

Manual of Procedures

Methods for adipose tissue and muscle biopsy

ESC – CALERIE phase 2

V 1.0

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I. Introduction / Contact information

The purpose of this MOP is to standardize the techniques and methods used to collect adipose tissue biopsies, prepare and store adipose tissue samples, ship and the samples to the Vermont repository.

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Chair, Emerging Science committee

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Contact information:

Vermont repository

Elaine Cornell

University of Vermont

Department of Pathology

Colchester Research Facility, Room T205

208 South Park Drive, Suite 2

Colchester, VT 05446

(802) 656-8963

II. Overview of tissue collection schema for adipose tissue

| TUBE # | ASSAY | committed to specified assay | open to ancillary investigators | TARGET (mg) | site | sample size (n=) | vials to repository | | | timeIDs | collection | shipping | storage | TUBE cap color | | | |
|-----------|--|------------------------------|---------------------------------|---------------------|----------------------------|------------------|---------------------|-----------|------|-----------|------------|-----------|-----------|----------------|----|-------|-------------------|
| | | | | | | | site | site | site | | | | | | | | |
| 1 | Gene expression - microarray (= 3.6ug RNA) | x | | 200 | all | all | P | 1 | T | 1 | W | 1 | BL,12, 24 | -80oC | DI | -80oC | |
| 2 | Gene expression - qRT-PCR (= 1.8ug RNA) | x | | 100 | all | all | P | 1 | T | 1 | W | 1 | BL,12, 24 | -80oC | DI | -80oC | |
| 3 | Gene expression - backup (= 0.9ug RNA) | x | | 50 | all | all | P | 1 | T | 1 | W | 1 | BL,12, 24 | -80oC | DI | -80oC | |
| 4 | IHC [content of macrophages] (+) | x | | ~ 50mg Do not weigh | all | all | P | 1 | T | 1 | W | 1 | BL,12, 24 | fresh | RT | RT | Buoins cassette ^ |
| 5,6,7,8,9 | open for ancillaries (5 x 100mg) | x | | 100 | all | all | P | 5 | T | 5 | W | 5 | BL,12, 24 | -80oC | DI | -80oC | |
| 10 | leftover / archive | x | | ~ | all | all | P | 2 | T | 2 | W | 2 | BL,12, 24 | -80oC | DI | -80oC | |
| | | | | 900mg | total sample weight | | | 10 | | 10 | | 10 | | | | | |

We have used this amount of tissue in several studies. The ordering of the sample collection is rearranged in order of priority. If difficulty in collecting the optimal quantity, then the biopsy will stop and samples will be processed in this order.

III. Overview of tissue collection schema for muscle

| TUBE # | ASSAY | open to ancillary investigators | | | TARGET (mg) | collection | shipping | long term-storage | |
|--------|------------------|---------------------------------|---|---|-------------|------------|------------|-------------------|-------|
| | | x | P | T | | | | | W |
| 1 | RNA | x | P | T | W | 50 | snap froz. | DI | -80oC |
| 2 | mounting for IHC | x | P | T | W | 15 | snap froz. | DI | -80oC |
| 3 | archive | x | P | T | W | 50 | snap froz. | DI | -80oC |
| 4 | archive | x | | T | | 50 | snap froz. | DI | -80oC |
| 5 | archive | x | | T | | 50 | snap froz. | DI | -80oC |
| 6 | archive | x | | T | | 50 | snap froz. | DI | -80oC |
| 7 | archive | x | | T | | 50 | snap froz. | DI | -80oC |
| 8 | archive leftover | x | P | T | W | ~ | snap froz. | DI | -80oC |
| | | | | | | 115 | PBRC | | |
| | | | | | | 315 | Tufts | | |
| | | | | | | 115 | Wash U. | | |

IV. Adipose tissue biopsy - supplies

Supplies for adipose tissue collection:

- Biopsy worksheet
- test tube rack (for the 50mL conical and pre-labeled cryovials)
- 2 ml pre-labeled cryovials (Corning 430659)
- Snap-seal container, 4 oz. (Corning 1730 4L) with 3oz Bouin's solution [Sigma-Aldrich HT10-1-32].
- Histoscreen cassettes (Fisher Scientific 22-045344 Richard-Allan HistoScreen* Tissue/Biopsy Cassettes)
- Bedsheet to cover the volunteer
- Sterile gloves
- Sterile saline irrigation (250mL bottle)
- Betadine surgical scrub
- lab coats (disposable preferred)
- Eye protection
- Mayo stand or table for instruments
- Sterile drape - fenestrated (Excel Health Care Products No. 1002 18" x 26" x 2 3/4" fenestration)
- Biopsy set - *sterilized in a pack or tray*
 - ✓ #11 disposable scalpel
 - ✓ small forceps
 - ✓ liposuction needle ± handle
 - ✓ 10cc syringe with #18 and #25 needles
 - ✓ lidocaine (2% WITHOUT epinephrine) (10cc)
 - ✓ two stacks of 4x4's
- 'Sharps' container
- 2 X 60cc plastic syringe (leur-lock)
- Syringe lock (part #)
- Leur-lock 16 inch extension set: Baxter 2C5643
- 1/4" x 3" Steri-strip™ for skin closure (3M No. R1541)
- Antibiotic cream (mupirocin; Bactroban™) and sterile cotton tip applicator
- Clear plastic adhesive (TegaDerm + Pad; 3M No.3582)
- Skin adhesive (Cavilon™ No Sting Barrier Film; 3M No.3343)

Source for Liposuction needles:

M.D. Resource

23392 Connecticut Street

Hayward CA 94545-1607

1-800-633-8423

510-732-9950

510-785-8182

www.mdresource.com

mel@mdresource.com

MACSTD315 Micro Aspiration Cannula Standard 3mm x 15cm/ Luer Lock Base

MACSTD415 Micro Aspiration Cannula Standard 4mm x 15cm/ Luer Lock Base

MACMRC315 Micro Aspiration Cannula Mercedes tip 3mm x 15cm/ Luer Lock Base

MACMRC415 Micro Aspiration Cannula Mercedes tip 4mm x 15cm/ Luer Lock Base

INFUSHDL-NC Infiltration Handle w/o stopcock control

BR1003 3mm Cannula Brush

BR1004 4mm Cannula Brush

Supplies for adipose tissue preparation:

- Powder-free Latex gloves
- Clean white lab coat
- Eye protection
- Table for adipose tissue preparation
- Clean covering for the table
- 60mL conical screw-on filters (part #)
- Balance (calibrated; measure to mg)
- Small (1cm) squares of aluminum foil for the balance
- Forceps (your choice)
- Disposable #11 scalpel
- Kim-wipes to blot adipose tissue sample (VWR No. 82003-820)
- Sterile PBS
(50ml in a 50mL conical tube - multiple aliquots - pre-warmed to 37°C).
- Fisher Scientific BD8341 Luer tip caps
- Corning sample vials (screw-top - supplied by the coordinating center)
- 'Sharps' container
- Netwells* Plate, 6-well cluster, 24mm dia. membrane, 500µm mesh size, Sterile Catalog Number 07-200-214 [Corning No.:3480]
- 60mL plastic conical tubes
- Bouin's solution (Sigma HT10-1-32)
- Histoscreen cassettes, tissue loc (Richard Allen Scientific)

- Snap-seal container, 4 oz. (Corning 1730 4L)
- 70% ethanol 1 L wide-mouth container for cassette storage (several cassettes can be stored in one container)

Supplies for storage:

- 100 count plastic cryo-box
- Liquid nitrogen
- Liquid nitrogen Dewar
- -70°C freezer

V. Skeletal muscle biopsy - supplies

- Mettler balance that can measure to accuracy of 1 mg *
- Bergstrom needle
- Forceps
Dumont #5SF, No. 11251-20, Fine Science Tools, 800-521-2109,
www.finescience.com
- Scalpel (#11)
- IV tubing with luer lock
- Sterile 4x4 gauze
- Fisher #03-388A Sample Vial, LDPE 5ml (144/cs) with needle hole punched
- Disposable base molds, #03020, 15x15x5 mm, case of 500, Surgipath Medical Industries, 1-800-225-3035
- Bedside, table top magnifier *
- Plastic cutting board *
- Bergstrom Needle, sterilized
- 60G Syringe
- Short Luer Lock IV tubing
- No 10 blade
- Disposable scalpel
- 2% Lidocaine solution, without epinephrine
- 3 cc syringe with 25G needle
- 10 cc syringe with 22 G needle
- Betadine swabs
- Disposable razor
- Sterile 4x4 gauze
- Steristrips
- Surgical bandage

- Athletic or phlebotomy tape
- Sterile drape
- Sterile surgical gloves
- OCT Compound - can be purchased from Fisher Scientific or TissueTek (#4583) *
- Gum (Sigma G-1128) *
- 3 small weigh boats *
- Isopentane *
- Liquid Nitrogen *
- No 5 flat forceps (without serrations) *
- Liquid nitrogen in dewer *
- Freezing container: can use urine sample container, with larger container placed in styrofoam base *

