


<b>Title:</b>	Lymph Node Biopsy Standard Operating Procedure		
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## **1 Purpose**

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

## **2 Scope**

Users of the ACTG/IMPAACT Lab Manual.

## **3 Background**

A majority of the body's lymphocytes exist in the lymphoid tissues of the lymph nodes (LN) and gut-associated lymphoid tissues (GALT). Removing samples from these sites provide information for HIV pathogenesis and persistence that cannot be obtained from peripheral blood. Chun et al showed that HIV persists in the LN even in patients on highly active antiretroviral therapy ([14.1](#)). Moreover, the HIV-specific immune responses in the LN may differ from that in the peripheral blood ([14.2](#)). The LN biopsy may be performed in order to quantify and characterize residual virus in lymph nodes, or to quantify and compare lymph node architecture in untreated subjects, HAART-suppressed subjects, elite controllers, and HIV-negative subjects. Biopsy of the inguinal LN is preferred over other biopsies (e.g. cervical, axillary) because it is less invasive and produces fewer complications. In most cases, the inguinal LN biopsy may be performed in an outpatient setting in about 1 hour under local anesthesia and provide an excellent, safe source of lymphoid tissue ([14.3](#)).

## **4 Authority and Responsibility**

- 4.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.
- 4.2 The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.
- 4.3 The Laboratory Director is responsible for the implementation of this LTC SOP or laboratory-specific SOP and for ensuring that all appropriate personnel are trained. A laboratory SOP must:
  - 4.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory
  - 4.3.2 Reference the current version of the LTC SOP
- 4.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described.
- 4.5 The site PI and designees are responsible for understanding and adhering to the patient preparation and specimen collection components.

## **5 Budgetary Considerations**

- 5.1 Room/facility fees

- 5.2 Salary support for surgeon and assistant
- 5.3 Procedural supplies
- 5.4 Subject reimbursement (~\$150 per LN biopsy)
- 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 LN biopsies, there should be a minimum interval of 4 weeks between LN biopsy time points if performing on the same side (left/right). A maximum of 2 LN biopsies should be performed per year.

### 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 Body mass index (BMI) 20-35 kg/m<sup>2</sup>.

Note: BMI (weight in kg/height<sup>2</sup> in m<sup>2</sup>) should be calculated by the site at screening to ensure eligibility. Thereafter, BMI will be calculated during data analysis using the height and weight data entered into the database by the site.

- 6.1.4 Willingness to undergo the Step 1 entry and week 48 lymphoid tissue biopsies.

### 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 Blood coagulation disorder (including bleeding tendency or problems in past with blood clots)
- 6.2.2 Pregnant
- 6.2.3 The following laboratory values obtained within 30 days prior to study entry by any US laboratory that has a CLIA certification or its equivalent:
  - Absolute neutrophil count (ANC)  $\leq 750$  cells/mm<sup>3</sup>
  - Hemoglobin  $\leq 10$  g/dL

- Platelet count  $\leq 75,000/\text{mm}^3$
- Calculated creatinine clearance (CrCl)  $< 50 \text{ mL/min}$ , as estimated by the Cockcroft-Gault equation
- Aspartate aminotransferase (AST) (SGOT)  $\geq 3 \times \text{ULN}$  (upper limit of normal)
- Alanine aminotransferase (ALT) (SGPT)  $\geq 3 \times \text{ULN}$
- Partial thromboplastin time (PTT)  $> 1.2 \times \text{ULN}$
- Prothrombin time (PT)  $> 1.2 \times \text{ULN}$

## 7 Equipment, Consumables and PPE

Refer to [Appendix A](#) for a full list of 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 LN biopsy by herself/himself)

#### 7.1.1 Personal protective equipment (PPE) appropriate to lymph node biopsy procedure

#### 7.1.2 Sterile lymph node biopsy tray (e.g., custom-ordered from Centurion)

- 7.1.2.1 Needleholder
- 7.1.2.2 Curved hemostats (2)
- 7.1.2.3 Straight scissor
- 7.1.2.4 Tenotomy scissor
- 7.1.2.5 Forceps
- 7.1.2.6 Right angle forceps
- 7.1.2.7 Retractor
- 7.1.2.8 Scalpel
- 7.1.2.9 Syringe/needles
- 7.1.2.10 Lidocaine
- 7.1.2.11 2-0 vicryl ties (2)
- 7.1.2.12 2-0 vicryl sutures
- 7.1.2.13 3-0 vicryl sutures
- 7.1.2.14 Sterile towel (3)
- 7.1.2.15 Gauze
- 7.1.2.16 Steristrips

