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1 Purpose

This Standard Operating Procedure (SOP) describes procedures for the collection, processing, storage and shipping of gut-associated lymphoid tissue (GALT) collected via colorectal biopsy.

2 Scope

Users of the ACTG/IMPAACT Lab Manual.

3 Background

The analysis of GALT is paramount to understanding HIV pathogenesis and persistence. Because the mucosal tissues of the gastrointestinal tract are a major portal of HIV transmission and a major site of the latent viral reservoir, the examination of GALT is essential for the development of preventative and therapeutic vaccines aimed at enhancing mucosal HIV-specific immunity, as well as for the assessment of the effectiveness of cure strategies.

Approximately 90% of the body's lymphocytes exist in lymphoid tissues, and sampling these lymphoid tissues may provide insights into events related to HIV pathogenesis and persistence that are not necessarily reflected in peripheral blood. For example, several studies have demonstrated that while peripheral blood CD4 counts remain in the normal range among the majority of patients with early HIV infection, at least half of the CD4+ T cells in the GALT are depleted within weeks of acute HIV infection; moreover, these cells are only minimally restored even after years of suppressive highly active antiretroviral therapy (HAART) [17.1-17.3]. In another study of long-term HAART-suppressed individuals, HIV DNA was 3-9 times higher in tissue samples along the gastrointestinal tract compared to peripheral blood mononuclear cells (PBMCs) [17.4], and the effects of intensification were observed in ileum but not in plasma or PBMC samples despite the use of ultrasensitive assays [17.5]. Finally, HIV-specific immune responses in peripheral blood may not reflect those in GALT, where most of the latent reservoir resides. For example, in one study of HAART-suppressed subjects, individuals who had stronger mucosal HIV-specific responses in GALT had lower measures of the latent reservoir; importantly, these associations were not observed with HIV-specific immune responses in peripheral blood [17.6].

Quantitation studies of GALT have demonstrated that there are approximately 15 lymphoid follicles/cm^3 in the colon and 25/cm^3 in the rectum. Multiple random biopsies can be obtained by sigmoidoscopy at a pre-determined, standardized depth of insertion of 10-20 cm. The procedure can be completed within 20 minutes with a simple pre-procedure enema. No additional pre-medication or sedation is required. This procedure can provide an easily accessible source of quantifiable lymphoid tissue which can be repeatedly, randomly sampled.

Measurements of drug and metabolite concentrations within GALT, and within lymphocytes found in GALT, are of interest in understanding effective treatment and prevention of HIV infections [17.7-17.11]. Details on sample collection, processing and storage are limited. Therefore, conservative precautions should be considered to ensure the stability of the drugs and/or metabolites of interest in the GALT specimen. Information on drug and metabolite stability in various tissues can sometimes be found in the investigators brochure. When drug and/or metabolite may be or are known to be light sensitive, samples should be protected from light.

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 gastroenterologist and assistant

5.3 Supplies (endoscope, forceps)

5.4 Subject reimbursement

5.5 Sample analyses: On-Site Laboratories or Central Laboratories

5.6 Shipping costs

6 Eligibility Requirements

6.1 Inclusion Criteria:

6.1.1 Able to give informed consent (See Appendix C: Sample Colorectal Biopsy Informed Consent Form)

6.2 Exclusion Criteria:

6.2.1 Known blood coagulation disorder

6.2.2 Platelets < 50,000/mm3

6.2.3 PTT > 2x ULN (PTT = partial thromboplastin time; ULN = upper limit of normal)

6.2.4 INR > 1.3 (INR = international normalized ratio)

6.2.5 Hemoglobin at less than 10 g/dL

6.2.6 Use of clopidrogel (e.g. Plavix®), warfarin (e.g. Coumadin®), or other blood thinners that cannot be stopped for clinical reasons for 5 days before and 5 days after the procedure.

6.2.7 Use of aspirin and/or non-steroidal anti-inflammatory drug (NSAIDs) that cannot be stopped for clinical reasons for a minimum of 3 days before and after the procedure

6.2.8 Pregnancy

6.2.9 Vaccination within 2 weeks of scheduled procedure

6.3 Screening Laboratory Tests:

6.3.1 Complete Blood Count (CBC)

6.3.2 PT/PTT (PT=Prothrombin Time; PPT=Partial Thromboplastin Time)

6.3.3 Urine/serum pregnancy test

6.4 Timing of Biopsy Sampling

6.4.1 Minimum interval of 4 weeks between gut biopsy time points

6.4.2 Maximum of 4 gut biopsies per year

6.4.3 Avoid any vaccines <2 weeks before procedure

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)

7.1.1 Flexible sigmoidoscope or colonoscope with 3.7mm working channel to accommodate maximum capacity biopsy forceps

7.1.2 Single-use Radial Jaw 3 maximum capacity with needle and 3.7mm minimum working channel (example: Boston Scientific Cat # M0051589)

7.1.3 Personal protective equipment (PPE) appropriate to sigmoidoscopy/colonoscopy procedure

7.1.4 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.

