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1 Purpose

This Standard Operating Procedure (SOP) describes procedures for the collection, processing, storage and shipping of adipose tissue biopsy specimens.

2 Scope

SOP is intended for ACTG and IMPAACT laboratories performing adipose tissue biopsy collection. This SOP is part of the ACTG/IMPAACT Laboratory Manual : (https://www.hanc.info/labs/labresources/procedures/Pages/actgImpaactLabManual.aspx).

3 Background

Both central fat accumulation and peripheral subcutaneous fat loss have been associated with increased inflammation and all-cause mortality in HIV-infected patients [14.1-14.6]. The mechanism(s) underlying this association is (are) not well understood, but may partially involve physiologic and structural changes in adipose tissue function. Therefore, exploratory studies are needed to describe the inflammatory and fibrotic environment of adipose tissue in HIV-infected patients.

4 Authority and Responsibility

4.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.

4.1.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory

4.1.2 Reference the current version of the LTC SOP

4.2 The Laboratory Director is responsible for the implementation of this ACTG/IMPAACT Laboratory Manual SOP or laboratory-specific SOP and for ensuring that all appropriate personnel are trained. A laboratory-specific SOP must:

4.2.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory

4.2.2 Reference the current version of the LTC SOP

4.3 All persons (surgeon, clinical staff, or laboratory staff) are responsible for reading and understanding this SOP prior to performing the procedures described.

5 Budgetary Considerations

5.1 Room/facility fees

5.2 Salary support for surgeon and assistant

5.3 Procedural supplies

5.4 Subject reimbursement

5.5 Reagent/Processing Kits

5.6 Sample analyses

6 Eligibility Requirements

The eligibility criteria must be clearly defined in the protocol. The following criteria are examples that can be considered when developing a protocol.

Note: If doing serial biopsies, there should be a minimum interval of 4 weeks between biopsy time points if performing on the same side (left/right). *Note*: For protocols requiring biopsies from more than one tissue type (e.g., adipose tissue and lymph node), consideration should be made as to whether or not all tissue types can be obtained from the same anatomic site (to minimize incision number).

6.1 Inclusion Criteria:

The exact inclusion requirements must be outlined in the protocol. Specific inclusion criteria must be clearly defined. The examples listed below are not intended to be inclusive.

6.1.1 Able to give informed consent (refer to the protocol for age-specific requirements)

6.1.2 No notable contraindication to surgical procedures

6.1.3 Willingness to undergo required tissue biopsies per the protocol schedule of events.

6.2 Exclusion Criteria:

The exact exclusion requirements must be outlined in the protocol. Specific laboratory test restrictions must be clearly defined. The examples listed below are not intended to be inclusive.

6.2.1 Pregnant (for abdominal fat biopsies)

6.2.2 The following laboratory values obtained within 30 days prior to study entry by any "approved" laboratory that has a CLIA certification or its equivalent:

- Absolute neutrophil count (ANC) ≤750 cells/mm^3
- Platelet count ≤75,000/mm^3
- Partial thromboplastin time (PTT) >1.2x ULN
- Prothrombin time (PT) >1.2x ULN

7 Equipment, Consumables and PPE

Refer to Appendix A for a list of example reagents and consumables.

7.1 Consumables for Tissue Collection (e.g. Surgical Suite)

Required equipment (this should be determined in consultation with the local surgeon, but the following list allows for 1 surgeon to perform the biopsy by herself/himself)

7.1.1 Personal protective equipment (PPE) appropriate to adipose tissue biopsy procedure

7.1.2 Sterile minor surgery tray

7.1.3 Large sterile drape

7.1.4 Sterile surgical gown/gloves

7.1.5 Electric clippers

7.1.6 Bovie/pad

7.1.7 Sterile scale or ruler if specific quantities of tissue are requested

7.1.8 Sutures or Dermabond and Steri-strips, depending upon surgeon preference

7.2 Consumables for Sample Processing (protocol-specific)

Note: adipose tissues must be sectioned by a qualified surgeon, pathologist, pathology technologist or other provider. Processing laboratory/site staff must not be responsible for identifying or sectioning adipose tissue. The site must coordinate the biopsy such that the appropriately labeled tubes and reagents are available for tissue collection and processing. Exact processing requirements must be defined by the protocol. If possible, the protocol can provide reagent kits with as many items pre-aliquoted as possible to minimize site burden.

7.2.1 General Equipment and Consumables

7.2.1.1 Cryogenic Vials: 1.8 to 2.0 mL screw cap with o-ring, sterile, polypropylene only, self-standing, leak-proof, and suitable for storage from cold refrigeration to LN2 vapor phase (-196 to 8˚C) (example: Sarstedt Cat # 72.694.006).

Note: Amber Cryovials for light sensitive PK analytes - 1.8 to 2.0mL screw cap with o-ring, sterile, polypropylene only, self-standing, leak-proof, and suitable for storage across a range of temperatures. The vials may need to be weighed before and after collection to determine the weight of the biopsy. These would only be required for studies that will measure drug levels in adipose tissue.

Note: the “amber” vials are opaque; it may be difficult to visualize samples within the vial. If these vials are required, they must be specified in the protocol laboratory processing chart (LPC) and a centralized purchase should be explored by the protocol team (example: E&K Scientific Products, Inc. Cat # 649020-A for tubes and 449010-A for caps; tubes are certified DNase, RNase and pyrogen-free).

