

Muscle Biopsy - General Instructions

A preliminary diagnosis can be obtained by calling Steven A. Moore, MD, PhD, at 319–384–9084. Final reports are faxed and final copies are mailed. If you have specific questions regarding the specimen preparation and/or shipping process, please contact our Histology Lab staff at 319-356-2140.

HOW TO SEND A MUSCLE BIOPSY:

1. Complete the Muscular Dystrophy Requisition Form (regardless of the clinical diagnosis)

- a. A completed requisition must accompany all requests. It should contain: the patient name, identification number, date of biopsy, date of birth, male/female, tissue source, biopsy site, clinical history, questions to be answered and differential diagnosis.
- b. Include pertinent history and findings, such as family history, neurological exam, and treatments.

2. Guidelines for Muscle Biopsy Preparation

Before proceeding with distributing muscle for freezing and/or fixation, an assessment must be made of the following parameters: patient history, the quantity of tissue submitted, the quality of tissue submitted.

a. If the biopsy is clearly intended to investigate inflammatory disease (terms like dermatomyositis, polymyositis, inflammatory myopathy, vasculitis, PAN-polyarteritis nodosa, fasciitis), the tissue should be divided for:

- i) freezing for enzyme histochemistry,
- ii) glutaraldehyde fixation for electron microscopy, and
- iii) formalin fixation for routine histology

(NOTE: When there is a scant amount tissue with this history, electron microscopy is less important.)

b. If the biopsy is clearly intended to investigate a muscular dystrophy (terms like DMD – Duchenne’s muscular dystrophy, BMD – Becker’s muscular dystrophy, LGMD – limb girdle muscular dystrophy, CMD – congenital muscular dystrophy, FSH – facioscapulohumeral dystrophy, DM1 or DM2 – myotonic dystrophy, dystrophinopathy, sarcoglycanopathy), the tissue should be divided for:

- i) freezing for enzyme histochemistry, immunohistochemistry, and biochemistry
- ii) glutaraldehyde fixation for electron microscopy, and
- iii) if the amount of tissue is large, a small amount can be placed in formalin

(NOTE: When there is a scant amount tissue with this history, err on the side of freezing ample tissue for immunostaining and biochemistry and do not fix any tissue in formalin.)

c. If the biopsy is clearly intended to investigate a metabolic abnormality or mitochondrial defect (terms like glycogen storage disease, McArdle’s disease, carnitine deficiency, MELAS, Kearns-Sayre syndrome, MERRF, lipid storage disease, mitochondrial myopathy), the tissue should be divided for:

- i) freezing for enzyme histochemistry and biochemistry
- ii) glutaraldehyde fixation for electron microscopy, and
- iii) if the amount of tissue is large, a small amount can be placed in formalin

(NOTE: When there is a scant amount tissue with this history, err on the side of freezing ample tissue for biochemistry and do not fix any tissue in formalin.)

d. For histories that are vague or that are not clearly inflammatory, dystrophy, or metabolic/mitochondrial and for which there is ample tissue, the tissue should be divided for:

- i) freezing for enzyme histochemistry and biochemistry,
- ii) glutaraldehyde fixation for electron microscopy, and
- iii) formalin fixation for routine histology

(NOTE: If there is scant tissue, err on the side of freezing ample tissue for enzyme histochemistry.)

3. Specimen Preparation

FRESH TISSUE

- a. If you are within 2-3 hours travel time, prepare for delivery of fresh tissue; if you are not within 2-3 hours travel time, then proceed to the FROZEN TISSUE preparation section below.
- b. At least one clamped biopsy is needed, measuring at least 0.5 cm in diameter and 1.0 cm in length. Two biopsies are preferred.
- c. Wrap the clamped muscle biopsy in gauze **slightly** moistened with normal saline. Do not immerse in saline, as this will produce an artifact which may interfere with interpretation.
- d. Immediately deliver to University of Iowa Diagnostic Laboratories on wet ice. Package the tissue so that it stays cold, but DOES NOT FREEZE.