7.1.5 Indelible marker (example: Fisher Scientific Cat # 13-379)

Note: specimens must be labeled (refer to11.2) using the LDMS. Ideally, specimens should be pre-labeled to prevent the need for hand written labels. If unavoidable, be sure the writing will not be affected by exposure to laboratory reagents such as ethanol.

7.2 Consumables for Sample Processing

Note: some of the processing may be done at the site of collection (e.g. in the surgical suite or nearby pathology suite) or it may be prepared for transport to the processing laboratory. The specific details must be worked out to meet local capabilities. Same-day

processing is extremely important to ensure specimen integrity. Processing should ideally be processed within 2-3 hours of collection.

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).

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.

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)

7.2.1.2 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 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 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 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

7.2.6 Additional Consumables for RNA*later*® Stabilization – immediately stabilizes RNA in tissues and allows for gene expression analyses of host and/or microbial signatures.

7.2.6.1 50 mL Polypropylene Conical Sterile Centrifuge Tubes (example: Thermo Scientific Cat # 339652) if transporting to processing laboratory.

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 (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 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. 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: the paraformaldehyde solution should ideally be made fresh the morning of the biopsy but may be stored at 4°C for up to 1 week.

8.3.2 10% Neutral Buffered Formalin (contains 3.7 – 4% Formaldehyde)

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 7.2.2.3) 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: Life Technologies Cat# AM7020)

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: Private outpatient clinic room or surgical suite

9.2 Required staff

9.2.1 One (1) gastroenterologist

9.2.2 One (1) assistant to help with endoscope and specimen handling

9.3 Subject preparation

9.3.1 No aspirin and/or non-steroidal anti-inflammatory drug (NSAIDs) for a minimum of 3 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)

9.3.3 Subjects may need to fast for at least 8 hours overnight (refer to protocol, water is allowed).

9.3.4 The pre-procedure enema should be administered immediately before or up to 30 minutes prior to the biopsy procedure. Saline enema (125 ml) is administered by the study staff or self-administered, held for 5 minutes, then expelled, as described in the protocol or MOPS. Hypertonic enemas (e.g. Fleet's) should be avoided [17.12]. Once the enema has been administered, the subjects should not eat anything until after the procedure has been completed (water is allowed).

9.4 Tissue Biopsy Procedure

9.4.1 No pre-medication or intravenous line is required.

9.4.2 A flexible endoscope is inserted to a depth of 20 cm from the anus.

9.4.3 Multiple random biopsies are obtained from 20 cm to 10 cm from the anus, with the intent of avoiding areas of mucosal inflammation and the distal rectum (in order to minimize any symptoms of urgency and irritation post-procedure). Up to 30 biopsy pieces may be removed (2 pieces per pass).

9.4.4 Rinse biopsy forceps with sterile PBS or distilled water after each biopsy. If distributing individual biopsies into vials of different media or reagents, new forceps may be recommended. The procedure should be completed within 20 minutes.

9.4.5 Sequence of Collections:

If the samples are to be separated in a Surgical Suite, follow the collection sequence below, with a forceps rinse of molecular biology grade water between each of the reagent steps (it may be necessary to use a sterile needle to dislodge the specimen from the forceps):

9.4.5.1 PK samples, dry and snap frozen

9.4.5.2 DPBS for transport to processing laboratory or for fresh cell preparation

9.4.5.3 Paraformaldehyde

9.4.5.4 RNA*later*® and OCT

9.4.6 Optimal collection and processing requires transfer of biopsy segments directly into the appropriate medium or reagent or snap freezing as required. If this practice is not feasible in the surgical suite, the non-PK biopsies should be placed into a suitable transport medium such as sterile Dulbecco's PBS and transported quickly to an appropriate processing area or directly to the laboratory for processing.

