European Reference Network

for rare or low prevalence complex diseases

Network Neuromuscular Diseases (ERN EURO-NMD)

EURO-NMD NEUROMUSCULAR PATHOLOGY WORKING GROUP

Recommended Standards for Muscle Biopsies; to be used by all partner laboratories

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Building bridges and breaking barriers in rare neuromuscular diseases

HOW TO SECURE BEST TISSUE QUALITY

Muscle biopsy samples should be handled very carefully at all stages to avoid artefacts. For cryostat sections, the samples need to be of a size and shape that allows an easy orientation in order to obtain transverse sections.

The samples for cryostat section should be frozen in isopentane cooled in liquid nitrogen; this should be done on site, immediately after collection. The time lapse between the collection of the specimen and freezing should not exceed 2 hours, or less than 15 minutes for biochemical studies of respiratory chain enzymes.

Frozen samples should be transported in dry ice or liquid nitrogen and stored at - 80° C. Any thawing during cryosectioning or during other procedures after freezing will damage the sample.

Before storage, specimens fixed in glutaraldehyde should be embedded in the resin for EM.

RECOMMENDED STANDARD LAB METHODS:

1. Specimen preparation

When processing the biopsy, samples for the following essential techniques should always be available:

- Histology
- Enzyme histochemistry **EHC**
- Immunohistochemistry **IHC**
- Western blotting
- Semithin sections of resin embedded muscle
- Electron microscopy

How to proceed:

- Mounted muscle sample - snap frozen in isopentane cooled in liquid nitrogen for 30 sec (longer in the case of a big sample), performing small circular movements.
- Glutaraldehyde-fixed muscle for electron microscopy - fixation best done immediately after removal of the tissue
- (+/- Formalin-fixed, paraffin-embedded muscle)
- Separate frozen muscle (without isopentane) for specific methods such as: Western blot, RNA-cDNA, enzyme biochemistry and +/-secondary paraffin embedding
- Optional: skin biopsy for fibroblast culture and muscle tissue for myoblast culture (specific protocols)

Cryostat Sections (Snap frozen sections)

- Cryostat at −23 to −25°C
- For histology, histochemistry and immunohistochemistry 7–10 µm sections

2. Routine stains for all new biopsies (frozen tissue):

Conventional histology, **with at least 2 serial sections from 2 different levels for each of the following staining:**

- H&E
- Gömöri Trichrome (modified)
- ORO / Sudan black
- PAS

EHC:

- NADH-TR
- COX-SDH
- Acid phosphatase
- ATPases: Type 1 and Type 2A, 2B and 2C fibres

➔ Alternatively, for fiber typing: Myosin heavy chain IHC with antibodies to slow beta and fast IIA with hematoxylin counterstain for Fiber types I, IIA, IIX, and hybrids I+IIA (corresponding to 1-2A-2B-2C with ATPase)

IHC – routine:

- Myosin heavy chain neonatal/fetal
- Myosin heavy chain developmental/embryonic
- Myosin heavy chain MyHC fast
- Myosin heavy chain MyHC slow/beta cardiac

  Optional: MyHC fast and slow when ATPase is used for fiber typing

- MHC-class1
- p62

Electron microscopy

To be used in samples without diagnostic findings by other methods, to clarify abnormalities observed or not visible on light microscopy. Particularly in: Congenital myopathies, unclear sarcoplasmic abnormalities, dysimmune, toxic, mitochondrial, metabolic myopathies and in neonatal muscle biopsies.

Optional: NMJ in myasthenic syndromes

3. Recommended extended methods – context dependent:

Methods that should be available or accessible for all EURO-NMD pathology labs as needed:

1. **Muscular dystrophy, congenital and progressive myopathies**

IHC: For sarcolemmal protein defects (use Beta Spectrin as the positive control): Dystrophin: N, rod and C domains, Utrophin, Sarcoglycans □□□□□□□, □DG glycosylated (always together with beta dystroglycan), □2-Laminin 80 &300 kDa, Caveolin-3, Emerin, Telethonin, COL6 (use together with perlecan or COL IV as control). Pediatric biopsies: laminin β1, laminin γ1 laminin alpha 5

Optional: nNOS

WB: DYS (at least 2 domains), dysferlin, Calpain3 (2 antibodies), aDG (with bDG)

Optional: sarcoglycans

2. **Immune mediated myopathies**

IHC: p62, CD68, CD8, CD20, C5b-9, CD31

EHC: alkaline phosphatase

Optional: van Gieson, MHC-class2, CD45/CD3, CD169, CD138, MUM1, MxA, ISG15

3. **Vacuolar and protein aggregate myopathies**

IHC: p62, TDP-43, Lamp2, Dys1, MHC class1, C5b-9, Desmin, Myotilin, Ubiquitin

EHC: Menadione NBT without substrate

Optional: LC3, FHL1, Filamin-C, BAG3, HSP70, HSP90, □B-Crystallin, CD68, CD45

4. **Congenital myopathies**

   IHC: RYR1, DHPR, SERCA1+2, α-actinin 2, phalloidin

   Optional: Sarcomeric and cardiac actin - for actinopathies using high salt for specificity

5. **Mitochondrial myopathies**

   EHC: COX and SDH (both techniques in isolation)

   Optional IHC: subunits of the mitochondrial respiratory chain complexes I-V

6. **Toxic myopathies**

   IHC: CD45, CD68, LC3

7. **Ion channel myopathies**

   Optional: IHC: CLCN1, RYR1, DHPR, SERCA1, SERCA2 STAC3

8. **Glycogenoses**

   EHC: Phosphofructokinase, Phosphorylase, LDH, PAS-D

   Semithin resin embedded sections: PAS

   Biochemical analysis of enzyme defects, which cannot be stained

9. **High CK and exercise intolerance, cramps**

   Histology: PAS-D

   EHC: Phosphofructokinase, Phosphorylase, Phosphorylase kinase A, B and A+B, LDH,

   IHC: Dystrophin, sarcoglycans, utrophin, beta spectrin, caveolin 3, dysferlin, laminins

   WB: calpain3 (2 antibodies), dysferlin

10. **Amyloid myopathy**

    HC: Congo red

    IHC: transthyretin, Immunoglobulin light chains κ, λ

11. **Myopathies with affected neuromuscular junctions (NMJ)**, require special biopsies, i.e. motor point biopsies or entire very short muscles and optionally EM

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