University of Rochester Neuromuscular Laboratory 575 Elmwood Avenue Rochester NY 14642 585 275-1330

SPECIMEN HANDLING, PROCESSING, AND STORAGE INSTRUCTIONS

Procedure for Punch Skin Biopsy:

A standard biopsy to look for a distal small fiber sensory neuropathy includes a total of two 3-mm punch biopsies – one performed on the upper lateral thigh, and one performed on the lateral distal leg, about 7-10 cm above the lateral malleolus. The tubes for the thigh will be labeled proximal thigh-hip. The lateral distal leg tubes will be labeled ankle. Note that skin biopsies will be performed on the same side as the pain and/or burning. Additional skin biopsies can be taken from more proximal sites, or the contralateral side depending on the clinical presentation.

The preparation consists of a thorough cleansing with 70% isopropyl alcohol.

1% lidocaine anesthetic with epinephrine (omit epinephrine if patients have had prior reactions to the epinephrine) is then infiltrated under the skin to raise a small bleb.

After the area is anesthetized, an Acupunch 3-mm punch is used to gently pull the skin plug up and away from the underlying subcutaneous fatty tissue.

A scalpel is used to cut any tissue ties.

Each specimen is then placed immediately in labeled specimen containers using PLP fixative for the anti PGP9.5 immunostaining and quantitation of the epidermal nerve fibers.

Handle the specimens carefully, do not crush or handle by the epidermis. Only handle (ie grasp with forceps by the dermis side)

Topical antibiotic ointment should be applied to the biopsy site. It should then be dressed with a gauze pressure dressing.

Important considerations in performing skin biopsy for nerve evaluation:

Is the person allergic to lidocaine?

Is the person on anticoagulants or have a coagulopathy?

Does the patient have a latex allergy?

Punches from Accuderm only need to penetrate half the distance of the metal head. Deeper will only create excessive bleeding and reach fat layers.

Only use 2% PLP fix. Formalin blocks the antigenic determinant sites.

Fixation time should be 12-24 hours for optimal immunohistochemistry results.

Forceps should **NEVER** contact the upper layers of the skin. Handle gently by the deeper reticular dermis ONLY. These fine unmyelinated epidermal fibers will be destroyed if crush artifact is introduced.

Specimens must never be allowed to air dry on gauze. They should be placed directly in fixative.

When placing specimens in refrigerator, check to see that they are submerged in fix and not stuck to the underside of the lid.

In determining side of subject to biopsy for peripheral neuropathy evaluation, do not biopsy distal to past surgical sites (i.e. past knee surgery). Do not biopsy skin

1% lidocaine injection should be close to the surface in order to raise a bleb. If injected only deep below the skin, the subject will still feel pain.

Stay away from lidocaine needle track when selecting punch site. Stay within bleb area but not including hole from needle.

Check person for anesthesia with a single needle tap away from site to be biopsied.

Place a 4x4 inch gauze pad over biopsy site to prevent potential blood leakage on pants.

You may batch ship fixed cryoprotected samples on wet ice after holding in the refrigerator.

You must make contact with the lab before sending specimens. Specimens should not be shipped to arrive on a holiday or on weekends.

If you have questions regarding these protocols, please call Don Henderson (585) 275-1330.

Sample Fixation and Preparation for Shipping:

This is a very critical step as proper fixation is essential for proper staining and interpretation. We will provide kits to all outside referring physicians that will include all the necessary tubes and solutions. These kits have a shelf life of 2 weeks. The tubes for the thigh will be labeled proximal thigh-hip. The lateral distal leg tubes will be labeled ankle.

The specimens should be placed in Tube A (PLP fixative) as soon as they are removed from the patient.

The specimens should remain in Tube A for 12-24 hours. The samples should be stored in the refrigerator during this time. If the samples stay in fixative for longer than 24 hours the results of the test will be compromised.

The specimens should be transferred to Tube B (Saline Solution) for 15 minutes.

The specimens should then be placed in Tube C (Shipping Buffer).

At this point the samples should be stored in the refrigerator until they can be shipped to Rochester.

Materials Needed for Skin Biopsy:

1. (1) Field drape-This is non fenestrated and is used to create a sterile field to place items needed for biopsy. We use the standard towel drapes

- 2. (1) Sterile gloves
- 3. (10) Alcohol pads/wipes
- 4. (1) Insulin syringe
- 5. (1) 3 mm biopsy punch
- 6. (1) Disposable scalpel #10 with handle
- 7. (1) Disposable forceps
- 8. (5) Sterile 4x4 cover sponges
- 9. Micropore tape
- 10. (6) Antibiotic ointment-Bacitracin double antibiotic
- 11. 1% Lidocaine with Epinephrine

Shipping Information:

Specimens must be sent overnight: Monday through Wednesday to:

Don Henderson University of Rochester Medical Center Dept. of Neurology Rm. 5-5329 575 Elmwood Avenue Rochester NY 14642

Contact Information:

Don Henderson E-mail don_Henderson@urmc.rochester.edu FAX: (585) 273-1255 Phone: (585) 275-1330