

**SOP J-NM-PR-20110401.026.02
MATERIALS AND METHODS FOR MUSCLE BIOPSY**

SUBMISSIONS TO NEUROMUSCULAR LAB

The muscle biopsied should be moderately affected, avoid muscle which is severely affected. Also avoid muscle which has had recent EMG testing or has been traumatized by injections or manipulation.

Three specimens are required: one fresh/frozen, one fixed in 10% neutral buffered formalin and one in 2.5% glutaraldehyde. All should be approximately 5mm in diameter and 8 to 16 mm long. If the fresh specimens for enzyme histochemical tests cannot be delivered within one hour of excision, they must be immediately frozen. Specimens being placed in fixative need to be placed in fixative immediately.

For each of the two specimens to be fixed, the surgeon should gently blunt-dissect free a cord of muscle, lift slightly on open scissors, clamp and then cut free the ends of the muscle cord to obtain a roughly cylindrical piece of muscle, held at rest along the length in the clamp. (See photographs)

1. The JPC must be contacted in advance prior to shipment of any muscle biopsy specimen.

- Muscle Laboratory- 301-295-7511 or
- JPC Customer Service phone number: 1-855-393-3904

2. All specimens must have a completely filled out JPC Contributor's Consultation Request Form Forms available at www.ipc.capmed.mil

Be sure to include identification of muscle biopsy site, date, time and relevant clinical history and also the name, address, telephone and fax numbers, and email address of the referring pathologist and/or physician.

3. Advance Preparation of Materials

a. Prepare 2 vials for submission of fixed specimen material. Each vial must have all required patient identification information and be of sufficient size to accommodate a muscle clamp and have the vial top fit securely. One vial should be filled with 10% neutral buffered formalin and one with 2.5% glutaraldehyde. The fixative volume should sufficiently cover the entire specimen when the clamp and specimen are placed in the vial.

b. Have available two sterilized isometric muscle biopsy clamps, preferably 8 mm and 15 mm.

c. Prepare a specimen bottle for the submission of the fresh/frozen specimen material. Place gauze dampened with balanced salt solution, 0.9% physiological saline or mammalian Ringer's solution in the specimen bottle. The gauze should be damp, not fully wet. Seal the specimen bottle and place in crushed ice.

d. If unable to ship a fresh specimen within one hour, have a ½ by 2 inch strip of aluminum foil available for handling a frozen specimen.

e. Prepare a shipping container for a frozen specimen, liquid nitrogen, isopentane and dry ice should be available. Put some crushed dry ice into the insulated shipping container and pre-cool the shipping tube or vial for the frozen specimen. Screw cap plastic centrifuge tubes 15 to 50 ml in size may be used.

4. Handling of Specimens

a. Place the specimen in 15 mm clamp in the vial containing 10% **neutral buffered formalin**. Clamped specimens should be fixed immediately at room temperature. Do not unclamp for one hour. Leave clamped. Include the patient name, surgical pathology number, site of biopsy, date and fixative on the label. The specimen should remain in the same fixative solution for shipment.

b. Place the specimen in 8 mm clamp in the vial containing 2.5% **glutaraldehyde**. Clamped specimens should be fixed immediately at room temperature. After one hour of fixation, open clamps and seal specimen into labeled vials for shipment. Include the patient name, surgical pathology number, site of biopsy, date and fixative on the label. The specimen should remain in the same fixative solution for shipment.

SHIP FIXED SPECIMENS SEPARATELY FROM THE FROZEN SPECIMEN. DO NOT FREEZE FIXED SPECIMENS.

c. **Local contributors may send specimens for freezing at JPC. A fresh/frozen specimen must be delivered within one hour after excision. If the specimen will not reach the JPC within one hour of excision, it must be frozen by the contributor for shipment.** If sending fresh tissue, wrap the muscle in a piece of saline-moistened gauze and place it the prepared specimen container. **DO NOT IMMERSE BIOPSY SAMPLES IN SALINE.** Place the specimen container in a Styrofoam shipping container with regular ice and send immediately by special messenger.

d. If the specimen cannot be delivered in one hour, then it **must be frozen immediately after excision**. Successful enzyme histochemical tests require muscle that has been very rapidly frozen, uncovered, without excess liquid and without matrix. Enzyme activity will be stable for about one hour, after which activity rapidly declines. The specimen must remain frozen until sectioned.

e. The specimen for freezing should be at least 10 mm long and 5 mm in diameter and kept on damp gauze in a cold sealed specimen vial until frozen. If the small (8 mm) clamp was placed toward one end of the dissected cord or bundle of fibers, the specimen for freezing can be the other end of the same cord. This free end can be cut off into the specimen bottle containing the damp gauze before the clamp is put into the fixative. The specimen for freezing should be handled, if necessary, by gently holding only the very end with tweezers.

f. Handling gently only the very end with tweezers, place the muscle tissue on a ½ " x 2" strip of aluminum foil that has been folded longitudinally to form a long narrow v-shaped trough. Orient the muscle biopsy so that the fibers are running along the length of the foil.

- g. Put about two inches of isopentane into a Pyrex beaker with a collar of Styrofoam or cork so that it can be put into a Dewar flask of liquid nitrogen for cooling.
- h. Cool isopentane to between -140° and -155° . If you do not have a thermometer, which registers that low, cool until isopentane becomes syrupy and white lumps start to form at the bottom and sides of the beaker.
- i. Hold the foil in tweezers and plunge it into the cooled isopentane for about ten seconds. Remove from the isopentane and immediately blot or drain off any excess isopentane and place the tissue in the pre-cooled specimen tube on dry ice.
- j. Close the tube securely and place in shipping container filled with dry ice.

DO NOT ALLOW SPECIMEN TO THAW.

Ship frozen specimens in an insulated box with enough dry ice for 4 days. The shipping label should read: RUSH/FROZEN TISSUE, DO NOT ALLOW TO THAW

Ship Monday through Thursday only. NO ONE IS AVAILABLE TO ACCEPT DELIVERIES AFTER HOURS, ON WEEKENDS OR FEDERAL HOLIDAYS.

**Ship to: Joint Pathology Center
Case/Accessions/Neuromuscular Laboratory
606 Stephen Sitter Avenue
Silver Spring, MD 20910**

The JPC highly recommends that cases be sent using a commercial express courier service (i.e. FedEx, UPS). All mail sent via routine US Postal Service routes will be irradiated prior to delivery at the JPC, this will result in delays and possible irreparable damage to enclosed pathological specimens which may result in the inability to render consultation.

CONTRIBUTORS ARE RESPONSIBLE FOR IDENTIFYING INFECTIOUS OR POTENTIALLY INFECTIOUS MATERIAL. FLAG ALL MATERIAL WITH A YELLOW STRIP AND WRITTEN LABELS (CREUTZFELDT-JAKOB, AIDS, HEPATITIS, ETC). Please refer to the JPC Contributor's Manual for additional guidance on packing and shipping of materials.