MUSCLE BIOPSY PROCESSING PROTOCOL

I  Clinical Information
Provide the clinical history, family history, EMG and nerve conduction results, CK level, and the site of
the muscle biopsy.

II  Muscle Biopsy Specimen
Three portions of muscle are required:

A. Muscle for enzyme histochemistry should be at least 1.0 - 1.5 cm in length and at least 0.5 cm in
cross-sectional diameter. There are two alternatives for handling and transporting this portion of the
biopsy:
   1. If the muscle can be transported within 2-4 hours and received at TNMC, prior to 4:30pm
      on weekdays, it can be sent fresh. Wrap the muscle (in the clamps) in saline moistened
      gauze (not floating in saline) and keep it cool with cold packs or wet ice (not in direct
      contact).
   2. If the delay will be longer that this, freeze the muscle as described below, and then transport
      on dry ice.

B. Muscle for electron microscopy should ideally be 1.0 cm in length and 0.3 cm in diameter;
maintained in an isometric state, preferably by clamping it in situ with a muscle clamp (Figure 1).
The muscle is fixed immediately in MPG fixative (2.5% glutaraldehyde/2% paraformaldehyde in
0.1 M phosphate buffer) or other routine EM fixative.

C. Muscle for paraffin embedding is taken as a separate specimen and then fixed in formalin.
   Alternatively, excess portions of muscle can be removed from the above specimens, but only if
   there is enough excess tissue to do so.

III. Freezing Technique
Trim the muscle to about 0.5 -1.0 cm in length and 0.5 cm in diameter. Place one end in 10% gum
tragacanth on a flat piece of cork so that the long axis is perpendicular to the cork (Figure 2). The
muscle should extend above the level of the gum. The cork and the specimen are then inverted and
submerged for 10 - 12 seconds in isopentane (2 methyl butane) cooled by liquid nitrogen (Figure 3).
Very quickly, working on dry ice or other very cold environment, wrap the frozen muscle in aluminum
foil, place the wrapped specimen in a biohazard bag, and place it immediately onto dry ice for shipping
or hold it in a -80°C freezer until it can be packed on dry ice for shipping (Figure 4). Do not allow any
opportunity for partial thawing.

Note: If gum tragacanth is not available, an O.C.T. type media may be used to attach the muscle to a
glass slide for freezing. Do not cover the muscle in O.C.T., just use a tiny amount on the glass slide and
lay the muscle longitudinally on top of it. Flash freeze the specimen, place the frozen specimen in a cold
biohazard bag then place it immediately on dry ice for shipping.

Or, roll/wrap the fresh muscle in aluminum foil (without O.C.T), flash freeze the wrapped specimen,
place the frozen specimen in a cold biohazard bag, then place it immediately on dry ice for shipping.
IV Shipping

If the muscle is transported fresh on wet ice, it must be completely sealed to prevent exposure to water. Specimens sent on wet ice cannot be in transit more than 2 hours. If the specimen is transported on dry ice, it must be packed in a Styrofoam shipping container with sufficient dry ice (ideally 4 lbs) to prevent any thawing of the tissue.

The formalin and MPG/Glutaraldehyde containers must be shipped in a separate container (preferably with a cool pack or wet ice). *Under no circumstances should they be placed in the container with the dry ice.* Freezing the fixed specimens creates an artifact which severely jeopardizes the diagnosis.

The tissue and clinical data should be sent to the following address to arrive prior to 3:00 p.m. on weekdays only. Specimens cannot be received on weekends.

University of Nebraska Medical Center
Regional Pathology Services
Department of Pathology and Microbiology
981180 Nebraska Medical Center
Omaha, NE 68198-1180
Tel: 402-559-6420
Attn: Neuropathology Laboratory
Tel: 402-559-5194

For technical or shipping questions please call Konnie Zeitner at 402-552-3132. For billing questions please call Regional Pathology Services at 402-559-6420.