## 7.1.2.17 Paper tape

7.1.3 Large sterile drape

7.1.4 Sterile surgical gown/gloves

7.1.5 Electric clippers

7.1.6 Bovie/pad

## 7.2 Consumables for Sample Processing

Note: lymph nodes must be sectioned by a qualified surgeon, pathologist or pathology technologist. Processing laboratory staff must not be responsible for identifying or sectioning lymphoid tissue. The site must coordinate the biopsy such that the appropriately labeled tubes are available for tissue collection and processing. Exact processing requirements must be defined by the protocol. It is extremely helpful if the protocol can provide reagent kits with as many items pre-aliquoted as possible.

## 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).

7.2.1.2 Note: Amber Cryovials for light sensitive PK analytes - 1.8 to 2.0 mL 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 lymph nodes.

7.2.1.3 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 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)

7.2.1.4 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 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.

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

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

- 7.2.1.7 Parafilm
- 7.2.1.8 Micropipettors and disposable tips with filter barriers
- 7.2.1.9 Serologic or volumetric pipets and laboratory ware for preparing reagents
- 7.2.1.10 Class II Biological Safety Cabinets (BSC) maintained per GCLP guidelines
- 7.2.1.11 2-8°C Refrigerator - maintained per GCLP guidelines
- 7.2.1.12 -80°C Freezer – maintained per GCLP guidelines
- 7.2.1.13 LN2 Freezer, if required – maintained per GCLP guidelines
- 7.2.1.14 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 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.
- 7.2.5 Additional Consumables for OCT (Optimal Cutting Temperature) 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 (product should be supplied by the team in “kits”)
- 7.2.5.2 Container for dry ice/ethanol bath (refer to 7.2.3.1)
- 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 ACTG Protocol Team and/or Central Supply. Refer to Appendix A for a full list of 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 ( $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  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 Paraformaldehyde fixation.

Note: Ideally, freshly prepared paraformaldehyde solution should be made rather than using commercially available formalin. 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. 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 may be purchased (example: Electron Microscopy Systems Cat # 157-4).



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 sample processing and shipping, and will benefit the comfort of fasting subjects.

- 9.1 Preferred environment: CTSI/GCRC Environment

- 9.2 Required staff

- 9.2.1 One (1) surgeon

- 9.2.2 If necessary, one pathologist or pathology technologist to section tissue

- 9.3 Subject preparation

- 9.3.1 No aspirin and/or non-steroidal anti-inflammatory drug (NSAIDs) for a minimum of 5-7 days before the procedure

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

- 9.4 Tissue Biopsy Procedure

- 9.4.1 Pre-sampling procedures

- 9.4.1.1 A physical examination of the groin will occur within four weeks of the anticipated date of LN biopsy.
- 9.4.1.2 No aspirin, NSAIDs, Plavix, Coumadin, or other blood thinners for 5 days before the procedure.
- 9.4.1.3 Biopsies should be conducted in the morning to allow for ample sample-day processing of fresh samples and because subjects will be fasting.
- 9.4.1.4 Subjects may need to fast for at least 8 hours overnight if specified in the protocol (water is allowed).
- 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 surgeon.
  - 9.4.2.4 First, the groin area will be cleaned with an antiseptic solution. Local anesthetics (Lidocaine) will be injected to numb the area. An incision between 1 and 3 inches will be made. The number of LN to be biopsied must be defined in the protocol. The surgeon will close the wound with dissolvable stitches, and then steristrips will be placed over the wound. These steristrips should remain in place until they fall off on their own, which usually takes 1-2 weeks (instruct subjects 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.
  - 9.4.3.2 At the completion of the procedure, subjects will remain for a length of time to be determined by the physician performing the biopsy and will be monitored for bleeding and stable vital signs prior to discharge.
  - 9.4.3.3 Subjects will be asked to refrain from strenuous activity until the following morning (no exercise, no lifting of heavy objects). Subjects should also wear loose fitting clothes and may consider bringing a pillow or something soft to cushion the lap belt portion of the seat belt from the incision site if they are driving.