9.4.7 To prevent dilution of PK analyte, do not immerse PK biopsies in PBS unless directed by the protocol. PK biopsies should be snap frozen in separate cryovials. Time of collection, time of transfer, and the pre-and post-collection weight of cryovials containing PK specimens must be documented on the appropriate requisition or CRF, and transferred with the specimen to the processing laboratory (the receiving laboratory must be able to differentiate between the pre- and post-collection weight for multiple PK vials). See Appendix B, GALT Specimen Flowchart, for overview of GALT specimen collection and initial processing.

9.4.8 Ideally, the post-collection weight should be performed just prior to snap freezing. Once frozen, the PK tissue must be kept frozen by transporting on dry ice (follow the appropriate regulations for handling specimens on dry ice). The cold chain must be maintained during all processing. If a post-collection weight must be determined after snap freezing, wipe the cryovials to remove any crystals that may have formed and quickly weigh the post-collection vial. Only remove one vial at a time to minimize the exposure to warming temperatures. Immediately return the vial to the dry ice after weighing. Recorded the post-collection weight as directed by protocol, LPC and/or CRF instructions.

9.4.9 Transfer biopsy samples to the laboratory immediately after collection for initial processing prior to shipping to central laboratory. If all samples were placed in a single tube of transport medium (i.e. DPBS or RPMI), the laboratory must document the time that each biopsy segment is transferred to protocol specified media or reagents.

9.4.10 The subject can be discharged from the outpatient unit immediately after completion of the procedure. The subject may also resume normal activities, including eating.

9.4.11 Post-sampling instructions

9.4.11.1 Instruct subject to not use aspirin or non-steroidal anti-inflammatory drug (NSAIDs) for a minimum of 3 days after the procedure.

9.4.11.2 Instruct subject to not use clopidrogel (e.g. Plavix®), warfarin (e.g. Coumadin®), or other blood thinners for 5 days after the procedure.

9.4.11.3 Instruct subject to avoid receptive anal intercourse or insertion of anything into the rectum for 7 days after the procedure.

9.5 Tissue Biopsy Considerations

9.5.1 A maximum of 30 biopsy pieces per gut biopsy shall be obtained at any given time point.

9.5.2 The Timing of gut biopsies

9.5.2.1 Minimum of 4 weeks between gut biopsy time points

9.5.2.2 Maximum of 4 gut biopsies per year

9.5.2.3 Avoid any vaccines <2 weeks before procedure

9.5.3 Distribution of gut biopsy pieces shall be clearly delineated in protocol language.

Division of biopsy pieces for collection and processing will depend upon the scientific question being addressed by the protocol and anticipated future needs. The following is an example of a typical 30 biopsy sampling that will permit virologic, immunologic and pathologic evaluations, including sufficient storage of whole biopsy pieces for future analyses (refer to 9.5.3.1 to 9.5.3.4). These numbers are only examples and should be tailored to the scientific questions of interest specified in the protocol. For example, if storage of whole biopsy pieces is of a lower priority, the 6 biopsy pieces that are listed for snap freezing may be added to the fresh samples. In order of priority:

9.5.3.1 Fresh samples (DPBS): 18 biopsy pieces for RMC (Rectal Mononuclear Cells) isolation and immunologic and virologic analyses.

9.5.3.2 Snap frozen samples: 6 biopsy pieces for virologic analyses. PK assays or future assays.

9.5.3.3 Paraformaldehyde and/or Formalin samples: 4 biopsy pieces for histopathology analyses.

9.5.3.4 The remaining 2 biopsy pieces for fresh frozen sections:

9.5.3.4.1 OCT samples: 2 biopsy pieces for histopathologic analyses.

9.5.3.4.2 RNA*later*®: 2 biopsy pieces for genomic profiling analyses.

10 Sample Transport

10.1 It is recommended that sample processing of snap frozen, RNA*later*®, paraformaldehyde, and OCT biopsies be completed immediately after time of collection when possible. 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 RNA*later*®: or in 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 medium or reagent.

10.2 The GALT 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*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 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 wild 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 30 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.

NOTE: post-collection weight should be done as quickly as possible and preferably before snap freezing.

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.

11.6.2 Add 1 mL of 4% Paraformaldehyde or (10% Neutral Buffered Formalin) to each cryovial and store at 4°C.

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

11.6.4 Ship samples overnight on the same day as collection to the specified central testing laboratory (refer to section 12 for shipping details).

