

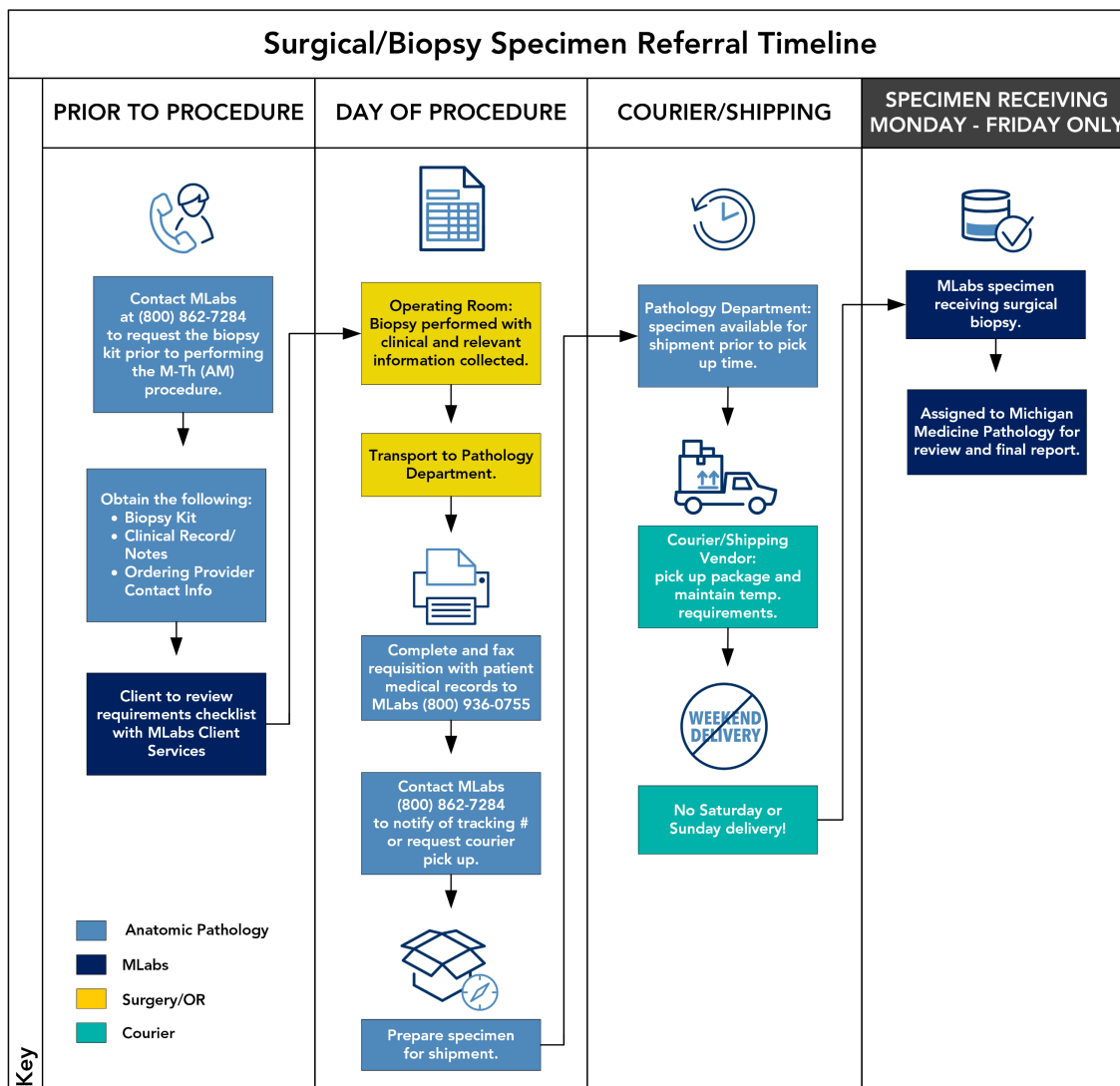
MUSCLE BIOPSY TISSUE SUBMISSION PROTOCOL



Please notify Michigan Medicine Laboratories (MLabs) Client Services prior to sending a muscle biopsy specimen. Muscle biopsy collection kits are available from MLabs and will be required in advance of the procedure. The glutaraldehyde fixative included in the kit must be refrigerated until ready for use; the entire kit may be refrigerated. MLabs Client Services can be reached at **800.862.7284** or **734.936.2598** (local). Please fax all associated paperwork to FAX **734.936.0755** prior to or at the time of notification to MLabs of the arriving specimen.