- 9.4.3.4 Do not shower until the morning after the procedure. After that, try not to get the wound too wet while showering. Subjects should not immerse the wound in water (do not take a soaking bath or swim) until the wound check 1-7 days after the procedure.
- 9.4.3.5 3-7 days after the LN biopsy, subjects will return to the clinic to have a brief examination and asked questions about pain, drainage from the wound, or discomfort.

## 10 Sample Transport

- 10.1 It is recommended that all sample processing must be completed within 10 minutes of collection when possible (the protocol must specify the exact processing timing constraints). If this process is 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, stored in RNAlater®: or in paraformaldehyde), as defined by the protocol. If 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 medium or reagent.

- 10.2 The lymph node 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 Case Report Form (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 and RNA<sup>later</sup>® 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 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

- 11.4 Refer to the protocol for how to process, store and transport specimens in order to obtain fresh cell suspensions.

### 11.5 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 wide array of options for tissue analysis.

- 11.5.1 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.

- 11.5.2 Combine dry ice and 200-proof ethanol in a container (refer to 7.2.3.1) to make a bath for snap freezing tissues.
- 11.5.3 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.
- 11.5.4 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.

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

#### 11.6 Paraformaldehyde Fixation

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

- 11.6.1 Using sterile forceps, transfer designated number of biopsy pieces into cryovial tubes, one piece per tube, taking care to ensure that biopsy is at the bottom of the tube.

Add 1 mL of 4% Paraformaldehyde or (10% Neutral Buffered Formalin) to each cryovial and store at 4°C overnight. After 24 hours at room temperature transfer the tissue to cold 70-80% ethanol (refer to protocol for specific processing and shipping details).

- 11.6.2 Secure cryovial lids tightly and seal tops with parafilm to prevent leakage in transit.
- 11.6.3 Ship samples via overnight courier to the specified central testing laboratory (refer to section 12 for shipping details).

#### 11.7 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 ISL or VSL for processing of blocks and/or slides.

- 11.7.1 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).
- 11.7.2 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.

11.7.3 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.

11.7.4 Freeze the specimens per protocol instructions.

#### 11.8 RNA<sup>later</sup>® Samples

Note: RNA<sup>later</sup>®-ICE may be used to stabilize snap frozen tissue as it is thawed. Refer to protocol for specific processing details.

11.8.1 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.

11.8.2 Add a protocol defined volume of RNA<sup>later</sup>® to each cryovial and store at a protocol defined temperature.

11.8.3 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 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)

12.1.4 Dry ice pellets if shipping frozen samples on dry ice

#### 12.2 Packaging of biopsy specimens for refrigerated shipments

Note: Specific packaging and shipping procedures must be followed in accordance with the US Department of Transportation and ICAO regulations. ACTG/IMPACT 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 RNAlater® cryovials in one bag with appropriate absorbent material
  - 12.2.4.3 Paraformaldehyde cryovials in one bag with appropriate absorbent material
  - 12.2.4.4 Place all of the above sealed bags into one Tyvek envelope and seal.
  - 12.2.4.5 Place two refrigerated gel packs on the bottom of the STP-309 insulated shipping chest.
  - 12.2.4.6 Place the sealed Tyvek envelope on top of the Saf-T-Temp gel packs.
  - 12.2.4.7 Place two more refrigerated gel packs on top of the sealed Tyvek envelope.
  - 12.2.4.8 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.
  - 12.2.4.9 Insert a shipping manifest and return airbill into a plastic sleeve and place on top of gel packs prior to closing and sealing box with packing tape.
  - 12.2.4.10 Follow all ACTG/IMPAACT shipping protocols and any protocol-specific instructions for receiving laboratory notifications and tracking of specimens.
  - 12.2.4.11 Ship overnight for next-day receipt. Protocol-specific shipping addresses and information can be found in the protocol Laboratory Processing Chart (LPC).
- 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 half full with dry ice pellets. Add the Tyvek envelope and fill the remainder of the box with dry ice.
  - 12.3.4.4 Insert a shipping manifest and return airbill 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 (LPC).