7.2.1.2 Cryogenic labels suitable for -80°C and LN2 temperatures and resistant to exposure to laboratory reagents such as ethanol (example Shamrock Labels Cat # ACTG-WAPC-1).

Note: All specimens must be labeled using the laboratory data management system (LDMS). Pre- and post-collection weight must be done using the same, labeled microtube. Labeled cryovials should be prepared by the laboratory and made available during specimen collection.

7.2.1.3 Appropriate PPE (gloves, gown/laboratory coat, goggles/safety glasses)

7.2.1.4 Dissecting Forceps, straight, nickel (example: Fisher Scientific Cat # 08-880)

7.2.1.5 Parafilm

7.2.1.6 Micropipettors and disposable tips with filter barriers

7.2.1.7 Serologic or volumetric pipets and laboratory ware for preparing reagents

7.2.1.8 Class II Biological Safety Cabinets (BSC) maintained per Good Clinical Laboratory Practice (GCLP) guidelines

7.2.1.9 2-8°C Refrigerator - maintained per GCLP guidelines

7.2.1.10 -80°C Freezer – maintained per GCLP guidelines

7.2.1.11 LN2 Freezer, if required – maintained per GCLP guidelines

7.2.1.12 Protocol-defined shipping supplies including supplies for transporting specimens to local processing laboratory

7.2.2 Additional Consumables if Transporting samples to Processing Laboratory for Fresh Cell Recovery or Subsequent Processing

7.2.2.1 50 mL Polypropylene Conical Sterile Centrifuge Tubes (example: Thermo Scientific Cat # 339652)

7.2.3 Additional Consumables for Snap Freezing – allow for analysis of whole biopsy pieces

7.2.3.1 Container for dry ice/ethanol bath (example: VWR Cat # 89198-950 [bucket], 89198-986 [floating rack])

7.2.3.2 Dry Ice pellets

7.2.3.3 PK Samples: Analytical Balance (example: Mettler Toledo Cat # MS204S) - calibrated and maintained per GCLP guidelines; accurate to 1mg. Pre- and Post-collection weight must be documented on the appropriate case report form (CRF) prior to snap freezing and sent with the samples.

Note: most laboratories will not have access to an analytical balance – so this process will need to be defined at the laboratory/site level.

7.2.4 Additional Consumables for Paraformaldehyde (or Formalin) Fixation – to permit histopathology

Note: Paraformaldehyde should be used if the samples are being stored for purposes of *in situ* hybridization. Formalin may be used for standard histological staining procedures (e.g. H&E).

7.2.4.1 50 mL Polypropylene Conical Sterile Centrifuge Tubes (example: Thermo Scientific Cat # 339652) if transporting to processing laboratory.

7.2.4.2 Tissue embedding cartridge (If applicable, product may be supplied by the team in "kits")

7.2.5 Additional Consumables for Optimal Cutting Temperature (OCT) Freezing – used for histopathology studies. Optional Site Laboratory Processing - Protocol dependent, may be deferred to central laboratory if deemed appropriate.

7.2.5.1 Tissue embedding cartridge (If applicable, product may be supplied by the team in "kits")

7.2.5.2 Container for dry ice/ethanol bath (refer to 7.3.3.1)

7.2.5.3 Dry ice pellets

8 Reagents and Reagent Preparation

Reagents for local laboratory processing will vary with protocol intent for the biopsies. Reagents are listed here to cover many potential uses for the samples. The protocol LPC will indicate the necessary reagents and processes required for the protocol. Some required reagents may be supplied by the ACTG Protocol Team and/or Central Supply. Refer to Appendix A for a list of example reagents and consumables.

8.1 Reagents for the transport of samples for viable cells:

8.1.1 Sterile 25mL Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium (Ca^{++} and Mg^{++} Free) or equivalent, contained in 1 x 50mL sterile conical polypropylene tube (example: Cellgro Cat# 21-031-CV)

8.1.2 RPMI-1640 may be supplemented with 10-15% fetal bovine serum (FBS) and antibiotics per protocol. Medium may be prepared ahead of time and stored at 2-8°C for 2-4 weeks.

8.2 Reagents for snap freeze process:

8.2.1 200 Proof Ethanol (anhydrous alcohol; example: VWR Cat # IB15720) – for use in creating dry ice/ethanol bath

8.3 Reagents for Formalin and Paraformaldehyde fixation.

Note: 10% neutral buffered formalin is a 1:10 dilution of 37-40% formaldehyde (final concentration 3.7 – 4% formaldehyde) in water. This is used for clinical pathology specimens but is less optimal for in situ hybridization analyses.

Ideally, freshly prepared paraformaldehyde solution should be made rather than using commercially available formalin. Making fresh paraformaldehyde from powder is not recommended because it is highly carcinogenic. Preparation and use of paraformaldehyde solutions carry significant risk. All work with paraformaldehyde must be performed in a fume hood. Preparers and users should be thoroughly familiar with all Safety Data Sheet information, particularly handling, storage, and disposal recommendations prior to using this

chemical. In addition, all appropriate site institutional training, regulations and procedures for handling should be followed carefully. Communication with institutional safety personnel is encouraged.

8.3.1 4% Paraformaldehyde (PFA) - Prepare from 16% Paraformaldehyde Stock Reagent (example: MP Biomedicals Cat # 0219998320) with DPBS (refer to 8.1.1).

Note: 4% PFA ampules may be purchased (example: Electron Microscopy Systems Cat # 157-4) and provided by the protocol to prevent reagent spoilage between biopsies.

