benefit to conducting these tests in Quebec.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues: Not assessed.

7. IN BRIEF

7.1 Clinical Relevance

Analysis of respiratory chain complexes and mtDNA is used to make or confirm the clinical diagnosis of a particular syndrome following the manifestation of symptoms affecting various organs. It can guide testing for mutations, and appropriate genetic counselling and prenatal diagnosis for families affected. No studies have assessed the effect on mortality, quality of life, etc., or on patient treatment.

7.2 Clinical Validity

Analysis of respiratory chain enzyme activity is not sensitive enough (although it is very specific) to establish a diagnosis for certain mitochondrial disorders. In some cases, abnormal muscle histochemistry seems to be a better indicator of mitochondrial myopathy than enzyme activity (sensitivity: 77% versus 39%, respectively; specificity: 100% versus 98%, respectively).

A few case studies show a genotype-phenotype connection. Although some mutations are commonly associated with a so-called classic syndrome, a few studies show that these mutations are also manifested under a wide variety of phenotypes. The genotype-phenotype correlation is weak due to the presence of mitochondrial and nuclear genomes, and the level of heteroplasmy, which can compromise diagnostic accuracy.

7.3 Analytical Validity

Sample preparation method and analytical conditions are the main causes of variation in spectrophotometric assays of respiratory chain complex enzyme activity. Intraday and interday variations below 7% were obtained. Interlaboratory concordance of 91% was reported for analysis of complex I to IV enzyme activity.
A single study was found on the analytical validity of mtDNA analysis. Results showed that MLPA cannot confirm all mtDNA deletions identified by PCR. MLPA identified deletions in 18% of samples compared with 55% for PCR. MLPA is low cost and easy to perform; it should always be followed by PCR confirmation of positive results. Although each test (biochemical and molecular) has limitations, an integrated approach ensures optimal diagnosis of mitochondrial disorders.

### 7.3 Recommendations from Other Organizations

CMGS stresses the importance of the type of tissue used to establish the molecular diagnosis of mitochondrial disorders and its effect on the likelihood of detecting a mutation for certain categories of clinical presentations. Mitochondrial genome sequencing is recommended for patients in whom investigations in affected tissue suggest a mitochondrial respiratory chain defect with no evidence of mtDNA rearrangements or common point mutations. Screening of nDNA-encoded genes is recommended for cases of isolated complex I, II, and IV deficiencies.

EFNS recommends a sequential procedure for genetic diagnosis of mitochondrial disorders. The test methods suggested depend on the type of phenotype observed (syndromic or non-syndromic) and the mode of presentation (sporadic, maternal or Mendelian transmission).
8. INESSS NOTICE IN BRIEF

Testing of Mitochondrial DNA and Mitochondrial Respiratory Chain Complexes

<table>
<thead>
<tr>
<th>Status of the Diagnostic Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>✗ Established</td>
</tr>
<tr>
<td>☐ Innovative</td>
</tr>
<tr>
<td>☐ Experimental (for research purposes only)</td>
</tr>
<tr>
<td>☐ Replacement for technology: ___________________ , which becomes obsolete</td>
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</table>

<table>
<thead>
<tr>
<th>INESSS Recommendation</th>
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<tbody>
<tr>
<td>✗ Include test in the Index</td>
</tr>
<tr>
<td>☐ Do not include test in the Index</td>
</tr>
<tr>
<td>☐ Reassess test</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ Draw connection with listing of drugs, if companion test</td>
</tr>
<tr>
<td>☐ Produce an optimal use manual</td>
</tr>
<tr>
<td>☐ Identify indicators, when monitoring is required</td>
</tr>
</tbody>
</table>
APPENDIX A
Algorithms

Figure A1: Integrated diagnostic management algorithms for samples from patients with suspected mitochondrial disease

Documentation provided by the requester (in French only).
Figure A2: Molecular diagnostic algorithm for mitochondrial DNA disorders

Documentation provided by the requester (in French only).
APPENDIX B
Diagnostic Strategy for Mitochondrial Disorders

Clinical and paraclinical tests that suggest mitochondrial pathologies:
- Biology:
  - Elevated lactate and L/P ratio, hyperlactatorachia
  - Elevated Krebs cycle derivatives
  - Metabolic work-up with no support for another metabolic pathology
- Cerebral MRI: basal ganglia hypersignals, stroke-like episodes, lactate peaks on MRI

Blood test
- Testing for mtDNA deletion (Pearson syndrome) or mutations responsible for Leber optic atrophy
- Testing for common mutations but the cost-benefit is low

Tissue biopsy
- Muscle, liver, kidney, fibroblasts...

Molecular testing
- Enzymology
- Histology

Testing for the most common mutations
- mtDNA deletion
- mtDNA depletion
- Single deletion (Keams-Sayre syndrome, PEO)
- Multiple deletions

Testing of mitochondrial (tissue) or nuclear (blood) genes corresponding to subunits of the complex with the deficiency and/or the clinical picture, or exhaustive mtDNA study by Surveyor or sequencing

Source: Adapted from Chaussenot et al., 2011.
APPENDIX C
Genetic Analysis Algorithm for Mitochondrial Disorders

Source: Finsterer et al., 2009.
REFERENCES


