INSTRUCTIONS FOR OBTAINING AND SENDING MUSCLE BIOPSIES

1. Obtain the following (2-4) pieces of muscle from a clinically-affected (but not end-stage) muscle group (usually quadriceps, biceps, or deltoid) that has not been recently subjected to EMG/NCS or other invasive procedures:
   
a. **One fresh piece of muscle tissue (up to 1 cm$^3$)**, for histology, Immunohistochemistry, and enzyme histochemistry. Wrap this fresh tissue in saline-dampened gauze or telfa pad and place in a water-tight, dry container; then place the container on wet ice or in a fridge. Do not place the muscle tissue directly on wet ice. **The gauze/pad must NOT be soaking wet**: wring out fully before letting it touch the tissue, and **NEVER immerse fresh muscle in liquid of any kind**: we may be unable to make a diagnosis because of swelling artifact. Do not let it dry out, either, or enzyme activity will be lost.
   
b. **One 3-5 mm thick piece of muscle**, clamped prior to resection to prevent retraction artifact. Place in cold glutaraldehyde immediately, keeping it in the clamp until fixed, for possible electron microscopic (EM) studies. You may prefer to keep this glutaraldehyde-fixed tissue at your own facility until needed. If you send it to us, we will embed it Epon and examine semi-thin pilot sections; full EM will be done only if necessary.
   
c. **For pediatric patients**, and adults who may have a metabolic/mitochondrial or other disorder that might require molecular/biochemical analysis, **obtain two more pieces of muscle (each up to 1 cm$^3$)**, and **freeze them right away** in liquid nitrogen, on dry ice, or in a non-frost-free freezer (do not embed them in gum or other embedding medium; freeze them in foil in a dry container). These will be held frozen for additional molecular/biochemical tests that might be requested by the treating physician. You may prefer to keep these at your own facility until needed.

2. Obtain **detailed clinical information** (recent complete history and physical, medication list, lab values, EMG/NCS reports, brain/spine/muscle MRI reports, and any neurology or rheumatology clinic notes). You may send a copy of the complete patient record if you are not sure what to send. The case will not be signed out without this information! Please provide a **phone number for the physician who requested the biopsy** and who will be treating the patient, so that we may call with results.
3. Complete a **Wet Tissue Laboratory Request form**, or use your own. Include the patient’s name, date of birth, social security number, home address, and home phone number. **Laboratory services will be invoiced to the submitting hospital.** Please provide a contact name, and address to where to send the invoice to.

4. **Sending muscle biopsies to Duke:**

   a. Deliveries can only be accepted Monday through Friday during business hours. Please **do not** ship specimens to arrive over the weekend or on holidays: no one will be available to receive them. If in doubt, please call us before you prepare the shipment.

   b. If the fresh biopsy can be delivered to us within a few hours or by the next day, it should be kept in saline-dampened gauze in a water-tight container, with the container on wet ice or in the fridge, until ready to go. Put the container with cold-packs in a Styrofoam box, and send it to us by courier/overnight shipping. During the summer months, be sure to use extra cold-packs. The glutaraldehyde specimen may be sent in the same chilled container. **DO NOT FREEZE; keep chilled only.**

   c. If fresh muscle tissue has to be held over the weekend before sending it to us, it may be snap-frozen in liquid nitrogen, on dry ice, or frozen in a NON-frost-free freezer. Do not embed it in gum or other embedding medium; freeze it in a dry container. Keep it frozen from that point on; do not thaw it for shipping.

   d. If you are sending any frozen muscle, ship it on abundant dry ice, **separately** from any fresh or glutaraldehyde-fixed tissue.

   e. Send to: **DUHS Clinical Laboratories / Immunochemistry Lab**

   4063 Duke South Clinics Yellow Zone
   40 Duke Medicine Circle, Trent Drive
   Durham, NC 27710
   Phone: 919-684-8431

5. If you have any questions at all, please contact the Duke Muscle Service: **musclepath@duke.edu** or phone: **(919) 681-9483.**
INSTRUCTIONS FOR SURGERY AND PATHOLOGY OF MUSCLE BIOPSIES

++PLEASE PLAN SURGERY TO AVOID WEEK END OR HOLIDAY SHIPPING++

Questions or concerns about collection: Call Dr. Buckley at any time at 415-317-5148 or email at anne.buckley@duke.edu

Obtain pieces of muscle from a clinically affected but not end-stage muscle group that has not been recently subjected to EMG/NCS or other invasive procedures. Quadriceps, biceps, and deltoids are the usual sites.

**TISSUE TO BE SENT FRESH: UNCLAMPED, ≤ 1cm³**
- FOR HISTOLOGY AND ENZYME HISTOCHEMISTRY
- Immediately wrap fresh muscle tissue in saline-dampened, not wet gauze. Do not place tissue directly on soaking wet gauze, wet ice, or immerse in liquid of any kind. Do not let the tissue dry out. Free liquid will cause swelling artifact and drying will cause a loss of enzyme activity.
- Send immediately to Pathology in the fresh state.