Follow hospital COVID-19 and infectious disease pre-screening guidelines and include notification if the patient is positive for COVID-19 or any other infectious etiology that may put technical personnel at risk.

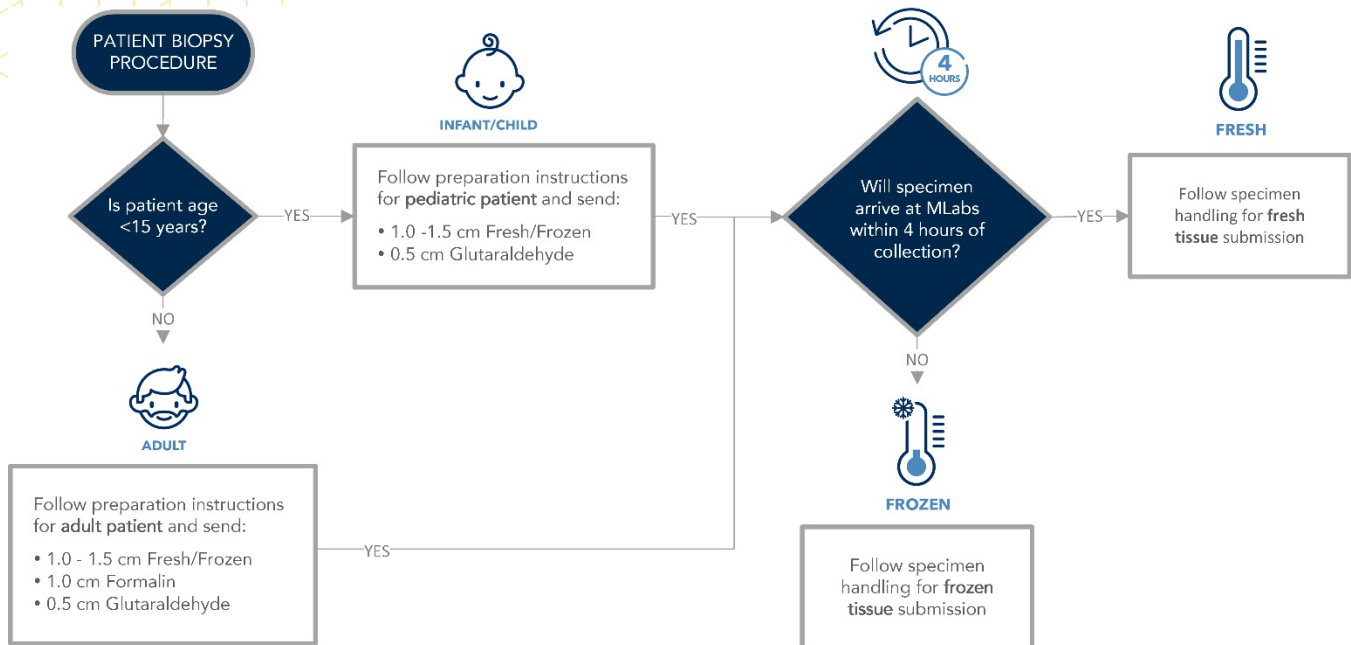
Skeletal muscle biopsy is performed to evaluate symptoms that may be due to inflammatory, autoimmune, metabolic, degenerative, or inheritable causes. The evaluation requires both specific surgical technique and unique handling that differs from routine tissue submitted for pathology evaluation. To ensure proper handling and shipment of skeletal muscle specimens, MLabs recommends that skeletal muscle biopsy procedures be performed Monday through Thursday and in the morning. This ensures that all specimen handling can be completed in advance of pick up at your location.



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The surgical procedure for acquiring skeletal muscle tissue, proper specimen handling and the submission protocol are determined by two primary factors: (1) the age of the patient, and (2) the elapsed time from when the skeletal muscle biopsy is performed and the skeletal muscle specimens arrive at the MLabs facility.

Please note these differences as you review the instructions in this submission protocol.



