



Pennington Biomedical Research Center
SOP – 2203
Biopsy Collection Translational Physiology Lab

Purpose

The Purpose of this SOP is to perform proper collection, processing, and storage of human subcutaneous adipose and human skeletal muscle of the Vastus Lateralis from biopsy procedures following biopsy collection.

Responsibility

It is the responsibility of the collector of biopsy specimens to ensure that all samples are accurately and quickly processed, handled in a careful way, and stored appropriately during processing, following processing while in the biopsy room, en route from the biopsy room to the lab, and after post-biopsy procedures. It is also the duty of the biopsy collector to ensure that all biohazard and sharps waste is disposed of properly, that the biopsy table for specimen processing is cleaned properly before leaving, and that all biopsy room supplies relevant to biopsy processing and collection are in adequate supply in the room and/or on the biopsy collection cart.

Procedure Steps

- A. Setting up the biopsy
- B. Biopsy Processing
- C. Supplies

Procedure Steps in Detail

A. Setting up the biopsy

- 1. Obtain tubes for appropriate study.
- 2. Label tubes according to the study requirements.
 - i. See Study MOP for details – list of potential items collected is provided in the pages below for adipose and for muscle tissue
- 3. Place blue pads (white side up) on the biopsy table



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4. Before going into the biopsy room pour liquid nitrogen into the small travel dewar
5. Place items from biopsy cart into the biopsy collection table. Place the osmium vial and rack farthest from main working area. As second collector will place the isopentane stock and waste bottle next to them for IHC collections.
6. Take out 1 large weigh boat and 1 small weigh boat from biopsy cart; place the small weigh boat inside of the large weigh boat; place an autoclaved mesh sheet (found on the biopsy cart) on top of the small weigh boat
7. Take out 2 autoclaved forceps (one for adipose biopsy processing and one for muscle biopsy processing) from biopsy cart.
8. Tare scale with piece of aluminum foil, according to **SOP 2204**.
9. Layer kimwipes and place them on biopsy table.
10. Fill out the physician/nurse practitioner and nurse assistant names on biopsy sheet.
11. Make sure there are enough petri dishes out (stacked in sets of 4 in front of the incubator).
12. Before the biopsy begins, pour Liquid Nitrogen into the Styrofoam container (outside the biopsy room) and place the autoclaved freezer clamp inside container (the freezer clamp handle will be hanging out of container); want to do this about 15 minutes before the biopsy begins.
13. Before and during hr biopsy make sure there are enough opened petri dishes near the surgical area for the nurse to place the collections.

B. Processing the biopsy

1. Participants are prepped and biopsy is performed according to **Clean Technique for Muscle and Fat Biopsies (SOP 906)**.
2. Adipose tissue is processed first.

Adipose Tissue Biopsy Collection:

Potential assays for sample collection and recommended order:

- a) Fat Cell Size (FCS; Osmium fixation)
- b) Electron Microscopy (EM; 2% glutaraldehyde, 2% paraformaldehyde, 1X Phosphate Buffered Saline)
- c) Light Microscopy (LM; 4% paraformaldehyde, 1X Phosphate Buffered Saline)
- d) Histology/Paraffin embedding (10% formalin)
- e) Conditioned Media/Cytokine Secretion Assay (M199 collection media)
- f) Snap Frozen tissues (RNA, DNA, Protein, and Archives)
- g) Adipose culture/Stromal-vascula fraction (SVF) isolation

Procedure:



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- 1) Biopsy will begin with Bergstrom technique – items “a” through “d” MUST be collected by the Bergstrom technique; items “e” through “g” can either be collected by the Bergstrom or the Mercedes Lipoaspirate Technique
 - 2) With each collection of adipose tissue, transfer from the Petri dish on the surgical/biopsy cart and place onto the mesh sheet covering the small weigh boat. Tissue should then be blotted on the stack of Kimwipes.
 - 3) Once ~40mg (but no more than 60mg) is obtained, collect for item “a”: Carefully open cap away from body, drop tissue into the bottle (or place on the inside lip of the lid), and quickly close the bottle. Shake the tissue down into the Osmium solution, and visually assess that the tissue begins to turn black.
 - 4) Item “b” will require only about 10mg. Place into collection tube with fixative and place on ice
 - 5) Item “c” will require between 150-250mg, based on how well biopsy collection is proceeding. Place into collection tube with fixative and place on ice
 - 6) Item “d” does not have to be weighed, but visually assess that ~30mg at least is present. Place into histology cassette, snap histology cassette closed, and place into container with fixative
 - 7) Once item “d” is collected, inform the physician/nurse practitioner to switch from the Bergstrom Biopsy technique to the Mercedes Lipoaspirate Technique
 - 8) After the first pass from Mercedes Technique is complete, the nurse/biopsy assistant will hand you the syringe and collection tubing. Visually assess if enough tissue is present (sometimes one pass is enough); if not, request another pass
 - 9) Open the plunger end of the syringe and inject sterile saline into the tube. Empty contents of the tube onto the mesh over the weigh boat
 - 10) Item “e” will require at least 250mg of tissue. Place tissue into M199 in 50mL conical and place on ice
 - 11) Item “f” should be weighed, placed in collection tubes, and frozen in liquid nitrogen
 - 12) Item “g” should not be weighed but visually assessed that at least 200mg of tissue is present. The tissue should be placed in the 50mL with the HEPES solution
3. Muscle Biopsy tissue is processed second, following the completion of adipose tissue biopsy. Should the Muscle Biopsy procedure occur before the Adipose tissue is completely processed, the adipose can remain on the mesh so long as it is moist with sterile saline. Muscle tissue is far more sensitive to degradation and must therefore be processed immediately.

Muscle Tissue Biopsy Collection:

Potential assays for sample collection and recommended order:



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- a) Freeze Clamp
- b) Electron Microscopy (EM; 2.5% glutaraldehyde in 1 X Phosphate Buffered Saline)
- c) Immunohistochemistry (IHC) frozen tissue (Embedded in Optimal Cutting Temperature (OCT) gel mixed with Tragacanth Powder)
- d) Snap Frozen Tissue (RNA, DNA, Protein, Enzymes, Archive)
- e) Primary Culture collection (Dulbecco's Modified Eagle's Media supplemented with Penicillin and Streptomycin)

Procedure:

- 1) Muscle tissue is only collected per the Bergstrom Biopsy technique, and item "a" should be collected first. Be careful to visually assess the first pieces of tissue removed are in fact skeletal muscle, because sometime the initial pieces are subcutaneous fat.
- 2) Item "a" is freeze clamped using the custom made freeze clamp tongs that have been cooled on the bench in liquid nitrogen. The physician/nurse practitioner will place the tissue piece directly between the two plates of the tongs. Immediately clamp the tissue down, allow the physician/nurse practitioner to pull out the tweezers holding the tissue, and then place the tissue and ends of the clamps down into the liquid nitrogen. Allow the tissue to remain in the liquid nitrogen for at least 15 seconds before placing into the appropriately labeled collection tube. This item does NOT need to be weighed.
- 3) Each subsequent piece will be obtained by having the physician/nurse practitioner place each biopsy specimen into a Petri dish on the biopsy cart. Samples are brought over to the biopsy processing table and blotted on Kimwipes before being processed. In the event of an excessively bloody sample, washing the tissue with sterile 1X Phosphate Buffered Saline can be performed (but note that washing with 0.9% saline is not advised).
- 4) Item "b" does not need to be weighed, but approximately 5-10mg is needed. Place tissue into tube containing fixative and place on ice.
- 5) Item "c" does not need to be weighed, but needs a specimen whereby visual assessment can discern orientation of muscle fiber direction; and approximately 30-80mg can be used. A mixture of OCT and tragacanth powder is made and vigorously mixed together until the consistency of "toothpaste" is obtained and the appearance of the mixture is white. This mixture is placed into the IHC base mold and then the tissue is laid into the base mold with muscle fibers being oriented longitudinally in a "north" to "south"/vertical direction. Isopentane (IUPAC designation: 2-methylbutane) is cooled over liquid nitrogen and then the base mold holding the tissue is placed inside. Allow the tissue to remain for about 30 seconds or more before removal. Once the base mold is removed, wrap in aluminum foil and place directly into liquid nitrogen.
- 6) Item "d" needs to be blotted and weighed before being placed into each appropriate cryovial. Make sure to get as close to the weight mark as possible for



