DIAGNOSTIC TESTS PERFORMED ROUTINELY OR AVAILABLE IF NEEDED

1. Muscle Biopsies

A. Histological Evaluations:

Routine stains (a) and histochemical evaluation of sections (b) are done on all specimens; additional Immunohistochemical (c) or/and ultrastructural studies (d) are done whenever needed for diagnostic evaluation.

- **Routine stains on cross & longitudinal sections from paraffin and frozen tissue blocks:** Hematoxylin & Eosin (H&E), Masson's trichrome (MT), Verhoeff-Van Gieson (VVG).
- **Histochemistry:** Modified Gomori's trichrome (GT), Oil Red O (ORO), PAS (PAS +/- Diastase digestion) are run on all cases, while Phosphorylase, Phosphofructokinase , Myoadenylate Deaminase, Acid Phosphatase, Non-specific Esterase (NSE) and other useful histochemical stains are only run when specific diagnoses of glycogenosis, neurogenic myopathy or abnormal protein metabolism are suspected clinically; **Mitochondrial enzymes activity panel** (NADH-TR, COX, SDH, COX-SDH, COX-NADH, ATPsynthase) and **Myosin ATPase** at pH 9.4 and 4.3 to assess differential proportions of myofiber types differentiation.
- **Immunohistochemistry**:
    1- **Antibodies to Dystrophy related proteins:** Dystrophin (Rod, C and N terminals), Utrophin, Sarcoglycans (A, B, D & E), Dysferlin, Calpain-3, Caveolin-3, Titin, Desmin, Myotilin, Lamin A/C, Emerin, Laminin, Merosin, Alpha and Beta Dystroglycans, Collagen IV and VI, Actin, Alpha-Actinin) and to ATPsynthase.
    2- **Immune panel to diverse inflammatory myopathies:** (MHCI, C5b-9, CD3, CD8, CD20, CD1a, CD10, CD68, CD45RO).
- **Morphometric evaluation** of myofiber size, type and distribution in plastic embedded sections, for the differential diagnosis of congenital and acquired myopathies.
- **Electron Microscopy:** Ultrastructural Study of Epon-embedded ultra-thin sections for the diagnosis of congenital storage, and metabolic diseases, and acquired infectious and degenerative processes.

B. **Mitochondrial Biochemistry**: Mitochondrial respiratory chain oxidative enzymes activities, and other non-oxidative mitochondrial enzymes activities are delineated. This study involves a detailed outline of the mitochondrial respiratory chain complexes (namely, CI, CII, CIII, CIV, CV, CI+III, CI+II) as well as the Citrate Synthase activity (which reflects total mitochondrial content of the muscle), and activities of other enzymes useful for interpretation of the observed changes (G3PDH, IDHs (NADP), and cytosolic LDH).
All above listed enzymatic activities are routinely assessed when an added request for this **Biochemical Study of Mitochondrial Activity** is received with the fresh muscle biopsy, using a routinely rapidly frozen portion of the muscle biopsy received. Such portion of the muscle biopsy is kept in deep freeze at -80°C to enable possible later requests for Biochemical Study in the diagnosis of Mitochondrial Dysfunction, or Multiplex Immunoblotting in the evaluation of Muscular Dystrophies (as detailed below).

C. **Muscle Dystrophy Multiplex Immunoblotting**: This electrophoretic migration procedure may be requested to further assess the steady state level of 6 skeletal muscle proteins most commonly implicated in muscular dystrophies namely, Dystrophin, Dysferlin, Calpain-3, Caveolin-3, Alpha-Sarcoglycan and Beta-Dystroglycan.

D. **Molecular Analysis:** Genetic sequencing procedures are used to assess the Mitochondrial Genome, or the Mitome (mitochondrial genome in addition to a set of genes) or panels of genes most frequently implicated in neuromuscular diseases.

2. Nerve Biopsies

A. **Routine stains and histochemistry:** H&E, Masson's trichrome (MT), LFB-PAS (Myelin stain)
Alcian blue, Crystal violet, Congo red, PAS+/-D, modified Gomori's trichrome (GT) and NSE.

B. **Immunohistochemistry (when needed)**: CD56, C5b9, CD3, CD8, CD20, CD68, IgM, IgA, IgG, Kappa and Lambda light chains, Transthyretin (TTR), CD1a, PGP 9.5.

C. **Morphometric Analysis performed on toluidine-stained semi thin plastic sections.**

D. **Myelinated Fibers evaluation on teased nerve fibers slides.**

E. **Ultrustructural analysis of photographs taken from ultrathin plastic sections.**