VI. adipose tissue biopsy

- Transfer the volunteer to the procedure room at least 15 minutes before starting the biopsy [play soft music and have the assistant go through the procedure with the participant one more time].
- After washing his/her hands, the physician puts on sterile gloves.
- The assistant opens the following items for the instrument set-up by the physician:
 - Sterile drape to place over the Mayo Stand/table
 - Instrument pack
 - Fenestrated drape
- The assistant can then pour Betadine scrub into a 4x4 gauze for application.
- The Betadine is scrubbed onto an area lateral to the umbilicus (about one hand width away).
- After cleaning the skin with Betadine, the fenestrated drape is used to establish a sterile field
- The second sterile urine cup is filled with Sterile Saline. Use a little of this saline on a 4x4 to wipe the iodine away.
- Use ~5-10ml of lidocaine to anesthetize the skin. Start with a wheal under the skin then move just under the skin in a radial pattern [from ~10 o'clock to 2-3 o'clock] advancing lidocaine as you go. It is not necessary to inject deeply. The total area anesthetized should be approximately 4 x 10cm.
- Wait 1-2 minutes.
- Incise the skin just enough to admit the liposuction needle.
- Insert the needle [which is already connected to the tubing - syringe]. Have the assistant apply suction with the syringe. The needle is moved in and

out at a rate of approximately 1Hz without breaking suction with a twisting motion as you go. The sampling continues until 900mg - 1g of tissue is removed. If you empty a pocket and stop collecting tissue, move to the next pocket.

- *Notes: The adipose tissue from some people is more fibrous and bloody than found in others. For the fibrous tissues (men and some centrally obese women) it may be easier to use a smaller needle. Stay away from the adipose tissue right under the skin: is often quite vascular. Stay away from the fascia superficialis: it is often quite vascular and the fascia is ripe with nerve endings which you have not anesthetized. The fascia can be close to the skin in some men, particularly those men with central obesity. You should be able to feel the fascia with the tip of your needle. Infiltrate with extra lidocaine as indicated if you have to work close to the fascia.*
- Remove the drape, clean the skin with saline/4X4's to remove the iodine, and apply Cavalon with the provided sterile stick/applicator.
- Allow to dry, apply the Steri-strip,
- Apply antibiotic cream with sterile stick
- **FILL OUT THE BIOPSY WORKSHEET COMPLETELY!**

VII. muscle biopsy

- Explain procedure to subject
- Prepare table:
- Place drape with sterile:
- Bergstrom needle
- Forceps
- Scalpel
- IV tubing with luer lock
- Sterile 4x4 gauze
- IHC Sample Vial with needle hole punched
- Disposable base molds
- Draw anesthetic into 3 ml and 10 ml syringes
- Position subject in reclining position
- Tape together feet for positioning
- Prepare biopsy site by shaving visible hair
- Clean with betadine swab
- Anesthetize skin with 3 ml syringe
- Anesthetize deep with 10 ml syringe
- Wait at least 10 minutes for anesthesia
- Make cutaneous incision with single pass, achieve hemostasis

- Make incision into fascia with single pass, achieve hemostasis
- Insert and position bioptome, connect IV tubing to 60 CC syringe
- Have assistance provide suction while.....
- Take biopsy
- Remove and process sample with forceps
- Repeat procedure as needed
- Achieve hemostasis in between passes
- Operator to dress wound while other cuts and measures samples
- FILL OUT THE BIOPSY WORKSHEET COMPLETELY!

VIII. Sample preparation - skeletal muscle

- set-up
 - Wear eye protection, a lab coat and powder-free gloves at all times.
 - Tare the scale
 - Record ID #, date, site, and your initials onto all of the cryovials
- snap-frozen samples
 - Place into appropriate tubes after recording weight and then into liquid nitrogen
- histology samples
 - Cut piece for histology cross section
 - Create slurry of isopentane and liquid nitrogen.
 - Place piece of muscle with fibers along length in the direction of an arrow written onto clear plastic disposable base mold (#03020, 15x15x5 mm, case of 500, Surgipath Medical Industries, 1-800-225-3035)
 - Place into isopentane slurry until plug is frozen. Slowly rotate in slurry to distribute heat to slurry from sample.
 - Place into sample vial
 - Store in liquid nitrogen until shipment to Vermont.

When complete, fill in your section of the **Biopsy Worksheet**.

