Submitting Muscle Biopsy Specimens

Introduction

A muscle biopsy is taken from patients with a history of weakness, sensory loss or reflex change. Diseases or circumstances that may be associated with these symptoms include diabetes, collagen vascular disease, metabolic disease, familiar neuropathies, a neoplasm or trauma. The biopsy may be from several different muscles, such as the deltoid, biceps or triceps of the arms, the gastrocnemius, rectus, tibial or vastus lateralis of the legs, or the abdominal rectus.

These specimens arrive at the laboratory either fresh from the operating room on saline-moistened Telfa gauze or from the Reference Lab. Those coming from the Reference Lab are either fresh on saline-moistened Telfa gauze at refrigerator temperature or partially processed with the frozen portion on dry ice and the refrigerated portion and room temperature portion on ice.

Muscle biopsy specimens must be accompanied by a completed requisition and muscle/nerve worksheet. Fresh specimens are placed into the refrigerator during accessioning for processing. Frozen specimens are placed in the –80° C freezer during accessioning for processing.

Specimen Requirements

1. Optimal conditions are a fresh specimen on saline-moistened Telfa gauze received at refrigerator temperature, Monday through Friday 7 a.m. – 5 p.m.
2. Processing of specimens received from outside facilities depends on the specimen portions received. Fresh or frozen portions canbe processed for enzyme histochemistry. Portions in glutaraldehyde can be processed by electron microscopy (EM). Portions in 10% neutral buffered formalin (NBF) can be processed by light microscopy or microscopic slides and/or paraffin blocks. Including all of the above portions ensures the most complete testing.
3. Specimens must be properly labeled and accompanied by a surgical pathology requisition completed by the originating clinician. The specimen container should be labeled with the patient's name, medical number (for Cleveland Clinic patients), date of birth, and social security number. (A minimum of two identifiers must be present on both the specimen container(s) and requisition.)
4. Specimen must be received with a muscle/nerve worksheet.

Specimen Preparation

Equipment

Vacuum-insulated flask, ½ to ¾ full of liquid nitrogen
Glass or plastic 50 cc test tube/centrifuge tube
Specimen chuck
Indelible pen
Specimen storage vial
Clean fine forceps
Long-handled forceps
Dental wax
Dissection microscope
Saline
Gem stars
Thunderbird
Microscope slides
Tickets

Reagents

2-methylbutane (isopentane) **
HAZARD CLASSIFICATION: FLAMMABLE, IRRITANT

Gum tragacanth **
HAZARD CLASSIFICATION: IRRITANT

Glutaraldehyde **
HAZARD CLASSIFICATION: SENSITIZER

Liquid nitrogen
HAZARD CLASSIFICATION: IRRITANT

***Store in the explosion-proof refrigerator. Bottle labeled with expiration date.*

Freezing for enzyme histochemistry (EH)

1. Select a large cross-section of the muscle with minimal fat.
2. Label a chuck with the patient's name, surgical number, and "muscle bx."
3. Label a vial cap with patient's name, surgical number, "muscle bx," and location within the capsule.
4. Mold a pyramid of gum tragacanth on the chuck.
5. Fill the vacuum flask with liquid nitrogen.
6. Fill the test tube with 15 cc of isopentane and promptly return to the explosion-proof refrigerator (*DO NOT LEAVE THE BOTTLE OUT WITH THE CAP ON.)
7. Use the fine forceps to place the specimen in the center of the gum tragacanth, orienting it so the cross section is up.
8. Place the test tube of isopentane (2-methylbutane) in the vacuum flask of liguid nitrogen, securing it with a plastic lid.
9. When crystals have formed on the bottom of the test tube, the isopentane (2-methylbutane) is ready to use.
10. Pick up the chuck with the long-handled forceps in an inverted position
11. Remove the test tube of isopentane, holding it while inverting the specimen into the tube. Do not completely submerge the specimen. Lower it until the muscle is covered.
12. Count one, one thousand, two, one thousand up until ten, one thousand.
13. Completely lower the chuck to the bottom of the tube.
14. Quickly remove the chuck.
15. Pick up the vial with the long-handled forceps and submerge into the liquid nitrogen. Pre-chill the cap of the vial in the same way.
16. Place the chuck into the vial and cap the vial.
17. Place the vial into the liquid nitrogen, using the long-handled forceps, for a second to complete freezing.
18. Place the vial into the –80°C freezer.
19. The frozen portion of the muscle should be sent to Cleveland Clinic on dry ice to maintain the frozen state. If the specimen does not maintain the frozen state, ice crystals will form and the specimen will not be optimal for diagnosis.

Submitting for light microscopy

1. Select a cross section and a longitudinal section of the sample.
2. Orient the muscle on a microscopic slide, gently stretching the longitudinal segment.
3. Fix the slide in 10% neutral buffered formalin (NBF).
4. Place the fixed tissue into a labeled container of 10% NBF for transport.
5. This portion of the specimen can be submitted at room temperature or refrigerator temperature.
6. H&E microscopic slides and the paraffin blocks may be sent as an alternative to the above procedure.

Submitting for electron microscopy (EM)

Tissue will be needed for mitochondrial disorders, metabolic disorders, storage disorders, inclusion body myositis (IBM), polymositis, and all pediatric cases. For other clinical histories, a piece is placed into glutaradehyde for EM hold, and a piece is not snap frozen

1. For EM / EM hold, a longitudinal segment is required.
2. Label a glutaraldehyde vial with the surgical pathology number, patient's name, "muscle bx," and EM.
3. Gently stretch the segment on a microscope slide.
4. Place the slide into a specimen cup, pouring the gluteraldehyde from the vial over the specimen.
5. After approximately 10 minutes, transfer the specimen and glutaraldehyde back to the vial.
6. Place the vial containing the EM specimen into the refrigerator until ready to transport.

For more information or assistance, please call:

Brad Skilton **216.444.3975** (Supervisor, Surgical Pathology)

MUSCLE & NERVE BIOPSY WORKSHEET

(This form must accompany all biopsy specimens)

Patient's Name _____ Age _____ Sex _____

Cleveland Clinic Medical Record Number _____ Date _____

Clinical diagnosis _____

_____

Reason for biopsy _____

Biopsy site(s) _____ Left _____ Right _____

Brief clinical history (include distribution of weakness and sensory loss, reflex change) _____

_____

_____

Past medical history (include diabetes, collagen vascular disease, metabolic disease, familial neuropathies, neoplasms, and trauma) _____

_____

_____

Drug therapy (current medications or drugs) _____

_____

Previous biopsy: Yes ___ No ___ (if yes, when, and where) _____

CPK _____ Aldolase _____ ESR _____ ANF _____ Other _____

Staff physicians to receive copy of report: Dr. _____

Dr. _____

Dr. _____

Special Instructions:

Please address questions regarding biopsy preparation to Dr. Richard Prayson at 216.444.8805. If there are special requirements for tissue handling (e.g. processing extra tissue for possible molecular biologic studies or enzyme assays), please contact Dr. Prayson or the Surgical Desk at 216.444.2836 before sending specimens.