## 13 Forms

- 13.1 Protocols must specify if a separate Lymph Node 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

- 14.1 Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, *et al.* Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997;**387**:183-188.
- 14.2 Hatano H, Hayes TL, Dahl V, Sinclair E, Lee TH, Hunt PW, *et al.* Raltegravir intensification in antiretroviral-treated patients exhibiting a suboptimal CD4+ T cell response [abstract 101LB]. In: Program and abstracts of the 17th Conference on Retroviruses and Opportunistic Infections (San Francisco, CA). In; 2010.
- 14.3 Skarda DE, Taylor JH, Chipman JG, Larson M, Baker JV, Schacker TW, *et al.* Inguinal lymph node biopsy in patients infected with the human immunodeficiency virus is safe. *Surg Infect (Larchmt)* 2007;**8**:173-178.



## **15 Acknowledgments**

- 15.1 ACTG Lymph Node (LN) Working Group. Hiroyu Hatano and Timothy Schacker. 2011. ACTG Reservoirs Sampling Focus Group; Inguinal Lymph Node (LN) SOP: pp1-7.

## **16 Appendices**

- 16.1 Appendix A: Example Reagents and Supplies
- 16.2 Appendix B: Sample Donor Consent Form

## **Appendix A: Example Regents and Supplies**

<b>Reagent/Supply</b>	<b>Example(s)</b>
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, Nunc CryoTubes™, internal thread, polypropylene (PP) tubes and screw cap #377267, 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
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-309SYS or DI or equivalent) 0°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 [xxxxxx]

**[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 a lymph node (immune system tissue) biopsy **[INSERT VISIT TIME POINTS]**. The lymph node 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.

You must agree **[INSERT ANY STUDY RESTRICTIONS]**.

The results of routine blood tests performed during your participation in this study will be returned to you as they are obtained. Your biopsy results 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.

#### 1<sup>st</sup> 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 1<sup>st</sup> of two entry evaluations. This visit will last about half a day and must occur within 7 days before the 2<sup>nd</sup> entry visit (the visit where you will be assigned to the telmisartan group or the control (no study medication) group).

- If there is a possibility that you are pregnant, you will have about 3 mL blood taken or 3 mL urine for a pregnancy test. If you are pregnant, you cannot enter the study.
- You will have the biopsies 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 groin area (the part of the body where your leg meets your hip) unless your doctor has another preference. You will have this done twice – once at the beginning of the study and once at the end of the study. The biopsies will be performed by a licensed physician. The skin above the lymph node will be numbed with local anesthetic (pain medication), an incision (cut) approximately 1-2 inches long will be made, and a lymph node will be surgically removed. If possible about a ½ teaspoon of fat will also be removed from the same cut. If you don't have enough fat in that area, the doctor might have to make a second cut in your lower abdomen (belly) area. If a lymph node cannot be found on one side, another cut may be needed on the other side to look for a lymph node. Also, if the lymph node is taken from an area other than the groin, you will need to have a second cut in your lower abdomen to obtain the fat tissue. When the procedure is performed in the groin area, most people will be able to have both biopsies with only one cut. 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 both lymph node at the 1st entry visit and one year (48 weeks) after starting the study.

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 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 45 minutes, the entire procedure, including the time you spend getting ready for the biopsy and resting afterwards, could take one half to one full day. However, it might be shorter.

You will have your 2<sup>nd</sup> 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 2<sup>nd</sup>

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.

### 2<sup>nd</sup> Entry Visit and On-Study Evaluations after 2<sup>nd</sup> 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 2<sup>nd</sup> entry visit. This 2<sup>nd</sup> entry visit will take about 60 minutes. You will be asked to come to this visit fasting (no food or liquids other than plain water and any medicines you are taking) for at least 8 hours, but preferably 12 hours, before the visit).

- **[INSERT VISIT REQUIREMENTS]**

At the 2<sup>nd</sup> 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]**

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

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 REQUIREMENTS]**

At the final visit (week XX), the biopsy procedure (see above for details) will be repeated for **[INSERT APPROPRIATE GROUP]**. After the second 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.

### 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. It is very important that you tell the study team any time you miss doses of your study drug, because it may affect the results of some

tests. In order to keep from running out of study drug, it is very important that you keep all of your study appointments.