IX. sample preparation - adipose tissue

set-up

- Wear eye protection, a lab coat and powder-free gloves at all times.
- Tare the scale with a piece of aluminum foil
- Record ID #, date, site, and your initials onto all of the cryovials [10]

snap-frozen
samples

- As the syringe(s) are transferred to you, remove the plunger and cap the syringe. Holding the syringe upright, add 20mL PBS, swish gently and pour into the 60mL conical. Repeat. Cap the 50ml conical. Gently invert several times to 'wash' the adipose tissue. Remove the cap. Put filter cap on the conical. Discard PBS through the filter.
- Carefully remove filter containing the adipose tissue. Using forceps, gently transfer the sample onto a short stack of Kim-wipes, blot free of any blood, dissect away large blood vessels
- Place ~100mg adipose tissue onto aluminum foil. Weigh and record on the cryovial (** or *** mg). Place sample into the cryovial and snap freeze by dropping into liquid N₂.
- Place ~50mg sample into a pre-labeled cassette and gently drop into Bouins solution [no splashing!].

ADIPOSE TISSUE COLLECTION IN BOUIN'S FIXATIVE FOR HISTOLOGY

Bouins
preservative

- Cassettes will com pre-labeled with participant information (double check!)
- Take a container of Bouin's solution (about half full) to the biopsy room. Solution can be reused several times.
- The sample will be washed as described above - transfer ~50mg tissue with a forceps to the cassette.
- Close the cassette and insert in the Bouin's fixative
- Transport the Bouin's container back to the lab and keep overnight (less than 24 hours).
- Transfer the cassette to a container of deionized H₂O. Perform several changes with deionized H₂O to wash most of the Bouin's. It is necessary to wash 6-7 times with water within 1-2 hours.
- Transfer the cassette to a 70% ethanol container and store at 4°C until ready to take to histology for paraffin blocking. Keep a log of samples that are sent for blocking and those return blocked.

When complete, fill in your section of the **Biopsy Worksheet**.

X. Aftercare:

Give the volunteer the following instructions:

- You may see some blood under the gauze. This is OK. If the blood comes out from under the plastic covering, it will need to be redressed. Call us: *place your contact information, here*
- Most people have no pain from the biopsy. If you do, it is OK to take Tylenol. If you need more than Tylenol to relieve the pain, call:
- It is OK to take a shower or bath. DO NOT soak in a hot-tub/whirlpool.
- If the biopsy site becomes red or swollen OR if you develop fever, call us: *place your contact information, here*
- After 48-72 hours, the plastic dressing will be removed by us.
- After the plastic dressing is removed, the Steri-strip will gradually come loose and fall off.

XI. Biopsy Log sheet

| # | Name | ID | Biopsy date | Shipping date |
|---|------|----|----------------|------------------|
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XII. Shipping of Tissue Biospecimens

Overview of Regulations

This shipping protocol follows the procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904. Frozen samples are shipped monthly to LCBR by Federal Express priority overnight delivery.

Materials Needed

ThermoSafe Styrofoam shipping container*
Phase-change material gel packs (for cassettes)
Rubber bands for freezer boxes
Ziplock plastic bags for freezer boxes
Absorbent material (i.e. paper towels, newspaper)
Packaging tape
Dry ice (~10 to 20 lbs per mailing container)
Federal Express Labels*
Biological Specimens Category B UN3373 labels*
Dry Ice Labels (class 9, UN1845)*
"Keep Frozen" labels*
Labeled freezer boxes with participant samples*
Completed Tissue Biopsy Forms*
Completed Shipping Forms (to be included in shipment and faxed)*

*Provided by LCBR

Frozen Tissue Shipping Procedure

For frozen shipments to LCBR, University of Vermont:

1. Line shipping container with absorbent material (i.e. lab mat, or paper toweling)
2. Place approximately ~5 to 10 lbs of dry ice on the bottom of the shipping container.

CALERIE phase 2 Tissue Biopsy MOP

3. Place another layer of absorbent material (i.e. lab mat) on top of the dry ice - so it will be between the dry ice and the freezer boxes.
4. Collect the freezer boxes containing samples to be shipped, and check the sample ID numbers against the Shipping Form for that shipment.
5. Wrap absorbent material around the box and secure with a rubber band around the box.
6. Place each freezer box in a ziplock plastic bag and seal tightly.
7. Place ziplocked freezer boxes in the shipping container. Note: the ziplock bags should NOT be in direct contact with the dry ice.
8. Add another layer of absorbent material on top of the freezer boxes in the shipping container.
9. Add remaining dry ice to the shipping container. Close and tape the Styrofoam lid.
10. Seal Tissue Biopsy Forms in a ziplock bag and place on top of the Styrofoam lid. Include a cover sheet with recipient address and contact information.
11. Close the top of the outer cardboard sleeve of the shipping container with packing tape.
12. Affix shipping labels (Fed Ex label, Biological Specimen Category B UN3373 label, Dry Ice Class 9 UN1845 label, and Keep Frozen label) to outside of shipping container.
13. Add extra shipping tape over the labels to ensure they will not fall off in transit.