Resources Needed for this Protocol

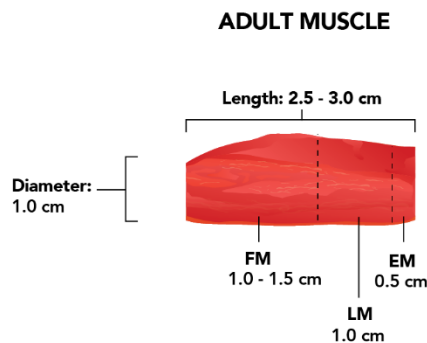
- MLabs Client Services phone number (800.862.7284 or 734.936.2598).
- HIPAA secure fax method
- Shipment or courier method
- MLabs muscle biopsy kit
 - Fresh kit (refrigerated)
 - Frozen kit (requires client-supplied dry ice)
- Items included in muscle biopsy kit
 - Test requisition form
 - Index card (3x5)
 - Patient specimen labels (3)
 - Specimen container (empty) for fresh or frozen tissue
 - Specimen container with 10% formalin fixative
 - Specimen container with glutaraldehyde fixative (store refrigerated)
 - Refrigerated specimen bag, absorbent pad, bubble wrap
 - Cold pack (refrigerate at 4°C for at least 24 hours prior to shipping)
 - Fresh kit includes MLabs Exempt Human Specimens transport bag
 - Frozen kit includes frozen specimen bag, Styrofoam shipping containers, and return service label
- Scalpel
- Skeletal muscle from patient biopsy
 - Adult: 2.5-3.0 cm length x **1.0 cm in diameter**

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- Pediatric: 1.5-2.0 cm in length x **0.5-1.0 cm in diameter**
- Telfa gauze pad(s)
- Sterile saline
- Refrigerator
- Forceps or tweezers
- For frozen specimens (elapsed time from biopsy to delivery to MLabs is greater than four (4) hours)
 - Liquid nitrogen
 - Isopentane
 - Nalgene or metal beaker
 - Cork board
 - O.C.T.
 - Tongs
 - Dry ice

Preparation Instructions: Adult Patient (age >15 years)

1. The muscle biopsy should be taken from the belly of a moderately affected muscle (avoid EMG and injection sites). Ideally, the muscle biopsy should be approximately 2.5-3.0 cm in length x **1.0 cm in diameter**. Divide the muscle into three (3) portions:
 - a. **FRESH** or **FROZEN TISSUE**: 1.0-1.5 cm in length by 1.0 cm in diameter **fresh** (ideal) tissue portion on a saline-moistened Tefla gauze (refrigerate or store on wet ice) (for Histochemical Stains).
 - b. **FORMALIN FIXED TISSUE**: 1.0 cm in length by 1.0 cm in diameter placed in 10% neutral buffered **formalin** (for Histology).
 - c. **GLUTARALDEHYDE FIXED TISSUE**: 0.5 cm length by 1.0 cm in diameter placed in **glutaraldehyde** (for Electron Microscopy).



FM: Fresh/Frozen Muscle
LM: Light Microscopy – Formalin
EM: Electron Microscopy – Glutaraldehyde

2. Fresh tissue should be sent only if the specimen will arrive at MLabs within four (4) hours of the biopsy during normal business hours. Otherwise, this specimen should be frozen by the client according to the procedure detailed below (see FROZEN TISSUE instructions page 6). This specimen is most important for diagnosis as it is used for histochemical stains.
3. Please complete the [MLabs Muscle Biopsy Requisition](#) that is provided in the muscle biopsy collection kit and send the completed requisition back to MLabs along with the specimens.

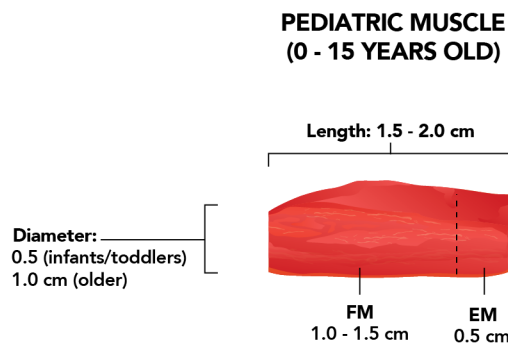
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4. See also Specimen Handling page 5, Packaging and Transport page 9, and Packaging Diagrams pages 11-12.

Preparation Instructions: Pediatric Patient (age 0 – 15 years)

1. The muscle biopsy should be taken from the belly of a moderately affected muscle (avoid EMG and injection sites). Ideally, the muscle biopsy should be approximately 1.5-2.0 cm in length x **0.5-1.0 cm in diameter (0.5 cm for infants and toddlers or 1.0 cm for older patients)**. Divide the muscle into two (2) portions:
 - a. **FRESH or FROZEN TISSUE:** 1.0-1.5 cm in length by 0.5-1.0 cm in diameter **fresh** (ideal) tissue portion on a saline-moistened Tefla gauze (refrigerate or store on wet ice) (for Histochemical Stains).
 - b. **GLUTARALDEHYDE FIXED TISSUE:** 0.5 cm in length by 0.5-1.0 cm in diameter portion placed in **glutaraldehyde** (for Electron Microscopy).