Note: the paraformaldehyde solution should ideally be made fresh the morning of the biopsy.

8.3.2 10% Neutral Buffered Formalin (contains 3.7 – 4% Formaldehyde; available in pre-filled containers)

Note: Paraformaldehyde should be used if the samples are being stored for purposes of *in situ* hybridization. Formalin may be used for standard histological staining procedures (e.g. H&E).

8.3.3 80% Ethanol Molecular Biology grade - Prepare using 100% Ethanol (200 Proof, refer to 8.2.1) with Molecular Biology grade water (example: Fisher Scientific Cat # BP2819-1)

8.4 Reagents for OCT Freezing Solution

8.4.1 200 Proof Ethanol (anhydrous alcohol – refer to 8.2.1)

8.5 Reagents for RNA*later*® samples:

8.5.1 Ambion RNA*later*® (example: Qiagen Cat # 76163, which comes in pre-aliquoted tubes).

8.5.2 Ambion RNA*later*® ICE (example: Life Technologies Cat # AM7030M)

9 Tissue Sampling Procedure

Biopsies should be conducted in the morning. This allows ample time for same-day sample processing and shipping.

9.1 Preferred environment: CTSI/GCRC (Clinical Translational Science Institute/ General Clinical Research Center) Environment

9.2 Required staff

9.2.1 One (1) surgeon or other qualified provider

9.2.2 If necessary, one pathologist or pathology technologist or other surgical assistant to section tissue

9.2.3 The study coordinator should be present to ensure accurate protocol-specific documentation and to assist the surgeon with non-procedural tasks (for example, call the lab or supply room in the event of supply needs during the procedure)

9.3 Participant preparation

9.3.1 No aspirin and/or non-steroidal anti-inflammatory drug (NSAIDs) for a minimum of 5 days before the procedure

9.3.2 No clopidrogel (e.g. Plavix®), warfarin (e.g. Coumadin®), or other blood thinners for 5 days before the procedure (or longer if indicated in the protocol)

Note: these medications should not be stopped without documented approval from the participant's prescribing physician.

9.4 Tissue Biopsy Procedure

9.4.1 Pre-sampling procedures

9.4.1.1 A physical examination of the area to be biopsied will occur within four weeks of the anticipated date of biopsy.

9.4.1.2 No aspirin, NSAIDs, Plavix, Coumadin, or other blood thinners for 5 days before the procedure (refer to the *Note* in 9.3.2).

9.4.1.3 Biopsies should be conducted in the morning to allow for same-day sample processing and shipping of fresh samples.

9.4.1.4 Check protocol to determine if fasting is required.

9.4.2 Procedure description

9.4.2.1 No intravenous line is required.

9.4.2.2 A general medical evaluation including determination of blood pressure, temperature, and heart rate will be determined prior to the start of procedure.

9.4.2.3 The biopsy will be done under local anesthesia by an experienced provider.

9.4.2.4 First, the biopsy area will be cleaned with an antiseptic solution of the surgeon's choosing. Local anesthesia will be used to numb the area prior to the biopsy. The surgeon/provider performing the procedure will close the wound with material of their choosing; this may include dissolvable or non-dissolvable sutures, Steristrips and/or Dermabond. If needed, sutures will be removed in follow-up by the site or surgeon, depending upon local preference. Steristrips should remain in place until they fall off on their own, which usually takes 1-2 weeks (instruct participants not to try to peel them off).

9.4.2.5 Place biopsy pieces into relevant media within 10 minutes of extraction (see below).

9.4.3 Post-sampling procedures

9.4.3.1 No aspirin, NSAIDs, Plavix, Coumadin, or other blood thinners for 5 days after the procedure (refer to the *Note* in 9.3.2).

9.4.3.2 At the completion of the procedure, participants will remain for a length of time to be determined by the clinician performing the biopsy and will be monitored for bleeding and stable vital signs prior to discharge.

9.4.3.3 The clinician performing the procedure will discuss restrictions for post-procedure activities with the participant and provide a list of the restrictions in writing.

9.4.3.4 Within 7 days after the biopsy, participants will return to the clinic to have a brief examination and be assessed for pain, drainage from the wound, discomfort or other complications. Participants should be instructed to notify the study team if any issues of concern regarding the wound arise prior to their scheduled wound check.

10 Sample Transport

10.1 It is recommended that all sample processing be completed within 10 minutes of collection when possible (the protocol must specify the exact processing timing constraints). If feasible, the laboratory shall supply a sterile conical tube containing an amount of transport medium defined by the protocol (DPBS, RPMI, etc.) for fresh processing, and a number of cryovials (one cryovial for each specimen to be snap frozen, or stored in RNA*later*®, formalin or paraformaldehyde), as defined by the protocol. PK specimen cryovials require pre-sample weight and post-sample weight, with or without transport media. Cryovial pre-collection weight must be documented prior to the collection and each individual vial must be uniquely linked to a pre-collection weight. Ideally, post-collection weight should be done prior to snap freezing, however, if this is not feasible, then the cold-chain must be adhered to for post-collection weighing. Any ice or liquid on the vial should be removed prior to weighing; and weighing must be done quickly to avoid sample thawing.

Note: Proper media, handling, and transport of samples are critical to specimen integrity and downstream processing. The number of biopsy pieces obtained will be dependent on study objectives and must be specified in the protocol-specific documents. The protocol will specify the number of biopsies to be placed in each cryovial which may contain a protocol-specific medium or reagent.