Cassette Shipping Procedure

All cassettes are shipped in temperature-regulated containers designed to maintain an internal ambient temperature range of 15°C to 30°C. *Cassettes may be batched and shipped monthly or every other month on the first Monday of the month.*

1. Collect the cassettes to be shipped, and check the sample ID numbers against the Shipping Form for that shipment. If cassettes are shipped monthly, the standard Sample Shipping Form will cover the cassettes,

tissue, and blood/urine samples. If cassettes are shipped separately every 2 months, an additional Shipping Form specific to the cassette IDs will be required.

2. Place cassettes to be shipped in a Ziploc bag and seal.
3. Place 2 PCM gel packs (which are at room temperature) into the bottom of the standard Styrofoam shipping container (ThermoSafe)
4. Place Ziplocked cassettes into the shipping container in between the gel packs.
5. Add filler material, such as newspaper or bubble pack, above the Ziploc bag to fill the space and decrease sample movement during transport.
6. Seal Tissue Biopsy Forms in a Ziploc bag and place on top of the Styrofoam lid. Include a cover sheet with recipient address and contact information.
7. Close the top of the outer cardboard sleeve of the shipping container with packing tape.
8. Affix shipping labels (Fed Ex label, Biological Specimen Category B UN3373 label) to outside of shipping container.
9. Add extra shipping tape over the labels to ensure they will not fall off in transit.

Notification of shipment

Fill out the Shipping Form(s) including the Fedex airbill #s and fax to LCBR at the University of Vermont at (802) 656-8965. Please complete 1 Shipping Form per package sent to the Vermont lab. The Shipping Form(s) are to be faxed the same day the samples are packaged and shipped. The Shipping Form lists all the participant sample sets (blood/urine as well and tissue or just tissue cassettes when applicable) contained in the shipment, by both their CALERIE ID# and the Sample ID#, and lists the Fed Ex tracking number for the shipment.

Mailing Address

University of Vermont
Department of Pathology
Colchester Research Facility, Room T205
208 South Park Drive, Suite 2
Colchester, VT 05446
Attn: Elaine Cornell
(802) 656-8963
(802) 656-8965 Fax

XIII. Biopsy worksheet and Tissue Shipping Form

Calerie

MUSCLE and ADIPOSE TISSUE BIOPSY FORM

CHECK VISIT: BaseLine 12 Month 24 Month

CHECK SITE: Tufts Pennington Washington

Calerie ID: Sample ID: (Affix Sample ID label here) Site ID:

DOB: / / DATE OF BIOPSIES: / /
D D M M M Y Y Y Y D D M M M Y Y Y Y

SKELETAL MUSCLE BIOPSY

Tissue samples collected:
(Enter weight of samples in mg)

01
02 (IHC Mounting sample)
03
04
05
06
07
08

ADIPOSE TISSUE BIOPSY

Tissue samples collected:
(Enter weight of samples in mg)

21
22
23
24 (Bouin's Cassette sample)
25
26
27
28
29
30

BIOPSY PERFORMED BY (please print):

Physician/Practitioner: _____ Assistant: _____

Sample Prep by: _____

Date Samples shipped to Vermont: ____/____/____ Date Form Faxed to Vermont: ____/____/____

Comments: _____

For LCBR use only:
Date Samples Received: ____/____/____

IHC Cassette Received at RT: ___ Yes ___ No
Samples Received Frozen: ___ Yes ___ No

Calerie - Shipping Form

Date of shipment: ___/___/___ Center: _____ Prepared by: _____

FAX TO LCBR @: 802-656-8965 Number of Pages: _____

FedEx Air Bill#: _____

Tissue Cassettes Shipped: ___ Yes ___ No

| Tissue Collection Date | Calerie ID | Sample ID |
|------------------------|------------|--------------------|
| | | (place label here) |
| | | (place label here) |
| | | (place label here) |
| | | (place label here) |
| | | (place label here) |
| | | (place label here) |
| | | (place label here) |
| | | (place label here) |

For LCBR use only:

Date Received at LCBR: _____ Frozen: Y / N

Tissue Cassettes RT: Y / N