

Collection and Processing of Human Skeletal Muscle Biopsies

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1 Objective

The objective of this SOP is to conduct a skeletal muscle biopsy on a patient and to preserve the sample for future analysis including mononuclear cell isolation, molecular analysis, immunohistochemistry, and immunofluorescence.

2 Applicability/Scope

This SOP is intended for use by trained physicians and/or researchers to collect muscle tissue biopsies for use in histological assessments.

3 Summary of the Procedure

The method is modified from Tarnopolsky *et al.*, 2011. After the participant is situated in the sterile field and consent for the procedure to proceed has been given, anaesthetic is administered to the area surrounding the site of incision, the skin is incised to provide access to the muscle tissue, and a Bergstrom biopsy needle is inserted and used to collect muscle tissue samples under suction. After the samples have been collected, the needle is withdrawn and the incision is closed. Muscle tissue samples can then be treated and stored for downstream use in a variety of analyses.

4 Definitions

None

5 Materials and Equipment Required

- Sterile gloves (Fisher, 19162227)
- Sterile drapes (Valuemed, VM500010)
- 50 ml syringe (Linen plus, VMEXEL-26300)
- Sterile tubing (MECP, 2C5645)
- Scalpels (EMRN, 4160)
- Sterile gauze (RW Medical, A2101-CH)
- Alcohol swabs (Canadian Safety Supplies, 100034)
- Sterilization liquid (United Canada, UCASOL-L0000017)
- Skin glue (Amazon)
- Steri-strips (Amazon)
- Wrap (Amazon)
- Tegaderm (Amazon)
- OCT (Fisher, 23730571)
- 5-mm Bergstrom muscle biopsy needle (Pelomi, Denmark)

6 Step-by-Step Procedure

Ethics Approval, Informed Consent, and Clinical Oversight:

Before initiating the muscle biopsy procedure, ensure that the study and biopsy protocol have received approval from the appropriate local Research Ethics Board (REB) or Institutional Review Board (IRB), and that written informed consent has been obtained from the participant. The procedure must be performed by appropriately trained and authorized personnel, either by a qualified physician or under physician supervision and/or authorization, as permitted by applicable institutional policies and local regulations.

Skeletal Muscle Collection:

1. Allow the participant to acclimatize to the room and get comfortable with research staff (~10-15 min). Engage in light conversation.
2. Remind the participant of the key steps in the procedure and associated risks.
3. Verbally reconfirm that the participant is consenting to the procedure and is ready to proceed.
4. Wash hands.
5. Ask the participant to flex their vastus lateralis by kicking up against resistance. Biopsies are collected from the vastus lateralis because it is a large, superficial muscle with limited large vessels and innervation.
6. Using a marker, make a line approximately 15 cm proximal to the top of the patella on the anterior, lateral portion of the thigh. This is where you will make your incision to gain access to the vastus lateralis.
7. Put on regular laboratory gloves. *Note:* Non-latex is best in case of allergies.
8. Sterilize the area with alcohol swabs. Start at the center of the mark you make and make consecutively larger circles moving outwards.
9. Inject a small amount (~6 ml) of local anesthetic (2% Xylocaine with 1% epinephrine) just beneath the skin using a 22-26G, 0.5-1.5" sterile needle. Briefly hold pressure with a sterile gauze to stop any bleeding. Next Inject a small amount (~6 ml) of local anesthetic (1% plain Xylocaine) using a 22-26G, 0.5-1.5" sterile needle deeper into the area. For the second injection, insert the needle until you feel resistance (this is the muscle fascia) and 'bounce' the needle off the fascia to determine distance of skeletal muscle from skin surface.
 - *Note:* The first injection contains epinephrine which is a vasoconstrictor and will help control bleeding. Epinephrine can alter skeletal muscle signaling and metabolism, so it is important that it is only used superficially. Xylocaine without epinephrine is used for the deeper injection as it will not induce signaling or changes in metabolism in skeletal muscle.
 - *Note:* Some patients require additional xylocaine to get fully numb. Participants are sometimes aware of this from previous experience (i.e., at the dentist). If this is the case, an additional 3 ml of xylocaine can be injected into the biopsy area. Do not exceed 15 ml total of xylocaine.