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 lymph node biopsies before stopping the study. The study staff will collect the leftover study drug from you.

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. You should discuss with your doctor whether the results have any relevance to your clinical care.

### Other

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

If you agree, some of your blood and 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

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About [XX] people will take part in this study

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about [XX].

WHY WOULD THE DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:

- the study is cancelled
- you are not able to complete the study procedures as required
- you are not able to take the study drug as required by the study

The study doctor may also need to take you off the study drug without your permission if:

- you are not able to attend the study visits
- continuing the study drug may be harmful to you
- continuing the study drug is not in your best interest
- you need a treatment that you may not take while on the study
- you become pregnant or are breastfeeding
- you start taking telmisartan outside of the study (or another drug in the same family as telmisartan)

If you must stop taking the study drug before the study is over, the study doctor may ask you to continue to be part of the study and return for some study visits and procedures.

If I have to permanently stop taking study-provided drug, or once I leave the study, how would drugs be provided?

During the study:

If you must permanently stop taking the study-provided drug before your study participation is over, the study staff will discuss other options that may be of benefit to you.

After the study:

After you have completed your study participation, the study will not be able to continue to provide you with the drug you received on the study. If you wish to continue to take this or a similar drug would be of benefit to you, the study staff will discuss how you may be able to obtain them.

## WHAT ARE THE RISKS OF THE STUDY?

The drug used in this study may have side effects, some of which are listed below. Please note that these lists do not include all possible side effects seen with this drug. These lists include the



more serious or common side effects with a known or possible relationship to **[INSERT DRUG NAME]**. If you have questions concerning additional study drug side effects please ask the medical staff at your site.

**[INSERT DRUG NAME]** may affect the way other medicines work, and other medicines may affect how telmisartan works. There may be a risk of serious and/or life-threatening side effects when non-study medications are taken with the study drugs. For your safety, you must tell the study doctor or nurse about all medications you are taking before you start the study and also before starting any new medications while on the study. These medications include but are not limited to prescription medications, over-the-counter medications, vitamins, minerals, herbs, and dietary supplements. This also includes medicines that you may not take daily, for example, naproxen or ibuprofen (also called non-steroidal anti-inflammatory agents, or NSAIDs), which are often used to treat pain and arthritis. You must also tell the study doctor or nurse before enrolling in any other clinical trials.

### Risks of **[INSERT DRUG NAME]**

#### **[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 lymph node 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 lymph node/fat biopsy for a few days, and a small scar may remain at the site of the lymph node/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 less than 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 do a lymph node biopsy we cannot find the lymph node that was felt before the procedure. This is unlikely to happen; however, if a lymph node cannot

be found during the biopsy procedure, 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.

### Risks of Drawing Blood

Having your blood drawn may cause discomfort, bleeding, and bruising where the blood is drawn. Occasionally, there is swelling in the area where the needle is placed in your body and there is a small risk of infection. There is also a risk of lightheadedness, fainting, and blood clots. These risks are no greater than when you normally have your blood drawn at your doctor's office.

### ARE THERE RISKS RELATED TO PREGNANCY?

Telmisartan may be unsafe for unborn babies. It may cause smaller amounts of the normal fluid surrounding the baby in the womb.

If you are a woman having sex that could lead to pregnancy, you must agree not to become pregnant. Because of the risk involved, you and your partner must use two methods of birth control that you discuss with the study staff. You must continue to use both methods until X months (XX weeks) after stopping study drug. You may choose two of the birth control methods listed below:

- Birth control drugs that prevent pregnancy given by pills, shots or placed under the skin
- Male or female condoms with or without a cream or gel that kills sperm
- Diaphragm or cervical cap with a cream or gel that kills sperm
- Intrauterine device (IUD)
- Tubal ligation
- NuvaRing

Not all of these methods of birth control prevent HIV transmission and some may increase the risk of HIV acquisition. If you can become pregnant, you must have a pregnancy test before you enter this study. The test must show that you are not pregnant. If you think you may be pregnant at any time during the study, tell your study staff right away. The study staff will talk to you about your choices.

If you become pregnant while on study, you must stop taking the study drug. 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 48 biopsies. 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](http://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 subjects.
- 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.

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Participant's Name (print)

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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)

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Witness's Signature and Date