**TISSUE TO BE SENT FROZEN: UNCLAMPED, UP TO 2 PIECES ≤ 1cm³**
- PEDIATRIC PATIENTS AND ADULTS WHO MAY HAVE A METABOLIC/MITOCHONDRIAL DISORDER, AND MAY REQUIRE MOLECULAR/BIOCHEMICAL ANALYSIS.
- Immediately wrap fresh muscle tissue in saline-dampened, not wet gauze. Do not place tissue directly on soaking wet gauze, wet ice, or immerse in liquid of any kind. Do not let the tissue dry out.
- Send immediately to Pathology to be rapidly frozen.

**MUSCLE FOR GLUTARALDEHYDE FIXATION: CLAMPED, ≈ 0.5 cm. thick**
- FOR POSSIBLE ELECTRON MICROSCOPY
- Clamp muscle prior to resection to prevent retraction artifact. Wrap the muscle, still in the clamp, in saline-dampened, not wet gauze. Do not place muscle directly on soaking wet gauze, wet ice, or immerse in liquid of any kind.
- Send immediately to Pathology to be immersed in glutaraldehyde.

CALL PATHOLOGY IMMEDIATELY FOR PICK-UP AND PROCESSING!

Pathologists: Please refer to sheet for “handling and sending muscle biopsies” for instructions on correct preparation of each type of sample. Obtain detailed clinical information: a recent complete history and physical, medication list, lab values, EMG/NCS reports, brain/spine/muscle MRI reports, and any neurology or rheumatology clinic reports. Send a copy of the complete patient record if unsure. Provide a phone number for the treating physician, so we may call with results. Fill out the Duke patient information form included with this kit. (Name, DOB, SSN, home address and phone number, and whom and how to bill.) **The case cannot be signed out without this information.**
INSTRUCTIONS FOR HANDLING AND SENDING MUSCLE BIOPSIES

**DELIVERIES CAN ONLY BE ACCEPTED MONDAY THROUGH FRIDAY DURING BUSINESS HOURS**

Questions about handling or shipping: Call the Lab at 919-684-2091 or Dr. Buckley at 415-317-5148 or Email Dr. Buckley at anne.buckley@duke.edu

FOR FRESH TISSUE

FRESH MUSCLE TISSUE

Immediately wrap tissue in saline dampened -NOT WET- clean gauze square. Place in dry container. Store in fridge or on wet ice until ready to ship. Freeze if specimen can't be shipped that day.

FOR FROZEN TISSUE

FRESH MUSCLE TISSUE

Immediately wrap tissue in foil. Freeze in LN2, dry ice, or non frost-free freezer until ready to ship. PACK IN SEPARATE SMALL FOAM CONTAINER WITH DRY ICE. SHIP SEPARATELY FROM FRESH OR GLUTARALDEHYDE FIXED TISSUE.

FOR GLUTARALDEHYDE FIXATION

CLAMPED MUSCLE

Immediately place in cold glutaraldehyde. Keep muscle in clamp a minimum of 4 hours. Keep sample cold during fixation and shipping, but do not freeze.

PACK FRESH OR GLUTARALDEHYDE FIXED TISSUE WITH COLD PACKS (USE EXTRA DURING HOT WEATHER). PACK FROZEN TISSUE WITH DRY ICE. PLACE IN STYROFOAM CONTAINER, AND PLACE STYROFOAM CONTAINER INSIDE SHIPPING CARTON. *DO NOT SHIP FROZEN TISSUE IN SAME CONTAINER WITH FRESH OR FIXED TISSUE.* ENCLOSE THE COMPLETED DUKE PATIENT INFORMATION FORM SUPPLIED WITH YOUR KIT: THE CASE CANNOT BE SIGNED OUT WITHOUT THIS INFORMATION. PROVIDE A PHONE NUMBER FOR THE REQUESTING PHYSICIAN SO WE MAY CALL WITH RESULTS.

**DO NOT SHIP SPECIMENS TO ARRIVE DURING WEEK ENDS AND HOLIDAYS**
Wet Tissue Laboratory Request Form

This form **MUST** be filled out **COMPLETELY** and **ACCURATELY** in order to proceed with the review of your case.

**ALL** cases must include a Pathology Report (gross only is fine) and an ICD-10

If you have any questions please contact **DUHS Immunochemistry Lab** at (919) 681-9483 or the Lab Manager at (919) 684-5822.

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<th>Patient Name: (last, first, middle)</th>
<th>Referring Physician (last, first):</th>
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<tr>
<td>Date of Birth (mm/dd/yyyy)</td>
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The submitting hospital is financially responsible for any charges related to requests for laboratory services. Please complete the information below for invoicing purposes.

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Revised 8/15/18