



VANDERBILT
PATHOLOGY
LABORATORY
SERVICES

VANDERBILT UNIVERSITY MEDICAL CENTER
Request for Neuropathology Consultation

Division of Neuropathology
C-2318 Medical Center North
Nashville, TN 37232-2561
Phone (615) 322-3998 — Fax (615) 343-7089

•••*Please fill in all spaces accurately to insure proper handling of specimen*•••

Type of tissue sent: Nerve_____ Muscle_____

Number of slides sent:_____ Number of paraffin blocks sent:_____

Fixed tissue sent:_____ Number of pertinent reports sent:_____

Frozen tissue sent:_____

Referring Clinician:

Name:_____

Phone number:_____

Contact Person (if more information is needed):

Name:_____

Title:_____

Phone number:_____

Hospital name:_____

Hospital address:_____

Requesting Pathologist:

Name:_____ UPIN number:_____

Phone number:_____ Fax number:_____

(For muscle biopsy) CPK = _____ EMG = _____

(For nerve biopsy) Conduction studies = _____

Preliminary diagnosis:_____

Clinical history:_____

Patient Information:

Billing: Insurance _____ Client _____

Name:_____ Social security number:_____

Age:_____ Date of Birth:_____ Sex:_____ Race:_____

PROTOCOL FOR PREPARATION AND SHIPPING OF MUSCLE AND NERVE BIOPSIES FOR VANDERBILT PATHOLOGY LABORATORY SERVICES

NERVE BIOPSIES

Technical Considerations

- 1) A 2-centimeter length of peripheral nerve (usually the sural nerve) should be gently exposed through a standard skin incision.
- 2) Using a sterile muscle clamp (see below), the nerve should be clamped *in situ* in order to maintain gentle stretching of the nerve during fixation; this is essential for proper preservation.
- 3) Holding the clamp by its "handle," cut the nerve at either outside end of the clamp being careful to leave 1- to 2-mm stump extending out from each end of the clamp.
- 4) When the nerve specimen is excised and the clamp containing the stretched nerve is free, lift the clamp away and drop it with nerve into 4% buffered glutaraldehyde. This should be done immediately to avoid any dessication of the nerve specimen prior to fixation.
- 5) Once the nerve specimen is placed in the glutaraldehyde container, the top should be tightly closed. The sealed container can be mailed (see below).

Shipping of nerve biopsies

- 1) The glutaraldehyde-fixed sample can be shipped in the screw-top container properly packed within a box or envelope. This specimen can be sent by regular or, preferably, overnight delivery.
- 2) Questions should be addressed to **Tom Peters**, 615-936-0510 or 1-800-551-5227.
- 3) Shipping address: VUMC
Department of Pathology
Division of Neuropathology
21st Avenue South
C-2318 MCN
Nashville, TN 37232-2561
615-322-3998

Equipment/material required for nerve biopsies (can be obtained through VPLS)

1. Rayport muscle biopsy clamp (Baxter Healthcare Corp., Cat. # SU 130-1111)
2. 4% glutaraldehyde

MUSCLE BIOPSIES

Technical Considerations

- 1) A muscle specimen of at least 1.0 cm in length and 0.5 cm in width and depth (approximately the size of a pencil eraser) should be removed by standard open biopsy approach.
- 2) The fresh muscle specimen should be divided into three portions (**if it cannot be transported within four hours**): approximately half for histochemistry, slightly less than half for routine paraffin-embedded light microscopy and the remaining small piece(s) for electron microscopy. See Procedure for Freezing for Histochemistry (below).
- 3) For histochemistry, the specimen should be sent to VPLS either in the fresh state or frozen, depending on how rapidly it can be transported. The following guide should be used:
 - a. If the muscle specimen can be transported to VPLS *within four hours* of removal from the patient, the specimen should be carefully wrapped in a saline-moistened (**not soaked or wet**, but only moist) gauze. The specimen in the moist gauze should be placed inside a plastic petri dish, securely closed with tape and placed in a small leak-proof ziplock bag. This can then be packed in regular ice (**not dry ice**) and sent to VPLS. The shipping container should be marked to indicate that this is perishable material.
 - b. If the transportation time will be **more than four hours**, the tissue should be specially frozen for shipping in dry ice. The section "NOTES ON SPECIAL FREEZING AND SHIPPING OF MUSCLE" provides specific details for this freezing procedure. Please contact us if there are any questions.
- 4) For routine light microscopy, muscle is placed in 10% buffered formalin and sealed for shipping.
- 5) For electron microscopy, small (1-mm square) pieces are placed in 2% glutaraldehyde and sealed for shipping. Any commercial or "routine" fixative for EM can be used; if this is not available, please notify us at VPLS and we can provide EM fixative to you.

SHIPPING OF MUSCLE BIOPSIES

- 1) If transport time to Vanderbilt is less than 4 hours, the fresh muscle can be packed in regular ice and shipped in an insulated (styrofoam) container (as described above). **PLEASE NOTE:** When packing a fresh muscle specimen for transport, the fresh muscle specimen should be placed in a saline-moistened (not soaked, not watery-wet) 4 x 4 gauze pad and wrapped carefully. This gauze-wrapped specimen should then be placed into a plastic petri dish; it does not have to be sterile. The petri dish should be taped shut (Scotch tape is fine) and put into a small ziplock plastic bag. Close the ziplock bag tightly to prevent water from leaking into it. Then place the ziplock bag (containing the petri dish) on a bed of regular ice (not dry ice) in the styrofoam shipping container. These four steps will prevent water-logging and subsequent ice-crystal artifact.

