PROTOCOL FOR MUSCLE & NERVE BIOPSIES

The Department of Pathology at the University of Louisville, in Louisville, Kentucky, currently offers histochemical evaluation of muscle biopsy specimens and electron microscopic evaluation if indicated. Proper handling of the muscle biopsy, especially proper freezing, will help ensure a correct diagnosis for the patient; therefore, the following protocol should be observed. Due to the limitations of our laboratory, we DO NOT accept fresh specimens containing INFECTIOUS AGENTS: such as, but not limited to, hepatitis, human immunodeficiency virus (HIV), tuberculosis (TB), or Creutzfeldt-Jakob disease (CJD).

MUSCLE BIOPSY

1. **SCHEDULING** - Notify the Special Procedures Laboratory (SPL), (502) 852-5519, 24 hours in advance of the biopsy. It is the responsibility of the surgeon or his office, the surgery department where the biopsy is being performed, and the submitting Pathology Laboratory to see that the muscle biopsy is scheduled with SPL. Due to changes in the Medicare Payment Schedule, all muscle/nerve biopsies must be accessioned in the requesting outpatient surgery/hospital’s pathology department. Muscles received without an accession number will be frozen but not processed until said accession number is received from the Pathology Department.

2. **COMMUNICATION** - This is of EXTREME IMPORTANCE. SPL is to be notified when the biopsy begins. This procedure should be SCHEDULED - not "to follow". The courier from the UofL Pathology Department will pick up muscle biopsies from the hospitals within the Medical Center complex ONLY if the biopsy is scheduled 24 hours in advance. **ALL SPECIMENS MUST BE LABELED WITH THE BIOHAZARD LABEL AS PER THE KENTUCKY BLOODBORNE PATHOGENS STANDARD #1910.1030. ALL SPECIMENS MUST BE LABELED WITH THE PATHOLOGY ACCESSION NUMBER.**

3. **AVAILABLE** - Biopsies may be scheduled between 8:00AM and 2:00 PM, Monday through Friday, excluding holidays. Specimens scheduled at other times are YOUR RESPONSIBILITY. To properly handle these 'emergency' or otherwise scheduled biopsies, please contact the SPL to schedule proper training of your personnel. This training will consist of correct orientation of specimen, flash/snap freezing, and storing of specimens until they can be sent to SPL.

4. **MUSCLE** - The biopsy specimen for frozen section enzyme histochemistry should be UNCLAMPED and UNDAMAGED (e.g., tied to tongue blades or squeezed with forceps,), UNCAUTERIZED, NOT PREVIOUSLY DISSECTED, and NOT CHOPPED. It should be placed on gauze that has been slightly dampened with saline. NOTE: Slightly dampened means that not even a drop of saline can be squeezed out of the gauze and the corners of the gauze are dry. A muscle biopsy 7-10 mm in diameter is optimal, comparable to a pencil eraser; the longitudinal verses cross fibers should be apparent and a minimum of 20mm in length. **DO NOT place muscle, at any time, in or near any gross area containing the liquid or fumes of formalin, glutaraldehyde, or alcohols as these will alter the enzymes.**

5. **EM** - The biopsy for electron microscopy should be clamped in situ and placed immediately in freshly prepared 3% glutaraldehyde.

6. **PATIENT INFORMATION** – As required by the College of American Pathologists (CAP), specimens must be accompanied with a completed requisition form, which includes patient identification, hospital numbers, pathology accession number, appropriate clinical information, and patient history. This ensures a proper diagnosis. Billing and insurance information is also necessary.
7. **CANCELATION** – Should a scheduled muscle biopsy be canceled, please notify the laboratory as soon as possible.

8. **STAINS** - The stains routinely performed for diagnosis include: H&E, modified Gomori trichrome, oil red O, Congo red, PAS, PAS-D, ATPase, SDH, NADH, COX, PFK, NE, APP, and AD.

9. **SNAP FREEZE** - Specimen will be frozen and stored at -70 °C according to CAP guidelines.

10. **REPORT** - In general, a verbal or written report will be available within 4 working days of receipt of the specimen.

**OTHER INFORMATION**

1. **Indications for muscle biopsy**
   a. Unexplained muscle weakness.
   b. Unexplained muscle pain.
   c. Evaluation of connective tissue disorder.
   d. Abnormality of muscle contraction such as myotonia or hypotonia.

2. **Before biopsy** - Before a muscle biopsy, certain points should be considered. The muscle selected for biopsy should be the least affected but definitely affected. Severely fibrotic or atrophic muscle should be avoided. Sites which have been previously traumatized by biopsy, injection, electromyography, or trauma should be avoided. Determine if muscle is to be obtained for special enzyme histochemistry studies, biochemical analysis, electron microscopy, routine paraffin processing, all of these, or any combination of these.

3. **At biopsy** - If muscle is to be obtained for routine studies, enzyme histochemistry, and electron microscopy, three cylindrical specimens are needed, each about the diameter of a pencil eraser. The length of the biopsies should range from 1/3 to 2/3 inch long. The biopsy specimen should not be infiltrated by the local anesthetic.

**NERVE BIOPSY**

1. Nerve biopsies should contain the same information/paperwork as muscle biopsies.
2. Nerve biopsies are to be sent fresh on barely moistened saline gauze.
3. Stains include: H&E, Masson trichrome, LFB/PAS, Congo red, and neurofilament via immunohistochemistry.
4. Electron microscopy with toluidine blue thick-sections will be performed.