NOTE: Samples may be removed from formalin or paraformaldehyde after 6-8 hours at room temperature and may be washed or stored in cold 70-80% ethanol (refer to protocol for specific processing 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 11.4.2). 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*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.

11.8.1 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*later®* 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/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 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 Example of a Colorectal Biopsy Informed Consent Form (Appendix C)

13.2 Example Sigmoidoscopy Patient Information (Appendix D)

14 Literature References

14.1 Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004,200:749-759.

14.2 Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol 2003,77:11708-11717.

14.3 Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med 2004,200:761-770.

14.4 Yukl SA, Gianella S, Sinclair E, Epling L, Li Q, Duan L, et al. Differences in HIV Burden and Immune Activation within the Gut of HIV-Positive Patients Receiving Suppressive Antiretroviral Therapy. J Infect Dis 2010,202:1553-1561.

14.5 Yukl SA, Shergill AK, McQuaid K, Gianella S, Lampiris H, Hare CB, et al. Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. Aids 2010,24:2451-2460.

14.6 Hatano H, Hayes TL, Dahl V, Sinclair E, Lee TH, Hoh R, et al. A randomized, controlled trial of raltegravir intensification in antiretroviral-treated, HIV-infected patients with a suboptimal CD4+ T cell response. J Infect Dis 2011,203: 960-968.

14.7 Patterson, Kristine B.; Prince, Heather A.; Stevens, Trenton; Shaheen, Nicholas J.; Dellon, Evan S.; Madanick, Ryan D.; Jennings, Steven; Cohen, Myron S.; Kashuba, Angela D.M. Differential penetration of raltegravir throughout gastrointestinal tissue: implications for eradication and cure. AIDS Vol. 27(9), 1 June 2013, p 1413–1419

14.8 Cory TJ, Schacker TW, Stevenson M, Fletcher CV. Overcoming pharmacologic sanctuaries. Curr Opin HIV AIDS. 2013 May; 8(3):190-59.

14.9 Asmuth DM. Kashuba AD, Albanese A, Li X-D, Troia-Cancio P, Pollard RB. Efavirenz, Tenofovir, and Emtricitabine Levels in Colonic Tissue 7th IAS Conference on HIV Pathogenesis, Treatment, and Prevention, June 30-Jul3 2013, Kuala Lumpur.

14.10 Walker DK, Bowers SJ, Mitchell RJ, Potchoiba MJ, C. M. Schroeder CM and Small HF. Preclinical assessment of the distribution of maraviroc to potential human immunodeficiency virus (HIV) sanctuary sites in the central nervous system (CNS) and gut-associated lymphoid tissue (GALT) 2008, Vol. 38, No. 10, Pages 1330-1339

14.11 van Marle G, Church DL, Nunweiler KD, Cannon K, Wainberg MA, Gill MJ. Higher levels of Zidovudine resistant HIV in the colon compared to blood and other gastrointestinal compartments in HIV infection. Retrovirology. 2010 Sep 13;7:74.

14.12 Fuchs EJ, Lee LA, Torbenson MS, Parsons TL, Bakshi RP, Guidos AM, Wahl RL, Hendrix CW. Hyperosmolar Sexual Lubricant Causes Epithelial Damage in the Distal Colon: Potential Implication for HIV Transmission. J Infect Dis 2007; 195:703–10.

14.13 [ACTG Gut-Associated Lymphoid Tissue Colorectal Biopsy SOP, ACTG Gut-Associated Lymphoid Tissue Working Group, 12 Dec 2011]

15 Acknowledgments

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15.2 ACTG HIV Reservoirs Sampling Focus Group

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15.4.4 Brian Claggett, Laboratory Technician, Case Western Reserve University

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15.4.7 Cheryl Jennings, VQA Laboratory Manager, Rush University Medical Center