FM: Fresh/Frozen Muscle
EM: Electron Microscopy – Glutaraldehyde

2. Fresh tissue should be sent only if the specimen will arrive at MLabs within four (4) hours of the biopsy during normal business hours. Otherwise, this specimen should be frozen by the client according to the procedure detailed below (see FROZEN TISSUE instructions page 6). This specimen is most important for diagnosis as it is used for histochemical stains.
3. Note that the formalin fixed specimen is omitted for pediatric patients.
4. Please complete the [MLabs Muscle Biopsy Requisition](#) that is provided in the muscle biopsy collection kit and send the completed requisition back to MLabs along with the specimens.
5. See also Specimen Handling page 5, Packaging and Transport page 9, and Packaging Diagrams page 11-12.

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Specimen Handling

A **fresh tissue** specimen is preferred, provided that the specimen can arrive at MLabs **within four (4) hours of the biopsy**, Monday through Friday 7:00 AM – 5:00 PM, excluding holidays.

The tissue must be frozen if it will be received outside of normal business hours (see Frozen Tissue instructions below). Frozen specimens should be shipped or transported to arrive Monday – Friday by 5:00 PM, excluding holidays. Specimens cannot be accepted during observed Michigan Medicine holidays. Please contact MLabs Client Services for observed holiday dates and hours. Specimens that cannot be shipped for arrival within these timeframes should be stored frozen on dry ice or in a -80 °C freezer until ready to ship.

Tissue transport time:	Follow guidelines below:
1 – 4 hours See FRESH tissue Packaging Diagram page 11	FRESH TISSUE FORMALIN FIXED TISSUE (adult patient only) GLUTARALDEHYDE FIXED TISSUE
Greater than 4 hours See FROZEN tissue Packaging Diagram page 12	FROZEN TISSUE FORMALIN FIXED TISSUE (adult patient only) GLUTARALDEHYDE FIXED TISSUE

FRESH TISSUE

Procedure to prepare fresh muscle tissue at your institution:

1. The fresh piece of muscle, approximately 1.0-1.5 cm in length by **1.0 cm in diameter (or for pediatric patients 1.0-1.5 cm in length by 0.5-1.0 cm in diameter)** will be used for histochemical stains.
2. Do not tease or pinch with forceps or let the tissue dry out.
3. Wrap the tissue in a saline-moistened Telfa pad and place in a screw cap container. **Do not immerse the tissue in saline. Do not let the tissue dry out at room temperature.**
4. The fresh tissue must be kept refrigerated using either the cold pack included with the kit (recommended) or with wet ice to transport to MLabs; **do not freeze.**
5. Refrigerate the cold pack supplied with the kit at 4°C for at least 24 hours before packaging the fresh tissue. If you must transport the fresh tissue with wet ice, double bag the wet ice separately from the bagged specimen.
6. If the specimen cannot arrive at MLabs within four (4) hours of the biopsy, or it is an observed Michigan Medicine holiday, it must be frozen as described below under FROZEN TISSUE.

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FROZEN TISSUE

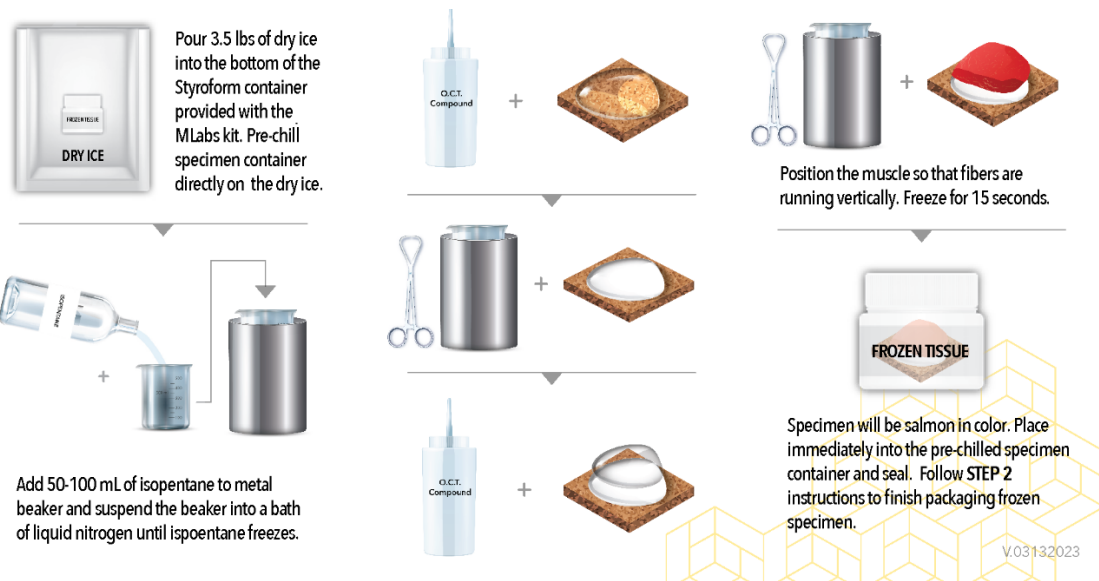
If the fresh specimen cannot arrive at MLabs within four (4) hours of the biopsy, or it is an observed Michigan Medicine holiday, it must be frozen in the following manner. Follow this procedure to freeze the muscle tissue at your institution:

1. Between two possible options, option A, isopentane method is preferred. Use option B only when isopentane is unavailable.

Option A: Isopentane method (PREFERRED):

- a. This method typically takes about ten minutes.
- b. Pour 3.5 pounds of dry ice into the bottom of the Styrofoam container provided with the MLabs kit. Pre-chill specimen **container** directly on the dry ice.
- c. Separately, add 50-100 mL of isopentane (2-methylbutane) to a Nalgene or metal beaker.
- d. Suspend the beaker in a bath of liquid nitrogen and wait until the isopentane freezes to a white, chalky substance.
- e. Select a small section of cork board that's slightly larger than size of the biopsy and cover the cork with O.C.T. Using tongs, immerse the cork and O.C.T. in the isopentane until the O.C.T. is frozen.
- f. Place a thin layer of room temperature O.C.T. on top of the frozen O.C.T. to act as an adhesive for the specimen.
- g. Carefully position the muscle onto the O.C.T. button, making sure the muscle is oriented so that the muscle fibers are running vertically; this will allow a cross section to be obtained during sectioning.
- h. Using tongs, carefully immerse the muscle sample into the isopentane to freeze for about 15 seconds (muscle will turn salmon pink in color). Once freezing is complete, remove the muscle from the isopentane and place the frozen muscle immediately into a pre-chilled specimen container.
- i. Finish packaging specimen with step 2.

STEP 1 FROZEN MUSCLE BIOPSY OPTION A: ISOPENTANE METHOD (PREFERRED)



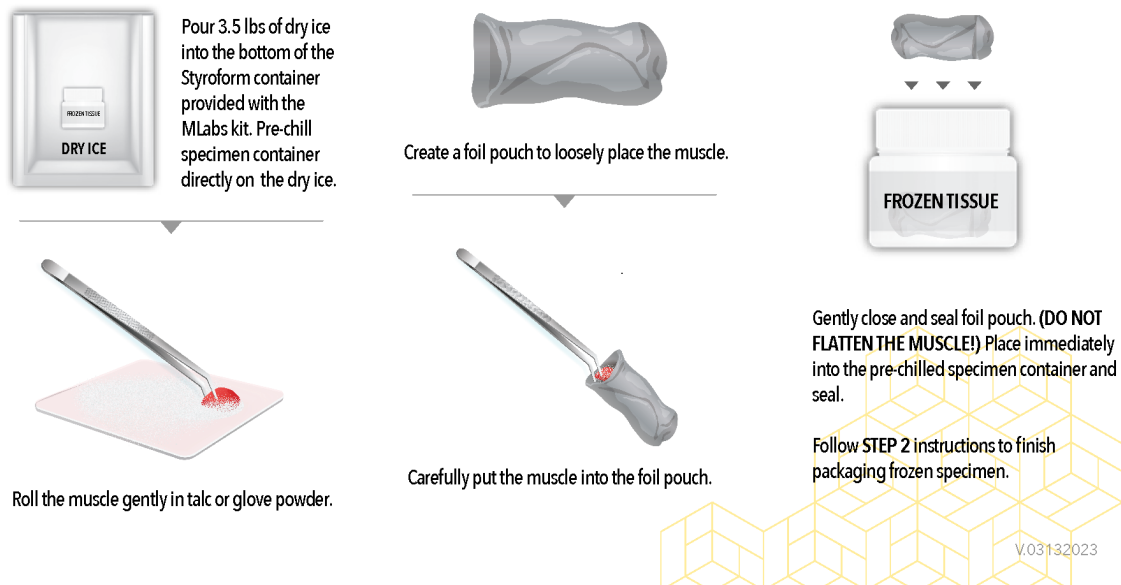
MUSCLE BIOPSY TISSUE SUBMISSION PROTOCOL



Option B: Alternative method if isopentane is not available.