- 2) If transport time to Vanderbilt is greater than 4 hours or overnight, the petri dish with rapidly-frozen muscle is placed in an insulated container **with dry ice**. This should be shipped by Federal Express or Emery Air Freight. For shipments with dry ice, federal regulations dictate that the following be visible on the outside of the container:

ORM-A-UN1845 DRY ICE FOR MEDICAL USE

- 3) Formalin and glutaraldehyde-fixed samples are mailed via standard means (i.e., in a box or shipping container by conventional mail or overnight delivery service).
- 4) Questions can be addressed to **Tom Peters**, 615-936-0510 or 1-800-551-5227.
- 5) Shipping address: VUMC
 Department of Pathology
Division of Neuropathology
 21st Avenue South
 C-2318 MCN
 Nashville, TN 37232-2561
 615-322-3998

NOTES ON SPECIAL FREEZING AND SHIPPING OF MUSCLE

Equipment/materials required for freezing

1. Insulated flask for liquid nitrogen
2. Liquid nitrogen—from a local source
3. Cork board or cardboard
4. Gum tragacanth (cold) or OCT
5. Can of dry unscented talc
6. Appropriate instruments (forceps, hemostats, etc.) for grasping/holding specimen while freezing

Procedure for freezing for histochemistry

1. Place liquid nitrogen into a metal or insulated flask; this flask should be about half full.
2. Place a small mound of the cold OCT-talcum paste in the center of a small square of cardboard or cork board (*see diagram 1*).
3. Take the piece of muscle to be frozen into a small pair of forceps and coat it completely in dry talcum.
4. Next, place the talcum-coated muscle specimen into the center of the mound of OCT-talcum paste on the cardboard.
5. Try to be certain that the muscle is oriented in cross-section in the mound of OCT-talcum and that a small portion is sticking up from the mound of OCT-talcum (*see diagram 2*).

6. With a pair of forceps, pick up the edge of the cardboard and submerge the whole specimen (board and mound of OCT-talcum holding the muscle) quickly into the liquid nitrogen (*see diagram 3*).
7. Keep it submerged until the liquid nitrogen stops bubbling.
8. Remove the frozen specimen and place it in a small sealable vial; place the vial on a bed of dry ice for shipping or into a freezer for storage until you are ready for shipping. **Do not allow the specimen to thaw.** Thawing occurs in the following situations:
 - a. Exposure to room air for more than 10 seconds.
 - b. Holding the specimen in the hand.
 - c. Placing the specimen in a container that has not been previously cooled to at least -10°C.

Improperly frozen specimens are specimens that are allowed to thaw and re-freeze. They sustain marked freezing artifact that renders the specimen uninterpretable.

Retaining some of the specimen in formalin is suggested so that anatomic pathology may be performed in the event freezing artifact has occurred.

Procedure for handling and shipping frozen tissue

Immediately remove the frozen tissue and place it in a cool (-70°C) airtight container (glass vial, etc.) and place in an ultrafreezer or on dry ice. Ship the frozen specimen in the airtight container on dry ice for next day delivery. **Please** always contact us before shipping to ensure that the specimen is anticipated.

Contact Persons

Tom Peters — Vanderbilt Pathology Laboratory Services

615-936-0510

800-551-5227

Division of Neuropathology

615-322-3998

Lab

615-343-0083

Diagram 1

Make a thick paste of Talc and OCT.



Place a dollop of paste on the cork or cardboard

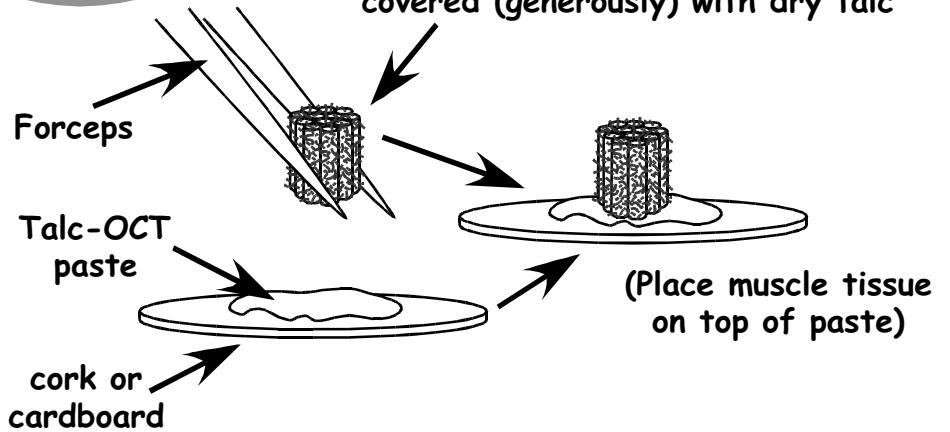


NOTES:

1. When covering specimen with dry talc, cover generously and do not shake off excess talc before placing into paste.

Diagram 2

Cross-section of fresh muscle tissue covered (generously) with dry talc



2. Immerse specimen quickly for freezing. After freezing, do not allow specimen to thaw.

3. Store the specimen immediately at -80°C or ship immediately in dry ice.

Diagram 3

Next, slide cork quickly into liquid nitrogen and completely immerse the entire specimen until liquid nitrogen stops bubbling.