10. Sterilize the area using an ample amount of antiseptic. Soak sterile gauze in antiseptic. Apply the antiseptic in expanding circles starting from the biopsy incision site until a large area (roughly the size of a dinner plate) is sterilized.
 - *Note:* This part of the participant is now sterile and should not be touched by the participant or any other unsterile researchers.
11. Wait approximately 10-15 min for the freezing to take effect. Once active, the freezing will last for several hours. *Note:* If blood draws are required, this is the optimal time to conduct them.
12. Wash hands and put on sterile surgical gloves.
13. Set up sterile field:
 - Lay a sterile drape across a cafeteria tray. This is the sterile area on which all sterile biopsy equipment will be placed. Inspect all tools to ensure they are in good working order and sterile.
 - Lay all sterile tools on the cafeteria tray.
 - *Note:* An assistant will open all packaging once you have put on your sterile surgical gloves. Make sure the gloves do not touch any non-sterile surfaces and that the assistant does not touch any sterile equipment.
 - Lay sterile drape below the participant's leg. This adds to the sterile field around the participant's biopsy site and protects sheets/bedding from any blood or liquids.
14. Make a small (~10 mm) stab incision at the pre-marked biopsy site using a sterile scalpel. Prior to making the incision, bounce the tip of the sterile scalpel off the participants skin and ask if they feel anything sharp. Obtain verbal consent to proceed and remind the participant that they should stop you if they feel anything sharp.
 - *Note:* If the participant does feel anything sharp at this point apply more anesthetic (see point 9) up to a total of 15 ml and wait an additional 10 min. If the participant continues to feel sharp pain, then end the biopsy procedure.
 - *Note:* In lean participants, the incision will go through the skin and muscle fascia. In participants with excess superficial adipose tissue, the scalpel will not reach the fascia.
15. Attach the biopsy needle to the tubing then pass the tubing to an assistant (without touching hands) and the assistant will attach the tubing to the 50 ml syringe. Ensure a tight fit between the tubing and needle/syringe.
16. Explain to the participant that you are about to collect the muscle biopsy and that they will feel deep pressure in their leg but should stop you if they feel anything sharp or painful. Verbally confirm the participant is ready to proceed.
17. Gently insert the needle and trocar through the incision in the skin and fascia until feeling slight increase in pressure/resistance indicating that the needle has entered the muscle. Continue to gently but firmly advance the needle (a slight twisting motion may be necessary) ~1 cm beyond the fascia so that the window in the needle is inside the muscle. Once the fascia is reached and passed, the participant will likely feel deep pressure which may cause the muscle to contract. Angle the needle ~45 degrees to the right with the window facing deep into the muscle to obtain a longitudinal piece of muscle.

- *Note:* In participants where the scalpel cannot reach the fascia (i.e., participants with excess superficial adipose tissue), the fascia will need to be breached with the biopsy needle by applying pressure and slight twisting. The same change in resistance will be detected once the needle is inside the muscle.
18. Pull back the inner trocar to expose the opening to the window in the needle.
 19. An assistant will pull suction (~15 ml) and hold the syringe while the operator closes the trocar while holding the end of the needle in place.
 20. Rotate the needle 90° clockwise 1-3 times to obtain another sample using the same procedure.
 - *Note:* Inserting the needle once with multiple clips is generally preferred to multiple needle insertions.
 21. Gently but firmly remove the needle from the leg and inspect the sample for adequacy.
 - *Note:* If insufficient sample is obtained, notify the participant and obtain verbal consent that the operator can reinsert the needle into the same incision for a second attempt at muscle collection.
 22. Pass the needle and sample to an assistant while maintaining sterility. The assistant will preserve the skeletal muscle sample for downstream analysis.
 23. An assistant will apply pressure to the biopsy site for approximately 10-15 min by pressing down forcefully (i.e., as if performing CPR) but not causing pain. Sterility will be maintained as the assistant will only touch the bottom side (i.e., non-sterile side) of the sterile drape with the sterile side contacting the participant. Apply pressure until the incision stops bleeding.
 24. Close the wound by applying a small amount (~3 ml) of skin glue. Once the skin glue dries, it forms an immediate scab to keep the incision sterile. Apply two steri-strips then cover the wound with Tegaderm.
 25. You may choose to wrap the leg with adhesive band aid. This would be important to maintain pressure to reduce bleeding if the participant is performing exercise after the biopsy. Otherwise, the wrap can be uncomfortable, and the participant may choose to forgo wrapping.
 26. Remind the participant of the biopsy care instructions and send the participant home with a biopsy care kit (see attachment, **Section 10**).
 27. Follow up with the participant within 24 hours, then as needed beyond to ensure there were no complications.

