



### **Muscle Biopsy - General Instructions**

The Division of Neuropathology, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, provides a full range of consultation services for muscle biopsies.

Clinical inquirers should be addressed to Dr. Mariarita Santi, M.D., Ph.D., Director of Neuromuscular Service at 215-590-3184. Final reports are faxed and final copies are mailed. Written reports indicate patient information, accession number, slide/blocks examined, stains performed and interpretation.

#### **How to send a Muscle Biopsy:**

- 1. Complete the Muscle Biopsy Requisition Form.** Questions about completing the requisition can be directed to: to Dr. Mariarita Santi, M.D., Ph.D., Director of Neuromuscular Service at 215-590-3184.
  - 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, question(s) to be answered and differential diagnosis.
  - b. Include other pertinent history and findings, such as neurologic history, imaging findings, family history, genetic testing results, prior biopsy diagnosis and/or treatment history.
  
- 2. Guidelines for Muscle Biopsy Preparation:**

For evaluation of muscular dystrophy (including congenital muscular dystrophy), congenital myopathy, metabolic myopathy, mitochondrial myopathy, and neurogenic disorder, the tissue should be divided for freezing for enzyme histochemistry/immunohistochemistry and freezing for biochemistry, and glutaraldehyde fixation.

For evaluation of suspected inflammatory myopathy in addition, a small amount of tissue, if available, should be fixed in formalin.

- 3. Specimen Preparation:**

#### FRESH TISSUE

- a. For muscle biopsies obtained from the Children's Hospital of Philadelphia, or from outside institutions within 2-3 hours travel time *and* lacking laboratory facilities to handle freezing; *if not, then go to FROZEN TISSUE (see below)*.

#### **CHOP Specimens (fresh)**

- b. One (1) biopsy measuring at least 0.5 cm in diameter and 1.0 cm in length. Wrap the muscle biopsy tissue in gauze slightly moistened with normal saline. Do not immerse in saline, as this will produce artifacts, which may interfere with interpretation. Immediately deliver to the Children's Hospital of Philadelphia, Department of Pathology and Laboratory Medicine on wet ice.

- c. Remaining biopsy specimen/specimens, should be directly frozen in liquid nitrogen in the OR and transported to the Children's Hospital of Philadelphia, Department of Pathology and Laboratory Medicine directly in liquid nitrogen in an appropriate flask.

**Outside Specimens (fresh)**

- b. Three (3) biopsies measuring at least 0.5 cm in diameter and 1.0 cm in length. Two biopsies are required for submission.
- c. Wrap the muscle biopsy tissue in gauze slightly moistened with normal saline. Do not immerse in saline, as this will produce artifacts, which may interfere with interpretation.
- d. Deliver to the Children's Hospital of Philadelphia, Department of Pathology and Laboratory Medicine on wet ice as soon as possible.

**FROZEN TISSUE**

Rationale: The best results from enzyme histochemistry and immunohistochemistry studies are obtained when tissues are frozen rapidly and kept frozen at -60 to -80°C until sectioned. Thawing and refreezing leads to ice crystal formation. This can impair morphologic detail, loss of enzymatic activity and antigenicity.

The methods of freezing are:

1. **Liquid Nitrogen** done directly in the OR at the time of tissue acquisition - tissue for biochemical and genetic studies
2. **Isopentane/liquid nitrogen** done in your pathology laboratory on fresh specimens received directly from the OR (see above for suggestions for handling fresh tissue from OR) - tissue for enzyme histochemistry and immunohistochemistry studies

Directions and guidelines for **Liquid Nitrogen** are:

- a. At least one piece of skeletal muscle tissue is required that measures at least 0.5 cm<sup>3</sup> (about the size of a pencil eraser).
- b. Always identify the specimen by writing with a waterproof marker on the aluminum foil.
- 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 the size of the tissue.
- e. Transport frozen tissue from OR to Pathology in liquid nitrogen.
- f. Place 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.
- g. When you are ready to ship, place the tissue in a small pre-cooled plastic Zip-lock bag to protect it from freeze-drying.
- h. Label the specimen with the patient name, identification number, tissue source and date.
- i. Write "Liquid Nitrogen" on the plastic bag.

Directions for **Isopentane/Liquid Nitrogen** are:

- a. At least one biopsy is needed, measuring 0.5 cm in diameter and 0.8-1.0 cm in length.
- b. Place a drop of O.C.T. embedding compound or Gum Guar in the center of a small cork.
- c. Place the biopsy in the O.C.T. or Gum Guar with the muscle fibers perpendicular to the surface of the cork [Please refer to: Color Atlas of muscle pathology, WJK Cumming pag8.]. However, do not cover the biopsy with OCT or Gum Guar.  
*(Preparation of Gum Guar solution: add 4 grams powdered gum guar to 50 mls DI water in a sterile container and mix until it is a firm gummy consistency. Store in the 5°C refrigerator to prevent overgrowth of mold).*
- d. Muscle tissue is prone to ice crystal artifact when frozen at temperatures quite adequate for other tissues. In order to consistently prevent this artifact and to preserve enzyme activity, it is important to follow the freezing protocol carefully.
- e. Cool 50 ml of isopentane (2-Methyl Butane) in a small metal container to -155°C in a liquid nitrogen bath.
- f. Stir continually to insure even cooling. If a cold temperature thermometer is not available, cool the isopentane until the outer area is frozen solid and the inner area is thick and slushy.
- g. Insert a probe into the cork (to use as a handle) and plunge the specimen into the isopentane for 30 seconds. More time may be needed based on the size of the tissue.
- h. Remove the specimen from the isopentane and place in a cryostat or freezer for several minutes.
- i. Remove the specimen
- j. Wrap in cold (pre-cooled) aluminum foil.
- k. 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.
- l. When you are ready to ship, place the tissue in a small pre-cooled plastic Zip-lock bag to protect it from freeze-drying.
- m. Always identify the specimen by writing with a waterproof marker on the foil and bag.
- n. Label the specimen with the patient name, identification number, tissue source and date.
- o. Write "EH" on the plastic bag.
- p. Ship tissue on dry ice. Tissue must be kept frozen.

#### ELECTRON MICROSCOPY

- a. Tissue may be cut from fresh portion prior to processing for enzyme histochemistry. Cut longitudinally at 0.1 cm intervals. One fragment 0.1 x 0.1 x 0.5 cm is sufficient, but if the biopsy is large, 2-3 fragments are better.
- 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

*For evaluation of suspected inflammatory myopathy in addition, a small amount of tissue, if available, should be fixed in formalin.*

- 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).
- c. Ship at ambient temperature. **DO NOT** freeze.

#### **4. Specimen Packaging**

##### WET TISSUE:

- a. Double bag the specimen container with the requisition on the inside 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 Courier services.

##### 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 the specimen containers and place into a 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 room temperature. **DO NOT FREEZE.**
- g. This container can be taped to the dry ice box for shipping as a single unit.

##### 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 from Monday to Thursday and never ship a frozen specimen over the weekend.

#### **5. Specimen Transportation**

Specimen transport may be done either through a courier services or through other transportation providers such as overnight carriers.

**Shipping address:**

Department of Pathology and Laboratory Medicine  
The Children's Hospital of Philadelphia  
34<sup>th</sup> Street and Civic Center Boulevard  
Main 5NW27, 5<sup>th</sup> Floor  
Philadelphia PA 19104-4318  
215-590-1728  
215-590-1736 FAX