10.2 The adipose tissue biopsies may need to be sent to a processing laboratory as defined in the protocol.

10.2.1 Properly label the specimens with the patient identification (PTID), protocol, visit identification (VID) and date of collection.

10.2.2 Document the collection and transport of the specimens on the corresponding CRF. Send copies of appropriate CRFs with specimens to the processing laboratory.

10.2.3 If samples have been processed (i.e. snap frozen) then the cold chain must be maintained during transport to the processing laboratory. Snap frozen tissue should be transported on dry ice – do not place dry ice pellets in a sealed container.

10.2.4 All samples must be transported according to local regulations. Secondary packaging must be used to prevent leakage during transport. Securing lids with parafilm will help to prevent caps from loosening during transport.

11 Processing

All specimens must be treated as potentially biohazardous materials. Proper PPE must always be used for all specimen handling. The use of chemicals such as paraformaldehyde, formalin and RNA*later*® and dry ice baths require that additional precautions be followed including the use of fume hoods and the use of insulated gloves when handling dry ice.

11.1 Documentation

11.1.1 Collection time must be documented on paperwork accompanying the samples. The laboratory will document the time each biopsy is transferred to the protocol-specified reagents and/or conditions. If any protocol-specific data are required, such as weighing of the sample or conditions of the sample, the laboratory must document as required.

Note: individual vials must be uniquely labeled for pre- and post-collection weighing.

11.2 Labeling

Generate cryovial labels using the Laboratory Data Management System (LDMS).

11.2.1 Follow protocol requirements for completing the data entry.

11.2.2 Proof each derivative type of cryovial label for data entry errors against the CRF PRIOR to labeling cryovial.

11.2.3 Visually inspect the label barcode and print area for alignment, and print quality.

11.2.4 Correct any data entry errors in LDMS and re-print labels as needed (making sure the appropriate global ID's are selected).

11.2.5 Apply the labels on the cryovials so that the information can be easily read and the contents of the tube can be clearly seen (it may not be possible to visualize specimens in amber microtubes).

11.3 Fresh Sample Processing

11.3.1 Refer to the protocol for how to process, store and transport specimens.

11.4 Snap Freezing

Care must be taken to avoid splashing the cold alcohol bath; do not handle dry ice without using appropriate thermal gloves. Never seal dry ice in a closed container. Snap freezing provides excellent specimen integrity and a wild array of options for tissue analysis.

11.4.1 Samples must be frozen as quickly as possible (immediately is preferred; up to within a maximum of 10 minutes of collection). If samples are transported to the processing laboratory frozen, on dry ice, and require post-collection weighing (refer to 10.1). If samples have already been weighed after collection, move to step 11.4.2.

11.4.2 Combine dry ice and 200-proof ethanol in a container (refer to 7.3.3.1) to make a bath for snap freezing tissues.

11.4.3 Using sterile forceps, transfer designated number of biopsy pieces into appropriately labeled and pre-weighed cryovials, one piece per vial, unless otherwise specified in the protocol or LPC. Take care to ensure that biopsy is at the bottom of the vial.

11.4.4 Immerse cryovials containing biopsies in ethanol ice bath for two minutes. If vial is hand-labeled with an indelible marker, protect the label by covering it in a material that is not corroded by ethanol.

Note: all specimens should be ultimately labeled with LDMS-generated labels.

11.4.5 Immediately store the specimens at -70°C or colder per the protocol instructions.

11.5 Paraformaldehyde and/or Formalin Fixation

Paraformaldehyde and formalin are toxic to the skin and respiratory tract must be handled in a chemical fume hood using appropriate PPE.

11.5.1 Using sterile forceps, transfer designated number of biopsy pieces into cryovial tubes, one piece per tube, taking care to ensure that the biopsy is at the bottom of the tube. Prepare the reagents and process the specimens as outlined in the LPC or manual of procedures (MOPS)

Note: for paraformaldehyde, the minimum volume:tissue ratio is 10:1; for formalin, the minimum volume:tissue ratio is 15:1.

11.5.2 Secure cryovial lids tightly and seal tops with parafilm to prevent leakage in transit.

11.5.3 Ship samples via overnight courier to the specified testing laboratory (refer to section 12 for shipping details).

Note: PFA samples may be transferred to ethanol prior to shipping; formalin fixed samples will not be transferred to ethanol.

11.6 OCT Frozen Sample

Tissue embedded in OCT compound followed by snap freezing not only preserves DNA, RNA and protein integrity, but also allows for section of the frozen tissue. Site specimen processing laboratories may not have the capacity to perform this processing unless the protocol team provides a processing kit and specific instructions. These samples (in DPBS) may be shipped

overnight at 2-8°C to a protocol-designated immunology specialty laboratory (ISL) or virology specialty laboratory (VSL) for processing of blocks and/or slides.

11.6.1 Fill each cryomold with OCT by slowly and carefully filling the mold to the top. It is important to avoid the formation of air bubbles and to ensure that the top surface of the OCT compound is completely level (avoid uneven surfaces).

11.6.2 Using sterile forceps or needle, transfer the specimen to the OCT-filled cryomold and gently submerge the tissue into the medium until it is completely covered. None of the tissue should remain exposed.

11.6.3 The OCT can be hardened by holding the cryomold with forceps over the dry ice/ethanol bath (refer to 11.5.2). Once the OCT has hardened, place the mold into a pre-labeled specimen bag.