15.4.8 Melissa B. Austin, Laboratory Project Manager, HIV/AIDS Network Coordination

16 Appendices

16.1 Appendix A: Example and Reagents

16.2 Appendix B: GALT Specimen Flowchart

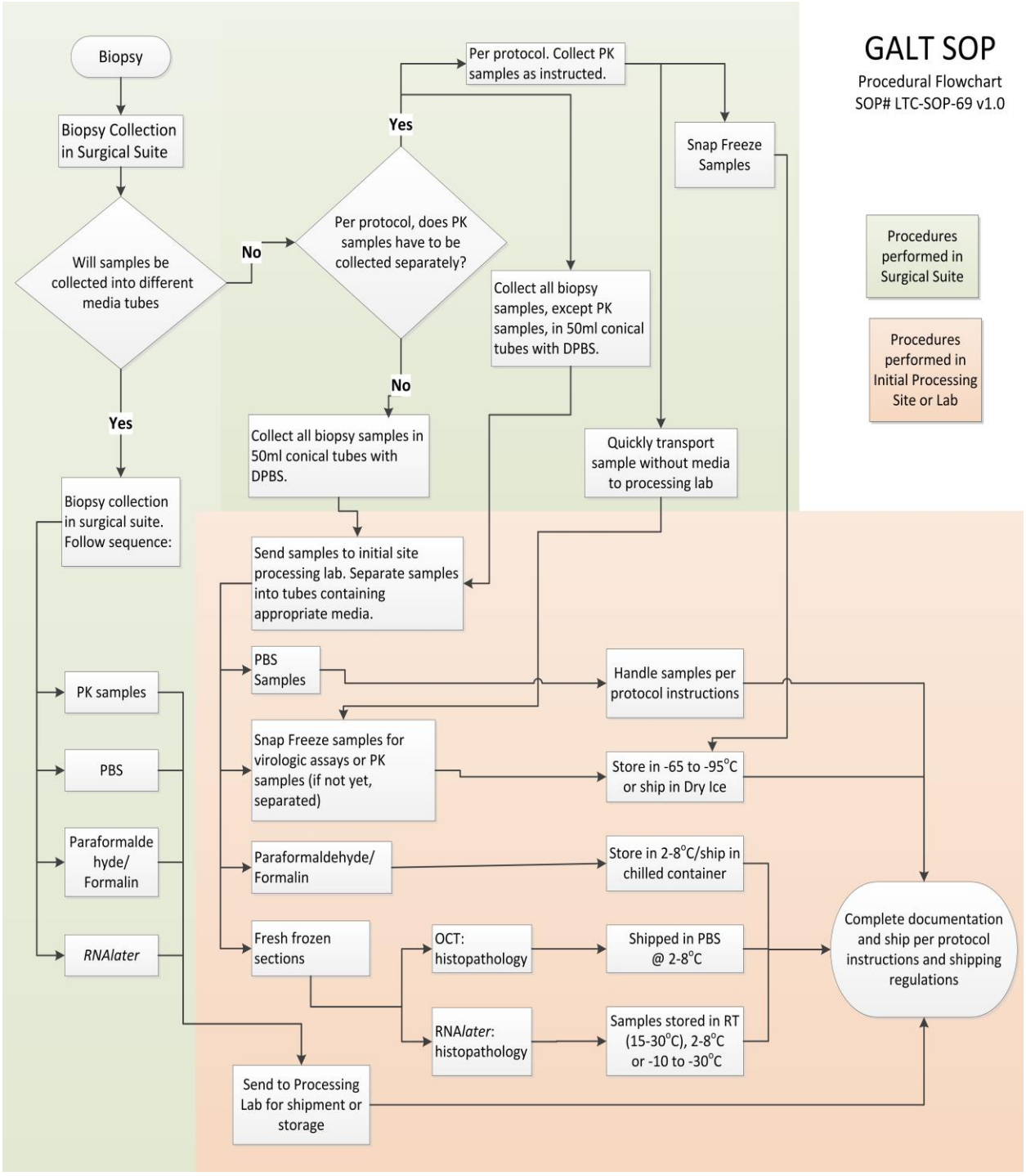
16.3 Appendix C: Example of a Colorectal Biopsy Informed Consent Form

16.4 Appendix D: Example Sigmoidoscopy Patient Information

Appendix A: Example Regents 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
16% Paraformaldehyde	MP Biomedicals Cat # 0219998320 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
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^{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: GALT Specimen Flowchart



Appendix C: Example of a Colorectal Biopsy Informed Consent Form

IRB # (if known):

Study Title:

Principal Researcher:

Revision Date:

Protocol Version:

CONSENT FORM: Persons with HIV-infection Ages 18 and up

Study Title: [Insert Drug Name] is used to treat gut/systemic inflammation in HIV+ people on suppressive ART

Principal Researcher:

The Research Team:

Name/Degree	Title	Department/Division	Phone Number
	Professor		
	Affiliate Associate Professor		
	Research Assistant Professor		
	Clinical Professor		
	Research Nurse		
	Health Care Specialist		
	Study Coordinator		

If you have questions about your rights as a research study participant, you can call the Institutional Review Board at **(XXX) XXX-XXXX**.