- a. Pour 3.5 pounds of dry ice into the bottom of the Styrofoam container provided with the MLabs kit. Pre-chill specimen **container** directly on the dry ice.
- b. If isopentane is not available, roll the muscle gently in talc or glove powder, just enough to cover the specimen, excessive talc will dry out the specimen.
- c. Create a foil pouch to loosely place the muscle.
- d. Carefully put the muscle into the foil pouch.
- e. Gently close and seal the foil pouch (**DO NOT FLATTEN OR PINCH THE MUSCLE!**)
- f. Place immediately into the pre-chilled specimen container and seal (handle the foil pouch by the ends to avoid squeezing the specimen).
- g. Finish packaging specimen with step 2.

STEP 1 FROZEN MUSCLE BIOPSY OPTION B: TALC/FOIL METHOD



2. Package the frozen muscle tissue.
 - a. Place the frozen tissue container into the frozen specimen bag and seal.
 - b. Set frozen specimen bag on top of 3.5 pounds of dry ice previously poured in step 1.
 - c. Fill with an additional 3.5 lbs of dry ice on top of the frozen specimen bag for a total of 7 pounds.
 - d. Place the lid onto the Styrofoam container.
 - e. See Packaging and Transport page 9, and Packaging Diagrams page 11-12.
3. To avoid specimen thawing, take care to store the specimen packaged with dry ice or in a -80°C freezer until ready to ship and to package with dry ice as described for shipping. Do not store in -20°C cryostat; the cryostat defrosts overnight, and the muscle will thaw. The specimen may not be salvageable if it thaws.

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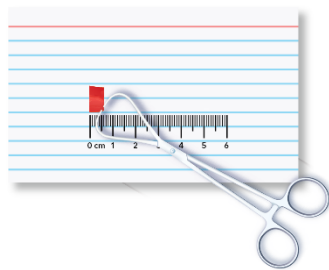
FORMALIN FIXED TISSUE

1. The formalin fixed portion of muscle, approximately 1.0 cm length by 1.0 cm in diameter (adult patient only) will be used for paraffin sections.
2. Place specimen in the container with 10% neutral buffered formalin (NBF). Ensure that the entire tissue specimen is fully submerged in fixative.
3. Refrigerate or store at room temperature until ready to transport. Do not freeze.

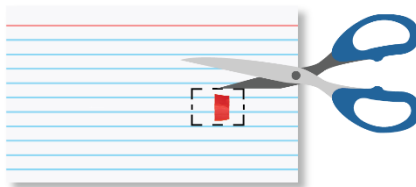
GLUTARALDEHYDE FIXED TISSUE

1. The glutaraldehyde fixed portion of muscle, approximately 0.5 cm length by 1.0 cm in diameter (or for pediatric patients 0.5 cm in length by 0.5-1.0 cm in diameter) will be used for electron microscopy (EM). The glutaraldehyde portion of muscle should be adhered to an index card to relax the tissue and keep its orientation. This is a crucial step to ensure precise cross and longitudinal direction for embedding.
2. Using forceps or tweezers, gently stretch the muscle longitudinally on an index card. The muscle will adhere to the dry surface immediately. Cut off excess index card around the adhered muscle.
3. Place specimen in the container with glutaraldehyde fixative. Ensure that the entire tissue specimen is fully submerged in fixative.
4. Refrigerate (4° C) until ready to transport. Do not freeze.

1. Using forceps, gently stretch the muscle longitudinally on an index card.



2. Remove the excess index card.



3. Place specimen in the container with **glutaraldehyde fixative** immediately. Ensure that the entire tissue specimen is fully submerged in fixative



MUSCLE BIOPSY TISSUE SUBMISSION PROTOCOL



Required Information

Please complete all documentation and labeling with indelible ink to preserve documentation during the shipping and handling process. Printed specimen labels are preferred to handwritten labels. Include the following documentation to accompany the specimens:

- Completed [Muscle Biopsy Requisition](#) form to include:
 - Client and Ward codes unique to your institution
 - Patient first name, last name, and date of birth
 - Patient medical record number (MRN) if available
 - Billing instruction and patient insurance information and demographics (or attached face sheet)
 - Clinician requesting the biopsy (e.g., neurologist, rheumatologist, or primary care provider)
 - Surgeon who performed the biopsy
 - Collection date
 - Laterality and anatomic site of the biopsy
- The name and telephone number of the clinician who requested the biopsy (e.g., neurologist, rheumatologist, or primary care provider). The surgeon's contact information alone is not sufficient. Failure to include proper clinical history and clinician contact information may delay or yield incomplete interpretation.
- A copy of the patient's most recent comprehensive medical record from the clinician that requested the biopsy, such as an outpatient clinical note or inpatient consultation note or discharge summary. The clinical record should include:
 - Chief complaint of the neuromuscular disorder, clinical impression, and differential diagnosis
 - Family history
 - Current medications
 - Creatine kinase (CK) laboratory test results
 - Rheumatologic and serologic laboratory test results as applicable (REQUIRED) including: myositis panel, rheumatoid factor (RF), antinuclear antibody (ANA), anti-Ro, anti-La, anti-dsDNA, anti-synthetase antibodies (e.g., anti-Jo, PL7, PL12, etc.), anti-Sm, ANCA, cryoglobulins, anti-SRP, anti-HMCGCR, anti-Mi2, anti-NXP2, anti-MDA5, anti-TIF1, anti-FHL1, anti-cN-1A, etc.
 - Imaging and nerve conduction studies
- Follow hospital COVID-19 and infectious disease pre-screening guidelines and include notification if the patient is positive for COVID-19 or any other infectious etiology that may put technical personnel at risk.
- Notify MLabs of arriving specimen by calling MLabs Client Services at **800.862.7284** or **734.936.2598** (local) prior to sending the muscle biopsy.
- Fax all paperwork to MLabs Client Services at FAX **734.936.0755** prior to sending the muscle biopsy.

Packaging and Transport

1. All specimen containers must be labeled with the following required information. Printed labels are preferred to handwritten labels.
 - a. Patient's first and last name.
 - b. A second patient identifier such as a date of birth or a medical record number.
 - c. Laterality and anatomic site of the biopsy.
2. Package specimens for transport to MLabs in the supplied muscle biopsy collection kit box for tracking.

MUSCLE BIOPSY TISSUE SUBMISSION PROTOCOL



3. If sending a FRESH TISSUE specimen:

- a. Once the formalin and glutaraldehyde fixed tissue specimens have been placed in their liquid containers and the tops secured, place these and the fresh tissue specimen in its container inside the refrigerated specimen bag with the absorbent sheet and seal. Secure specimen bag in bubble wrap. Place cold pack into the box along with wrapped specimen bag, completed requisition and clinical history documentation.
- b. Please be careful to not contaminate the outside of the transport bags, paperwork, or box when handling.
- c. Transport refrigerated via courier to arrive at MLabs **within four (4) hours of the biopsy**, Monday through Friday 7:00 AM – 5:00 PM, excluding holidays.

4. If sending FROZEN TISSUE specimen:

- a. Put the frozen specimen into the frozen tissue container. Place the frozen tissue container into the frozen specimen bag and seal. Pour 3.5 lbs of dry ice into the bottom of the larger Styrofoam container (Box A). Place frozen specimen bag on top of dry ice. Fill with an additional 3.5 lbs of dry ice on top of the frozen specimen bag. Place the lid onto the Styrofoam container and secure with rubber band. Place Styrofoam Box A at the bottom of the cardboard shipping box.
- b. The formalin and glutaraldehyde fixed tissue specimens must be shipped refrigerated separate from the frozen tissue transported on dry ice. Care must be taken that these specimens do not freeze. Do not place these in the larger Styrofoam container (Box A) with the dry ice. Refrigerate the cold pack supplied with the frozen muscle kit for 24 hours prior to transporting the specimens.
- c. Once the formalin and glutaraldehyde fixed tissue specimens have been placed in their liquid containers and the tops secured, place them inside the refrigerated specimen bag with the absorbent sheet and seal. Secure the specimen bag in bubble wrap. Set a refrigerated 4°C cold pack at the bottom of the smaller Styrofoam box (Box B). Put the wrapped specimen bag on top of the refrigerated cold pack, place additional bubble wrap square inside the box to minimize shifting of the contents, secure the Styrofoam lid on top of the Styrofoam box and secure with rubber band. Place Styrofoam Box B on top of Box A, then place the requisition form, clinical history, and other paperwork on top of Box B.
- d. Seal the cardboard outer container with packaging tape. **NOTE:** *Frozen specimens for two different patients may be shipped in the same Styrofoam shipping container but MUST be packaged individually. It is recommended to use two separate kits, if possible.*
- e. If shipping by **UPS**, call 800.742.5877 to schedule a pickup. Specimens should arrive Monday – Friday by 5:00 PM, excluding Michigan Medicine holidays. Do not send specimens outside of these timeframes unless prior arrangements are made through MLabs Client Services.