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each tissue request on the biopsy sheet. After tissue is secure in the cryovial it will be dropped into the small travel dewar with liquid nitrogen.

- 7) Item "e" does NOT need to be weighed. Bring the 50mL collection conical to the biopsy/surgical field. The physician/nurse practitioner will place the tissue directly into the conical. Between 50-150mg of tissue can be used, and this item should be the last item collected from the biopsy.
1. Following Muscle biopsy, remove all biohazardous waste into the trash (dirty kimwipes, gloves, paper towels, aluminium foil for tissue weighing, weigh boats and mesh for adipose collection, blue drape pads etc).
2. Return all biopsy specimens (both in liquid nitrogen, at room temp, and on ice) back to the biopsy cart.
3. Collect all tools and instruments (tweezers, freeze clamp tongs, spatulas etc) into a glove or kimwipe and bring to cart—these will be washed and autoclaved
4. Discard any remaining opened Petri dishes into the biohazard waste.

C. Supplies

- Labeled tubes/vials
- Biopsy Sheets
- Small ice bucket
- Eye protection
- Mesh squares
- Large, medium weigh boats
- Autoclaved tissue clamp
- Styrofoam container
- Isopentane (IUPAC name: 2-methylbutane) stock and waste bottles)
- IHC tools
- Safety Glasses
- Base molds and foil
- Long forceps
- Liquid Nitrogen dewar
- Liquid Nitrogen Travel Dewars (red, medium blue)
- Aluminum can w/Styrofoam float ring and funnel (on biopsy cart)
- Large weigh boat lined w/foil (in biopsy room)
- Bouin's fixative (yellow)
- Bouin's cassette
- EM collection tube
- Ice
- Liquid Nitrogen
- Kimwipes
- Petri dishes



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- Scale
- Hepes (5ml in a 50ml conical)

Reference Documents

- SOP 2204 – Tare Scale
- SOP 906 – Clean technique for muscle and fat biopsies
- Biopsy MOP



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SOP 906

Clean Technique for Muscle and Fat Biopsies

Purpose

This SOP describes the clean technique, a modified aseptic technique, used to avoid introducing organisms into the muscle and fat biopsy sites and to prevent transfer of organisms to other sites. Sterile materials are required but the process of sterile technique has been greatly modified. This SOP also describes the general steps for a fat or muscle biopsy.

Responsibility

This SOP is the responsibility of the members of the research team who prepare for and/or perform the biopsy procedure.

Procedure Steps

- A. Clean Technique Preparation
- B. Collection of fat and muscle samples
- C. Supplies

** All procedures are to be completed according to the SOP unless otherwise indicated by the specific protocol.

Procedure Steps in Detail

A. Clean Technique Preparation

1. Wash hands for 10-15 seconds using soap and running water.
2. Assemble all equipment and supplies needed:
 - a. Before adding sterile supplies to the sterile field, inspect them for proper packaging, processing, seal, and package integrity.
 - b. To assure proper sterilization, once the supplies have been opened, verify that the sterilization indicator in the autoclaved tray has turned a brown color.
3. Two team members set up the sterile field. The first team member opens the sterile supplies maintaining sterility of the items and hands them to a second