11.6.4 Freeze the specimens per protocol instructions.

11.7 RNA*later*® Samples

Note: RNA*later*®-Ice may be used to stabilize snap frozen tissue as it is thawed. Refer to protocol for specific processing details.

11.7.1 Using sterile forceps, transfer designated number of biopsy pieces into cryovials, one piece per vial, taking care to ensure that biopsy is at the bottom of the tube.

11.7.2 Add a sufficient volume of RNA*later*® to each cryovial to achieve a 10X greater volume of solution to tissue and store at a protocol defined temperature.

11.7.3 Samples can be stored at 2-8°C for one month, 25°C for one week, or -20°C indefinitely per manufacturer's recommendation or as specified by the protocol or LPC.

12 Real-Time Sample Shipping

12.1 Packaging and shipping materials for ambient/refrigerated shipments

12.1.1 Secondary containment (example: SafTPak Cat # STP-710): Includes an inner leak-proof bag, Tyvek envelope, and absorbent material (example: SafTPak STP-151)

12.1.2 Insulated category B shipper (example: SafTPak Cat # STP-309SYS or 309DI)

Note: Individual components of a given shipping system are designed, tested and certified to be used as specified by the vendor. DO NOT mix and match individual components from different systems.

12.1.3 0°C Gel Packs (example: SafTPak, Cat # STP-400), refrigerated (2-8°C, refrigerated shipments only).

12.2 Packaging of biopsy specimens for ambient/refrigerated shipments

Note: Specific packaging and shipping procedures must be followed in accordance with the US Department of Transportation and ICAO regulations. ACTG/IMPAACT Shipping Guidelines may be found at the following websites:

https://www.hanc.info/labs/labresources/procedures/Pages/actnShippingDemo.aspx

https://member.actgnetwork.org/labs#profile=1

12.2.1 Perform initial processing as described in this SOP and protocol-specific documents.

12.2.2 Ensure that all specimens are properly logged and labeled according to LDMS conventions.

12.2.3 Wrap the caps of all tubes and cryovials with parafilm prior to packaging for shipment.

12.2.4 Place tubes into STP-710 leak-proof bags as follows:

12.2.4.1 One 50mL (or 15mL) conical tube per bag with appropriate absorbent material

12.2.4.2 RNA*later*® cryovials in one bag with appropriate absorbent material

12.2.4.3 Paraformaldehyde or formalin-containing cryovials in one bag with appropriate absorbent material.

Note: formalin-fixed samples must be shipped ambient; if other specimens require refrigeration then they will have to be shipped in separate shipments.

12.2.4.4 Place all of the above sealed bags into one Tyvek envelope and seal. Place the Tyvek envelopes into a fiberboard inner box to help secure them and to protect them from being cracked by the refrigerated gel packs in transit.

12.2.4.5 If applicable, place 4-8 refrigerated gel packs around the inner box with an insulated shipping chest (e.g., STP309-DI or equivalent) based on the size of the gel packs and the packaging used.

Note: do not use gel packs for ambient shipments.

12.2.4.6 Fill any extra space with packing material to prevent shifting. Controlled room temperature thermal packs or other insulation material may be used when outdoor temperatures are expected to be particularly cold. Extra refrigerated gel packs may be necessary if outdoor temperatures are expected to be particularly warm.

12.2.4.7 Insert a shipping manifest and return air waybill into a plastic sleeve and place on top of gel packs prior to closing and sealing box with packing tape.

12.2.4.8 Follow all ACTG/IMPAACT shipping protocols and any protocol-specific instructions for receiving laboratory notifications and tracking of specimens.

12.2.4.9 Ship overnight for next-day receipt, unless the protocol specifies otherwise. Protocol-specific shipping addresses and information can be found in the protocol Laboratory Processing Chart .

12.3 Packaging of biopsy specimens for frozen shipments

Note: Specific packaging and shipping procedures must be followed in accordance with the US Department of Transportation and ICAO regulations. ACTG/IMPAACT Shipping Guidelines may be found at the following websites:

https://www.hanc.info/labs/labresources/procedures/Pages/actnShippingDemo.aspx

https://member.actgnetwork.org/labs#profile=1

12.3.1 Perform initial processing as described in this SOP and protocol-specific documents.

12.3.2 Ensure that all specimens are properly logged and labeled according to LDMS conventions.

12.3.3 Wrap the caps of all tubes and cryovials with parafilm prior to packaging for shipment.

12.3.4 Place tubes into STP-710 leak-proof bags as follows:

12.3.4.1 Snap frozen cryovials in one bag with appropriate absorbent material

12.3.4.2 Place into one Tyvek envelope and seal. Place the sealed tyvek bag into the inner box.

12.3.4.3 Fill the packaging with dry ice pellets, ensuring the inner box is secure.

12.3.4.4 Insert a shipping manifest and return air waybill into a plastic sleeve and place on top of Styrofoam lid prior to closing and sealing box with packing tape.

12.3.4.5 Follow all ACTG/IMPAACT shipping protocols and any protocol-specific instructions for receiving laboratory notifications and tracking of specimens.

12.3.4.6 Ship overnight for next-day receipt. Protocol-specific shipping addresses and information can be found in the protocol Laboratory Processing Chart .

13 Forms

13.1 Protocols must specify if a separate Adipose tissue biopsy informed Consent Form (ICF) is required or if the procedure will be outlined in the main ICF and the subject will sign a minor procedure consent form at the time of the procedure.

