

UW MEDICINE

NEUROPATHOLOGY

Harborview Medical Center

Department of Pathology, Neuropathology Division

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www.pathology.washington.edu/clinical/neuropath/clinical.php

MUSCLE BIOPSY – FROZEN (muscle frozen at referring institution)

Instructions for Tissue Preparation, Packaging and Shipping

Human Tissue: Always use universal precautions

1. NOTIFY NEUROPATHOLOGY HISTOLOGY LAB

Please call 206-744-3910 at least 24 hours before the biopsy is performed. **The frozen specimen should arrive in our laboratory between 9am and 4pm, Monday to Thursday (no later than 4pm).**

If a planned biopsy is cancelled/postponed, please let us know.

2. SPECIMEN SPECIFICATIONS

The muscle biopsy specimen should be a longitudinal piece of **muscle 0.8 to 1.0 cm in diameter** (just thicker than a pencil) and at least **3 cm in length**.

The surgeon should be instructed to place the specimen on a tongue blade or stiff paper and cover the muscle with Telfa or gauze moistened with saline. The specimen must **NOT** be immersed in saline. Muscle clamps should not be used unless the surgeon is experienced with their use. If the muscle contracts before it is placed on the surface, obtain an additional sample so that there is 3cm total length.

3. SPECIMEN HANDLING

The specimen will be divided in four ways:

- A) **snap freezing on embedding medium**
- B) **snap freezing directly**
- C) **electron microscopy (EM) fixation**
- D) **formalin fixation**

A) SNAP-FREEZING MUSCLE ON EMBEDDING MEDIUM FOR HISTOCHEMISTRY:

1. Prepare a 0.5 to 1.0 cm long sample of muscle maintaining the orientation to allow cross sections.
2. Immerse the bottom half of a small plastic beaker containing isopentane into liquid nitrogen. When the isopentane becomes slightly viscous and forms solid white laminate lining the inside of the beaker (temperature: -160 degrees C) then it is ready for use.
3. Freeze some embedding medium (e.g. OCT) on a chuck (cork, wooden block, or test tube cap) or in a cryomold by dipping it briefly (10 sec) into isopentane. Alternatively, a cryomold filled with embedding medium can be prepared ahead of time in a freezer.
4. Place a **small** amount of fresh embedding medium on the frozen embedding medium and carefully touch the end portion of the fresh muscle to the embedding medium. The muscle will stick. **It is important NOT to surround the muscle with embedding medium. It is important to maintain the cross sectional orientation.**
5. Lower the chuck with the attached muscle into the isopentane bath. The usual freezing time is 7-15 seconds, depending on specimen size and composition. Immersion in the freezing solution should not last more than is needed to completely freeze the specimen. Larger fatty specimens usually take longer (15 seconds), smaller dense muscle takes a shorter time (7 seconds). Freezing too long will fracture the tissue block, too short will cause ice crystal formation. A well-frozen specimen should be chalky white.
6. Once the specimen is frozen it should be placed in a small plastic bag and **immediately** stored in a deep freezer at -70C or placed in dry ice for shipping or storing at -70C.

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Specimen Handling Instructions

B. SNAP FREEZE A PIECE OF MUSCLE DIRECTLY

1. Place a 1 cm length of muscle directly in liquid nitrogen. The tissue may be held with forceps or hemostat.
2. Remove the frozen muscle to a small plastic bag pre-labeled with the patient's name or accession number.
3. Place the bag in dry ice until transfer to -70C freezer or shipping container.

C. FOR ELECTRON MICROSCOPY (EM)

For embedding in epoxy resin, sectioning and possible electron microscopy, place a small piece of muscle in EM fixative (e.g. Karnovsky's or Truumps).

D. FOR ROUTINE STAINS (FORMALIN FIXATION)

Muscle and other soft tissue remaining should be fixed by immersion in buffered formalin.

4. COMPLETE NEUROPATHOLOGY SERVICE REQUEST FORM

Complete this form carefully to avoid possible delay in processing or results. Provide **Clinical Information and telephone number for the clinician**. Be sure that the specimen is labeled with patient name, patient number, hospital, date and specimen description. Please also send medical history including the history and physical (H&P) from the surgeon and clinic note from neurologist, rheumatologist, or primary care provider who requested the biopsy. Other clinical information may also be sent if pertinent.

If a preliminary telephone report is desired, indicate so and include the name and phone number of the physician to be contacted.

5. SHIPPING

- **Important:** Delivery must be arranged **to arrive before 4 pm Mon-Thurs**.
- Pack the frozen muscle tissue in an ample **Styrofoam container with AT LEAST 5 LBS DRY ICE**.
- Place **FIXED TISSUE** outside of Styrofoam container containing dry ice or ship separately.

Overnight or Next Day Service (deliver Mon-Thurs only)

HMC Pathology, 2NJ-244 Neuropath
RECEIVING DOCK
908 Jefferson St
Seattle, WA 98104
206-744-3910

LABEL ALL PACKAGES: **“DIAGNOSTIC TISSUE SPECIMEN”** NOTIFY 744-3910 or 744-6315 immediately upon arrival.

6. REMEMBER TO CALL

Call 206-744-3910 to inform the Neurohistology laboratory that shipment is coming.

QUESTIONS?

To reach Neurohistology lab dial 206-744-3910. Reach Dr. Donald Born via tele 206-744-6315 or pager 206-982-4958. To reach Neuropathologist On-Call dial 206-744-6315 or the paging operator at 206-744-3000.

*revised 5/10
Protocol prepared by Donald Born, M.D., Ph.D.*