PLEASE BE ADVISED: The client is responsible for following all applicable U.S. Department of Transportation (DOT), International Air Transport Association (IATA), and International Civil Aviation Administration (ICAO) shipping guidelines for safety and compliance. Training and certification are available from Michigan Medicine via the links below and is required every two years to remain compliant.

- Create a '[Friend Account](#)' at University of Michigan
- [REGULATIONS FOR SHIPPING BIOLOGICS - INFECTIOUS SUBSTANCES CATEGORY B](#)
- [REQUIREMENTS FOR SHIPPING NON-DANGEROUS GOODS WITH DRY ICE](#)
- Contact MLabs Client Services if you have questions.

MUSCLE BIOPSY TISSUE SUBMISSION PROTOCOL

Packaging Diagrams

If submitting **FRESH TISSUE** (fresh muscle biopsy kit):

FRESH MUSCLE BIOPSY KIT STORE REFRIGERATED / SHIP REFRIGERATED



SPECIMENS MUST ARRIVE AT MLABS WITHIN **4 HOURS** OF COLLECTION.



Place the specimen containers into the refrigerated specimen bag.



Secure specimen bag in bubble wrap.



Place frozen cold pack* into box along with wrapped specimen bag, completed requisition and clinical history documentation. Transport specimens in this box for tracking.

***FREEZE COLD PACK 24 HOURS PRIOR TO TRANSPORTING SPECIMENS**

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MUSCLE BIOPSY TISSUE SUBMISSION PROTOCOL

If submitting **FROZEN TISSUE** (frozen muscle biopsy kit):

STEP 1: REFER TO PROTOCOL: SPECIMEN HANDLING, FROZEN TISSUE STEP 2: BOX A FROZEN MUSCLE BIOPSY KIT

 **REFRIGERATE (4°C) GLUTARALDEHYDE / COLD PACK IMMEDIATELY UPON ARRIVAL**



PACK FROZEN SPECIMEN

- STEP 1:** Refer to protocol: Specimen handling, frozen tissue, step 1.
- Set frozen specimen bag on top of 3.5lbs of dry ice
 - Fill with additional 3.5lbs of dry ice on top of the frozen specimen bag for a total of 7lbs.
 - Place the lid onto the Styrofoam Box A, and secure with rubberband.



PACK REFRIGERATED SPECIMEN

- Place the formalin fixative and glutaraldehyde fixative containers into the refrigerated specimen bag and seal.
- Secure specimen bag in bubble wrap.
- Insert refrigerated cold pack and bubble wrapped specimen bag into the Styrofoam Box B and, secure with rubber band.



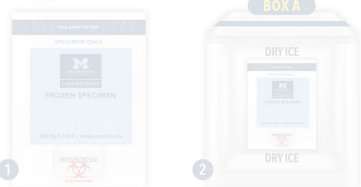
PACK SHIPPING CONTAINER

- Place Styrofoam Box A at bottom of shipping box.
- Stack Styrofoam Box B on top of Box A.
- Place the requisition, and clinical history ON TOP of Styrofoam Box B.
- Transport in shipping container provided for specimen tracking.

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STEP 3: BOX B FROZEN MUSCLE BIOPSY KIT

 **REFRIGERATE (4°C) GLUTARALDEHYDE / COLD PACK IMMEDIATELY UPON ARRIVAL**



PACK FROZEN SPECIMEN

- STEP 1:** Refer to protocol: Specimen handling, frozen tissue, step 1.
- Set frozen specimen bag on top of 3.5lbs of dry ice
 - Fill with additional 3.5lbs of dry ice on top of the frozen specimen bag for a total of 7lbs.
 - Place the lid onto the Styrofoam Box A, and secure with rubberband.



PACK REFRIGERATED SPECIMEN

- Place the formalin fixative and glutaraldehyde fixative containers into the refrigerated specimen bag and seal.
- Secure specimen bag in bubble wrap.
- Layer specimen bag, cold pack and additional bubble wrap into the Styrofoam Box B and secure with rubber band.



PACK SHIPPING CONTAINER

- Place Styrofoam Box A at bottom of shipping box.
- Stack Styrofoam Box B on top of Box A.
- Place the requisition, and clinical history ON TOP of Styrofoam Box B.
- Transport in shipping container provided for specimen tracking.

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MUSCLE BIOPSY TISSUE SUBMISSION PROTOCOL



Questions

Call MLabs Client Services at **800.862.7284** or visit our website **mlabs.umich.edu**.