14 Literature References

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15 Acknowledgments

15.1 Jordan E. Lake, MD, MSc and Netanya G. Sandler, MD and the A5317 protocol.

16 Appendices

16.1 Appendix A: Example Reagents and Supplies

16.2 Appendix B: Sample Donor Consent Form

16.3 Appendix C: Sample Adipose Tissue Biopsy Information Sheet

Appendix A: Example Reagents and Supplies

Reagent/Supply	Example(s)
10% neutral Buffered Formalin	Sigma Aldrich Cat# HT 5011 or equivalent
200 Proof Ethanol	VWR Cat# IB15720 or equivalent
4-16% Paraformaldehyde	MP Biomedicals Cat # 0219998320 (16%) or equivalent Electron Microscopy Systems Cat # 157-4 (4%) or equivalent
Amber Cryogenic Vials	E&K Scientific Products, Inc. Cat # 649020-A for tubes and 449010-A for caps; tubes are certified DNase, RNase and pyrogen-free, or Analytical Sales Cat# 16401A - Cat# 16501A - 2.0mL Amber Self-standing MicroTube with TFE O-ring for leak-proof seal; Cat# 16561 - Amber Caps with O-ring, or equivalent
Analytical Balance	Mettler Toledo Cat# MS204S, or equivalent
Cryogenic labels	Cryo-Tags® and Cryo-Babies® Brady B461 or B490, or Shamrock freezer labels # ACTG-WAPC-1, or equivalent
Cryovials	Corning® 2mL external thread polypropylene cryogenic vial, self-standing with round bottom #430659, WHEATON Cryule® Plastic Cryogenic Vials, external thread, #985742, or SARSTEDT Screw cap micro tube, external thread #72.694.006, or equivalent
Dissecting Forceps, straight, nickel	Fisher Scientific Cat# 08-880, or equivalent
Dulbecco's Phosphate Buffered Saline (DPBS)	Cellgro Cat# 21-031-CV, or equivalent
Marking pens	Fisher Scientific Fisherbrand Marking Pens cat#13-379, or Nalgene® Lab Pen/Lab Marker #6310/#6311, or equivalent
Molecular Grade Water	Fisher Scientific Cat # BP2819-1 or equivalent
RNAlater®	Invitrogen Cat# AM7020 (bulk) Qiagen Cat # 76163 (pre-aliquoted)
Shipping materials	SafTPak Secondary containment (STP-710 or equivalent) STP-151 absorbent material or equivalent category B shipper (STP-309DI, STP-320 or equivalent) 0^{o}C Gel Packs (STP-400 or equivalent) Dry ice
Single-use Radial Jaw 3 maximum capacity with needle and 3.7mm minimum working channel	Boston Scientific Cat # M0051589), or equivalent

Appendix B: Sample Donor Consent Form

APPENDIX I SAMPLE INFORMED CONSENT

DIVISION OF AIDS

AIDS CLINICAL TRIALS GROUP (ACTG)

SAMPLE INFORMED CONSENT

For protocol **[xxxxx]**

[INSERT PROTOCOL TITLE]

SHORT TITLE FOR THE STUDY: **[INSERT SHORT TITLE]**

INTRODUCTION

You are being asked to take part in this research study because you are **[INSERT TARGETED POPULATION]**. This study is sponsored by the National Institutes of Health (NIH). The doctor in charge of this study at this site is: **[insert name of Principal Investigator]**. Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

[INSERT STUDY RATIONALE]

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

This study is **[INSERT STUDY DURATION AND # OF VISITS].**

If you decide to join the study, you will be **[INSERT STUDY DESIGN FOR RANDOMIZATION].**

Your study nurse or doctor will explain the study to you, and ask if you have any questions. Please ask your study nurse or doctor to explain anything that you do not fully understand. After learning about this study, if you wish to participate you will be asked to sign this consent form. After you have signed this form, if you wish to take part in this study, you will be asked some questions and will undergo some tests to see if you qualify for the study. Discussing the study and completing the tests will take about 60 minutes.

If you decide to take part in this study, you will be asked to undergo an adipose tissue biopsy **[INSERT VISIT TIME POINTS]**. The adipose tissue biopsy will not affect your ability to fight infection. If you do not want to have the biopsies, you will not be able to take part in the study. The biopsies are explained in more detail below.

[**Define the results that**] will be returned to you after the study is over. Although we do not yet know whether the results obtained from these biopsies will be helpful in guiding your medical care, we want you to have the results available to you and your doctor.

The results of other information obtained during this study will be provided to you in a summary of study results, but you, the study staff, and your doctors will not receive your specific results. This is because we do not yet know whether these tests are useful in guiding the medical treatment of people with HIV or other medical conditions caused by inflammation, and these blood tests results should not be used to change your medical treatment during or after the study.

At Screening

[INSERT SCREENING VISIT REQUIREMENTS]

If you do not enroll into the study

If you decide not to take part in this study or if you do not meet the eligibility requirements, we will still use some of your information. As part of this screening visit, some demographic (for example, age, gender, race), clinical (for example, medications you make take and medical problems you may have), and laboratory (for example, CD4+ T cell count, HIV viral load) information is being collected from you so that ACTG researchers may help determine whether there are patterns or common reasons why people do not join a study.