Skeletal Muscle Preservation:

1. After receiving the needle containing the skeletal muscle sample, the assistant will retrieve the skeletal muscle from the needle using tweezers and/or the plunger.
2. ~100-350 mg of skeletal muscle (i.e., the size of an eraser on a pencil) is obtained from a typical biopsy. Clear samples of any visible fat or fascia.
3. The sample can be divided for different downstream assays:
 - *For molecular analysis:* a piece (~50 mg) can be placed in a sterile tube and flash frozen in liquid nitrogen until long-term storage at -80°C.
 - *For IHC/IF:* The sample can be mounted on OCT and frozen in liquid nitrogen-cooled isopentane then transferred to a sterile tube and placed on liquid nitrogen until long-term

storage at -80°C. *Note:* Samples preserved for IHC/IF (~25-50 mg) should be intact and minimally handled.

- *For mononuclear cell isolation:* The largest piece can be placed in media (i.e., 5-20% FBS with DMEM or PBS) then placed on ice until returned to the lab for immediate processing.

7 Quality Assurance and Quality Control

Dependent on downstream assays.

8 Safety Precautions

Potential Harms, Discomforts and Risks Involved: Pain (burning or stinging) during the administration of the local anesthetic. Delayed soreness comparable to a 'Charlie Horse' for one to three days following the biopsy. Internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin. On occasion a small lump may form under the site of the incision, but this normally disappears within 2-3 months. As with any incision, there is also a slight risk of infection. If the incision does not heal within a few days or if the participant is concerned about inflammation or infection, they will be instructed to contact the researchers immediately. Rarely, there can be damage to a superficial sensory nerve, which will result in numbness in the area that is typically temporary. Permanent motor nerve damage has been reported but is not common. Allergic reaction to the local anesthetic is a risk; the real incidence of lidocaine allergy is unknown. Feeling lightheaded or fainting. Anxiety about the procedure is also a risk.

The lab should be equipped with emergency equipment including an automated external defibrillator (AED). All medical and research staff should be trained on proper use of the AED, first aid, and CPR. Telephones should be available for any adverse events that require emergency assistance. In such instances, emergency services (911) should be contacted immediately. Also provide all people involved in the procedure with the phone numbers for study leads and the physician providing medical oversight. Muscle biopsies will only be conducted by trained personnel under the auspices of a physician. Infection risk will be minimized by completing all invasive procedures under sterile conditions using sterilized instruments.

9 References

Tarnopolsky, M.A., Pearce, E., Smith, K., and Lach, B. Suction-modified Bergstrom muscle biopsy technique: experience with 13,500 procedures. (2011). *Muscle and Nerve* 43(5):717-25. PMID: 21462204.

10 Attachments/Checklists

Biopsy Care Instructions for participants

Activity (for instance walking or biking) is good for your muscle after the biopsy, this reduces stiffness. Your muscle may feel sore for a few days, as if you have performed unaccustomed exercise; however, this is normal and should pass.

A pressure wrap has been applied over the biopsy site. Keep this on for 24 hours or as long as tolerable. The bandage (Tegaderm) should be left on for 24 hours. Steristrips (the strips that keep the incision closed together) and glue have been applied under the bandage to keep the incision closed. To reduce the chance of infection after your muscle biopsy, please shower every day. After you have showered PAT the area dry with a clean towel. Please use the alcohol swabs included to clean the area AROUND the biopsy starting at the biopsy site and moving in a circular motion creating bigger circles as you move away from the site. After the first day a band aid or similar cover for the incision is NOT necessary, but DO NOT remove the steristrips. The glue and steristrips will keep the incision bonded and will dry up and flake off. Please DO NOT shave your legs in the area of the biopsy for at least 20 days after the biopsy. Use ice or pain relievers such as Tylenol™ (acetaminophen) to help alleviate any soreness. To allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 4 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure.

If you have any questions or problems with severe soreness, bleeding, or if the biopsy site becomes red or warm to the touch, please call [INSERT CONTACT INFORMATION FOR STUDY LEADS]. If no other option exists, go to your nearest emergency department.