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Clean Technique for Muscle and Fat Biopsies

team member who has applied sterile gloves. Utilizing sterile technique, the second team member will:

- a. Place a sterile drape on table for work area
- b. Place instrument tray on sterile field
- c. Draw up local anesthetic with 10 ml syringe and filter needle
- d. Replace filter needle with the 25 ga 2 in. needle
- e. Arrange all other sterile supplies including the biopsy needle on sterile field
- f. When the sterile field set up is complete, cover the tray with a sterile drape
- g. After the sterile field is set up, it must be used within 2 hours

B. Collection of fat and muscle tissue samples

1. All personnel assisting with the procedure should wear clean lab coats/disposable coats and eye protection for each case:
 - a. The eyewear will be cleaned between each use with the appropriate solution
 - b. Wearing a mask/face shield is optional
 - c. The mask and disposable coats will be discarded after each use
 - d. Ensure all non-sterile lab coat sleeves are not touching the gloves or sterile field
2. Limit the number of persons near the table and the traffic near the sterile field.
3. Non-sterile personnel should not touch any part of the sterile field. If materials need to be collected from the field the collection containers must be placed on a non-sterile portion of the work surface.
4. Identify the participant, verify procedure and correct site, obtain vital signs, confirm fasting status, note allergies, verify recent medications and document all needed pre-procedure information on the biopsy checklist. Notify the coordinator of any abnormal findings.
5. Explain the procedure to the participant.
6. Position participant in bed appropriately and expose the site needed for the biopsy.
7. The MD/NP/PA will:
 - a. Apply sterile gloves and prep the site with betadine (or hibiclens if allergic to betadine or iodine)
 - b. Apply a fenestrated drape over site
 - c. Inject local anesthetic into the biopsy area
 - d. Make an incision using the scalpel
 - e. Insert the biopsy needle to obtain the specimen(s). Another team member will attach a syringe with tubing to the appropriate port of the biopsy needle at intervals to provide suction.
 - f. Provide tissue sample to appropriate lab for collection and preparation
 - g. Apply pressure to site with sterile gauze for 5 minutes after all samples have been obtained



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- h. Clean site, apply skin protectant, close the incision with steristrips or sutures and apply tegaderm dressing
 - i. An Ace bandage will be applied over a muscle biopsy site after dressing applied
8. Obtain vital signs
9. Complete the documentation of the procedure on the **Biopsy Checklist**.
10. Escort participant back to his room or to his next procedure.
11. Provide participant with **Biopsy Care Information Sheet**.
12. All sharps will be discarded into an approved sharps container, biohazard materials and disposable supplies will be discarded appropriately, reusable instruments will be sent for cleaning and sterilization per **SOP 913** and the durable equipment cleaned.

C. Supplies

- 10 cc syringe
- 20 G filter needle
- 25 G red needle
- 250 NS bottle
- 4x4 Sterile Gauze
- 60 cc syringe
- Bactroban
- Betadine
- Bupivacaine
- Fenestrated drapes
- Sterile drapes
- Gloves
- Green pads
- Lollipops
- Q-Tip
- Scapels
- Sterile Gloves
- Steri-strips
- Syringe tip
- Syringe tubing
- Tegaderm
- Towel drapes
- Urine cups
- Ace wrap
- Sterile Tray
- Bergstrom Needles
- Mercedes Needle and Blue Handle
- Blue Syringe Clip
- Biohazard bags



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SOP 906

Clean Technique for Muscle and Fat Biopsies

Referenced Documents, SOPS and Forms

- **SOP 913** – Autoclave Procedure
- **Form – 906.A** Biopsy Care Information Sheet
- **Form – 906.B** Fat/Muscle Biopsy Checklist
- **Form – 906.C** Fat Biopsy Checklist
- **Form – 906.D** Muscle Biopsy Checklist