1st Entry Visit

If you meet all the requirements and choose to participate in the study, you will come to the clinic within **[XX]** days after the screening visit for the 1st of two entry evaluations. The 1st entry visit will last about half a day and must occur within X days before the 2nd entry visit (the visit where you will be assigned to the study medication group or the control (no study medication) group).

- You will have the biopsy described below.

Biopsies

A biopsy is a procedure in which a sample of tissue will be taken from your body. The biopsies will likely be done in your XX (insert protocol-specific anatomic site) area (the XX part of the body) unless your doctor has another preference. You will have this done XX times – once at the beginning of the study and XX. The biopsies will be performed by a licensed physician. The skin above the adipose tissue will be numbed with local anesthetic (pain medication), an incision (cut) approximately 1-2 inches long will be made, and adipose tissue will be surgically removed. If possible about XX teaspoon of fat will be removed. If you don't have enough fat in that area, the doctor might have to make a second cut to obtain the fat. If the doctor needs to make a second cut, he/she will tell you first, and you will get more numbing medication. By signing this consent form, you are agreeing to have adipose collections at weeks xx and yy per the study requirements.

Because the risk of infection with these biopsies is low (<2%), taking antibiotics before the biopsy to prevent infection is not indicated. Therefore, if you request and/or are given a prescription for an antibiotic to take before the procedure, you will be responsible for paying for the antibiotic. Because the antibiotic is optional, the cost of it is not covered by the study.

After the biopsies, the incision site(s) will be closed by stitches or glue (depending on what the physician performing the procedure recommends). If you have stitches, they will remain in your skin for about 5-10 days. Depending on the type of stitch used, they will either be removed by your regular doctor, the doctor who performed the procedure, or the study staff. If a type of suture that absorbs into your skin was used, then the sutures will not require removal. You will be informed of which type of suture you have.

After being observed for a few hours (the exact amount of time will depend on how long the physician performing the biopsy thinks is safest for you), you will be allowed to go home. You will be asked to rest (no strenuous activity) until the next morning. You will also be told what else you should do (or avoid) to help the biopsy site heal well, and be provided with a list of these recommendations. You and the doctor will discuss what medicines to take for pain in case you need them, and how to keep your incision clean. While the biopsy itself only takes about 15-30 minutes, the entire procedure, including the time you spend getting ready for the biopsy and resting afterwards, could take up to half a day. However, it might be shorter.

2nd Entry Visit

If you have met all the requirements to take part in the study (including having the biopsies done), you will come to the clinic for the 2nd entry visit. This 2nd entry visit will take about 60 minutes. You will have your 2nd entry visit within **[X]** days after the biopsy. The study doctor will check to make sure your incision is healing well, and will ask you questions about pain, drainage from the wound, or discomfort. If you have any questions or concerns about the incision before the 2nd entry visit, you should notify the study staff so that you can have your incision examined. If there is any sign of infection you will be followed closely, be given wound care instructions, and possibly given antibiotics or another appropriate treatment. Treatment prescribed as a result of a biopsy side effect will be paid for according to local guidelines. The study staff will discuss these guidelines with you.

- **[INSERT VISIT REQUIREMENTS]**

At the 2nd entry visit, you will be assigned randomly (by chance) to **[INSERT STUDY DESIGN FOR RANDOMIZATION].**

If you were unable to provide **[INSERT ANY ELIGIBILITY CRITERIA OR RESTRICTIONS THAT COULD INHIBIT ENROLLMENT]**

On-Study Evaluations after 2nd Entry Visit

You will come to the clinic for post-entry visits at weeks **[INSERT VISIT SCHEDULE]**.

You will have the following evaluations done at most of these visits, and if you have to stop taking part in the study.

- **[INSERT STUDY REQUIRMENTS]**

At the final visit (week XX) [**or as outlined in the protocol**], the biopsy procedure (see above for details) will be repeated for **[INSERT APPROPRIATE GROUP]**. After the XX biopsy, there are no more required study visits, but you should let the study team know if you are having any side effects from the biopsy and/or if you think the wound is not healing well. The study team will continue to follow you, if needed, until any issues related to the study resolve.

Extra study visits may be required if **[INSERT IF NECESSARY]**

IF YOU DECIDE TO STOP THE STUDY DRUG OR THE STUDY

If you stop taking the study medication before the end of the study, you will still be asked to complete the rest of the study visits and evaluations, including the biopsy.

If you stop taking part in the study before the end of the study, you will come into the clinic and have most of the week **[XX]** visit evaluations. If you have taken the study drug for at least **[XX]** weeks, you will be asked to have your final adipose tissue biopsies before stopping the study.

At the end of the study, you and your doctor will receive a summary of the study's results, in addition to your biopsy results (as defined above). You should discuss with your doctor whether the results have any relevance to your clinical care.

Other

Some of your biopsy samples will be stored and used for immunologic and virologic testing that is required for this study.

If you agree, some of your biopsy samples that are left over after all required study testing is done may be stored and used for future research that is not yet planned. These samples will be kept indefinitely and will not identify you by name. You do not have to give permission for storage of these samples and you may withdraw your permission at any time. This will not affect your participation in the study. Initial and check below whether you agree or disagree to have your leftover samples stored for future research.