FROZEN TISSUE

- a. The best results from enzyme histochemistry and immunostaining (especially immunofluorescence) studies are obtained when tissues are frozen rapidly and kept frozen at -60 to -80°C until sectioned. Any thawing which takes place and is followed by refreezing leads to ice crystal formation with loss of morphologic detail and cell membrane (hence antigenic) integrity. Enzymatic activity can also be lost.
- b. The preferred methods of freezing are:
 - i. Liquid Nitrogen – see **Tissue for Biochemical studies** below
 - ii. Isopentane/Liquid Nitrogen – see **Tissue for Enzyme Histochemistry and Immunohistochemistry studies** below

Tissue for Biochemical Studies - Directions for Liquid Nitrogen:

- a. One piece of skeletal muscle tissue is required that measures at least 0.5 cm³ (size of a pencil eraser).
- b. Cut the specimen from the clamp.
- c. Wrap the tissue in aluminum foil and carefully immerse into the liquid nitrogen.
- d. Leave the tissue in the liquid nitrogen for 1 minute. More time may be needed based on size of tissue.
- e. Place wrapped tissue in a freezer or cryostat while you prepare the packing/shipping. A freezer must be used for storage of more than a few minutes.
- f. When ready to ship, place tissue in a pre-cooled plastic Zip-Lock bag to protect it from freeze-drying.
- g. Always identify the specimen by writing with a waterproof marker on the foil and bag.
- h. Label the specimen with the patient name, identification number, tissue source and date.
- i. Write "Biochem" on the plastic bag.

Tissue for Enzyme Histochemistry and Immunohistochemistry Studies - Directions for Isopentane/Liquid Nitrogen (a pdf file illustrating the protocol is available on request):

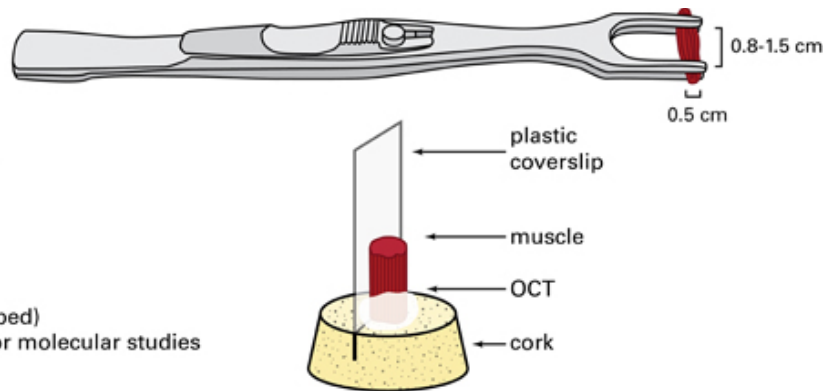
- a. At least one clamped biopsy is needed, measuring at least 0.5 cm in diameter and approximately 1.0 cm in length. See illustration on next page.
- b. Prepare a cork with vertically oriented plastic coverslip.
- c. Cut the muscle specimen from the clamp.
- d. Place a drop of O.C.T. embedding compound on one side of the coverslip near the center of the cork.
- e. Place the biopsy in the O.C.T. with the muscle fibers perpendicular to the surface of the cork. The bottom of the muscle should be 1-2 mm away from the surface of the cork. The gap between the muscle and the cork will be filled with O.C.T. The upper portion of the muscle should not be covered with O.C.T. (Note: The muscle may be oriented before adding the O.C.T.)

First priority:

- 1) Cut biopsy from between forks of clamp
- 2) Trim 0.1 cm diameter strip for glutaraldehyde
- 3) Mount remainder on cork
- 4) Freeze in isopentane at -160°C

If there is sufficient tissue:

- 5) Fix a portion in formalin (clamped or unclamped)
- 6) Freeze a portion in liquid N_2 for biochemical or molecular studies