_____ YES _____ NO

[INSERT ANY KNOWN RISKS ASSOCIATED WITH THE STUDY DRUG]

Risks of Biopsies

The primary risks associated with biopsies are bleeding, pain, and infection. Rarely, damage to tissues, nerves, or blood vessels may occur. The anesthetic (pain medication) injection may be accompanied by mild discomfort, and, rarely, patients may have an allergic reaction to the local anesthetic. There is no good evidence that antibiotics will prevent infection in people getting adipose tissue biopsies, but some doctors may prefer to prescribe them. Antibiotics can sometimes also cause diarrhea. This diarrhea may be due to the antibiotics themselves, or, rarely, to the overgrowth of a gut bacteria called *Clostridium difficile*. Diarrhea from *Clostridium difficile* overgrowth requires additional antibiotic therapy. Because any antibiotics taken at the time of biopsy are optional and not required by the study, the study will also not pay for the antibiotics or for treatment of diarrhea due to *Clostridium difficile* overgrowth. If you take antibiotics at the time of your biopsy and get diarrhea, you should see your primary care doctor so he/she can determine the cause and what treatment (if any) is best for you.

Mild discomfort may occur at the site of the fat biopsy for a few days, and a small scar may remain at the site of the fat biopsy. Medications to help control the pain associated with the procedures can be prescribed for you but will not be provided free of charge (During the procedure, you will be given pain medication, and you may be given a prescription for additional pain medication).

The biopsy procedure may cause pain, even though you have been given an anesthetic. There may be bleeding associated with the procedure. The risk of infection is <2 percent. There is the possibility you might develop a seroma, which is a collection of fluid under the skin and around the incision. It is also possible that you may experience bruising of the skin around the incision. Lastly, sometimes, when we sufficient adipose tissue cannot be found from the location in which case, you will be asked if you are willing to do the procedure again. If you are not, you will be unable to continue in the study.

ARE THERE RISKS RELATED TO PREGNANCY?

If you become pregnant while on study, you will be asked to continue in the study and still have the evaluations done, but you will not be asked to have the week XX biopsies (if obtained from the abdomen). Additionally, the study staff would like to obtain information from you about the outcome of the pregnancy (even if it is after your participation in the study ends).

If you are taking HIV drugs when you become pregnant, your pregnancy will be reported to an international database that collects information about pregnancies in women taking HIV drugs. This report will not use your name or other information that could be used to identify you.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

If you take part in this study, there may be a direct benefit to you, but no guarantee can be made. It is also possible that you may receive no benefit from being in this study. Information learned from this study may help others who have HIV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:

- treatment with prescription drugs available to you
- treatment with experimental drugs, if you qualify
- no treatment

Please talk to your doctor about these and other choices available to you. Your doctor will explain the risks and benefits of these choices.

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the U.S. Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Also, any publication of this study will not use your name or identify you personally.

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

Your records may be reviewed by the ACTG, Office for Human Research Protection, **[insert name of site]** institutional review board, National Institutes of Health (NIH), other government agencies, study staff, and study monitors.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by U.S. law. This Web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

WHAT ARE THE COSTS TO ME?

Taking part in this study may lead to added costs to you and your insurance company. In some cases it is possible that your insurance company will not pay for these costs because you are taking part in a research study.

WILL I RECEIVE ANY PAYMENT?

You will receive compensation to help cover the costs of missing work. You will receive **(*site to insert site-specific compensation amounts*)** for all visits, plus (***site to insert site-specific compensation amounts***) for the additional time and burden of undergoing a biopsy. You will not be compensated for visits and procedures you do not complete. The maximum total compensation for this study is **(*site to insert site-specific compensation amounts*).** You will not receive final compensation until the second biopsy has been performed.

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. The cost for this treatment will be charged to you or your insurance company, or managed as per local institutional policy. There is no program for compensation through the National Institutes of Health. You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH or your clinic and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- name of the investigator or other study staff
- telephone number of above

For questions about your rights as a research subject, contact:

- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site. The IRB is a committee that watches over the safety and rights of research participants.
  - telephone number of above

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Participant's Legal Representative (print) (As appropriate)	Legal Representative's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date

Appendix C: Sample Adipose Tissue Biopsy Information Sheet

What Should Participants, undergoing an Adipose Tissue Biopsy Expect?

Immediately after the biopsy

How you feel after the biopsy depends on what sort of procedure was performed. Generally, you can expect after a biopsy:

- Some pain around the biopsy site. This should resolve, or at least ease over the next two or three days.
- Your doctor will prescribe appropriate pain relieving medication.
- Nurses monitor your condition for some hours and check for bleeding from the biopsy site.
- You may have a blood count test to double-check that you are not bleeding internally from the biopsy site.
- You may undergo other tests to make sure all is well: for example, a chest x-ray if you had a lung biopsy.
- Surgical medications can sometimes make you drowsy, so you should not drive yourself home. Ask a friend or relative to pick you up or take a taxi.

Potential Complications from a biopsy

- Depending on the biopsy procedure, possible complications include:
- Excessive bleeding (hemorrhage)
- Infection
- Puncture damage to nearby tissue or organs
- Skin numbness around the biopsy site.

Taking care of yourself at home

- Be guided by your doctor or surgeon, but general suggestions include:
- Rest as much as you can in the next day or two.
- Limit using the body part, if this is possible. For example, if you had a biopsy performed on your arm or leg, try to rest and raise the limb for the next day or so.
- Avoid vigorous exercise and lifting heavy objects.
- You may need to keep your wound (and its dressing) dry for around one week to 10 days. If possible, hold the affected area out of the shower or bath, or cover the dressing in plastic wrap. Alternatively, it may be easier to sponge bath for the first few days.
- Dressings can usually be removed one week to 10 days after the procedure.
- Long-term outlook