- f. Muscle tissue is prone to ice crystal artifact when frozen at temperatures quite adequate for other tissues. To prevent this artifact and preserve enzyme activity, follow the freezing protocol carefully.
- g. Cool 50 ml of isopentane (2-Methyl Butane) in a small metal container to -160°C in a liquid nitrogen bath.
- h. Stir continually to insure even cooling. If a cold temperature thermometer is not available, cool the isopentane until the outer layer is frozen solid to the sides and bottom of the container and the inner area is thick and slushy.
- i. Insert a probe into the cork (to use as a handle) and plunge the specimen into the isopentane for 30 seconds.
- j. Remove the specimen from the isopentane and place in a cryostat or freezer for several minutes.
- k. When the specimen has reached the temperature of the cryostat (approximately -20°C), remove the coverslip (it will pull out easily), then use a single edged razorblade to cut through the O.C.T. between the muscle and the cork.
- l. Wrap the frozen muscle in cold (pre-cooled) aluminum foil.
- m. Leave the wrapped tissue in a freezer or cryostat while you prepare the packing/shipping. A freezer must be used for storage of more than a few minutes.
- n. When ready to ship, place tissue in small pre-cooled plastic Zip-lock bag to protect from freeze-drying.
- o. Always identify the specimen by writing with a waterproof marker on the foil and bag.
- p. Label the specimen with the patient name, identification number, tissue source and date.
- q. Write "EH" on the plastic bag.
- r. Ship tissue on dry ice. Tissue must be kept frozen.

ELECTRON MICROSCOPY

- a. Tissue may be cut from clamped specimens prior to processing them for enzyme histochemistry or routine light microscopy. Cut longitudinally at 0.1 cm intervals. One fragment 0.1 to 0.2 cm diameter x 0.5 cm long is sufficient, but if the biopsy is large, 2-3 fragments are better. Tissue larger than 0.1 to 0.2 cm diameter will fix poorly.
- b. Immerse in pre-cooled electron microscopy (2.5% glutaraldehyde) fixative and refrigerate until delivery.
- c. Ship at ambient temperature. **DO NOT FREEZE.**

ROUTINE LIGHT MICROSCOPY

- a. Residual tissue not used in above protocols may be placed in formalin for routine histology.
- b. Immerse the tissue in 10% neutral buffered formalin (NBF). Muscle may be fixed while still clamped.
- c. If the biopsy is fixed in the clamp, remove all of the tissue from the clamp after at least 4 hours and return to 10% NBF before delivery.
- d. Ship in 10% NBF at ambient temperature. **DO NOT FREEZE.**

4. Specimen Packaging

Wet Tissue:

- a. Double bag the specimen container with the requisition on the outside of the plastic bag.
- b. Place the double-bagged specimen into a Styrofoam box (primary container) with adequate wet ice to keep specimen cool during transport.
- c. Securely seal the primary container.
- d. Place the primary container in a secondary shipping container, which should contain enough absorbent material to prevent any leakage from escaping outside the container.
- e. These specimens can be sent by our Courier services within our service area.

Formalin-Fixed Tissue, Glutaraldehyde-Fixed Tissue:

- a. Immerse the tissue in the appropriate fixative and container.
- b. Label containers with "Formaldehyde precaution" or "Glutaraldehyde precaution".
- c. Double-bag specimen containers and place in box with adequate absorbent material in case of leakage.
- d. Securely seal the box.
- e. Indicate on the exterior of the box "Formaldehyde Precaution" and/or "Glutaraldehyde Precaution".
- f. Ship at ambient temperature. **DO NOT FREEZE and do not place in or next to dry ice.** Ship ambient temperature specimens in their own separate box with their own separate airbill.

Frozen Tissue:

- a. Place the double-bagged specimen into a Styrofoam container (primary container) with adequate dry ice.
- b. Use at least 6-8 pounds dry ice. Use more in the summer months. **DO NOT** use wet ice, or coolants (i.e., Cool Packs).
- c. Make certain the requisition is placed in the box, but on the outside of the double bag.
- d. Securely seal the container and label with "Frozen Tissue - Do Not Thaw".
- e. To avoid delivery problems due to prolonged transit time, please try to ship specimens on Monday, Tuesday, or Wednesday and never ship a frozen specimen over the weekend.
- f. Call Client Services at 866-844-2522 for special situations such as weekend or holiday instructions.
- g. Specimens can be sent via courier if you reside within our service area or by overnight express airmail.
- h. If using overnight express service, place a Dry Ice label on the outside of the outer most box.

5. Specimen Transportation

UIDL can provide specimen transport either through our courier services or through overnight carriers. If you have questions, please contact Client Services: 319-384-7212 (local) 1-866-844-2522 (toll free).

Shipping address: UI Diagnostic Laboratories
Department of Pathology
200 Hawkins Drive, Room 5231 RCP
Iowa City, IA 52242-1087