24 hour Emergency Contact Number(s): **Page AIDS research/ACTU On-Call at XXX-XXX-XXXX**

1. Researchers' Statement:

You have the option to take part in a research study. The goals of this form are to give you information about what would happen in the study and to help you decide if you want to participate in the study.

Feel free to take notes, write questions or highlight any part of this form. This is a consent form. It provides a summary of the information the research team will discuss with you. If you decide that you

would like to take part in this research study, you would sign this form to confirm your decision. If you sign this form, you will receive a signed copy of this form for your records.

2. What you should know about this study:

- This form explains what would happen if you join this research study.
- Please read it carefully. Take as much time as you need.
- Please ask the research team questions about anything that is not clear.
- You can ask questions about the study at any time.
- If you choose not to be in the study, it will not affect your care at **[Insert your Institution Name]**.
- If you say 'Yes' now, you can still change your mind later.
- You can quit the study at anytime.
- You would not lose benefits or be penalized if you decide not to take part in the study now or to quit the study later.

3. What is the goal of this study?

The goal of any research study is to answer questions. We (the research team listed on the front of this form and our staff) are doing this research study to answer questions about human immunodeficiency virus (HIV) infection. HIV is the virus that causes AIDS. HIV infection damages the intestine. This damage allows small amounts of material from bacteria that normally live in your intestine to pass into your blood stream. This event may cause your body to have an increased amount of inflammation. Inflammation is a reaction where some of the body's cells and chemical messages are activated. Long-term inflammation may increase a person's risk of heart attacks or strokes or other medical problems. Antiretroviral therapy decreases inflammation caused by HIV infection, but the inflammation does not go away completely. HIV-1 also persists in the body even on very effective antiretroviral therapy. HIV-1 is latent (sleeping) in CD4 cells and many of these cells are present in the intestine.

- The main questions that this study will answer are whether **[X weeks]** of the oral medicine called **[insert drug name]** will decrease inflammation in the blood and the gut.
  OR – The main questions that this study will answer are whether HIV is still present in the lymphocytes (T cells) in the gut (intestines), how much HIV is still present and what type of lymphocytes (T cells) have HIV in them
- If **[insert drug name]** decreases inflammation, we want to see if the effect lasts at least **[X weeks]** after **[insert drug name]** is stopped OR if **insert drug name]** effects the amount of HIV in the gut we want to see if the effects lasts
- We also want to study if **[insert drug name]** has any effects on the levels of antiretroviral medicines and the levels of HIV in your blood.
- We will also study the safety and tolerability of **[insert drug name]**.
- Another question we want to answer is how **[insert drug name]** decreases inflammation.

4. Why do I have the option of joining the study?

You have the option to take part in this research study because you have HIV infection. You have also taken antiretroviral medicines that have suppressed HIV to undetectable levels in your blood for at least **XX weeks**. However, the number of one of the cells important for your body defenses (CD4+ cells or T-cells) is still low.

5. How many people will take part in the study?

We think that about **XX people** with HIV infection will take part in this research study at **XXXXXXXXXXX**.

6. If I agree to join this study, what would I need to do?

If you join the study, you would have clinic visits with exam and tests. All the research study visits you would need to make are described below.

Screening Visit:

The first visit would be a screening visit to see if you qualify to be in the study. This visit would last about **XXX minutes.**

- We would explain the study. We would ask you questions to be sure that you understand the study. We would ask you to sign the consent form for the study and give you a copy to keep.
- We would ask you about your medical history, medication history, symptoms, smoking history, and your contact information.
- We would do a physical exam, including measuring your height, weight, blood pressure, and pulse.
- We would draw blood (**insert amount**) from a vein in your arm. Your blood would be tested for a routine **blood count, chemistries, kidney, and liver function tests, lipids, blood clotting tests, [insert or modify tests to be done]**.
- If you are female, you would have a **pregnancy test**.
- You **[may]** would be asked to provide a urine sample for a routine **urine test**.
- We would ask you to complete a questionnaire about **[add topic or delete]**. You can refuse to answer any questions that make you uncomfortable.
- We would ask you for permission to obtain medical records from your primary care provider and ask you to sign a release so that we could send information to your primary care provider.

The results from the screening visit will determine if you are eligible to continue in the study. About one week after the screening visit, we will call you or ask you to call us to tell you about your test results. If you are eligible, we will invite you to schedule the first group of study visits.

If you are not eligible to continue in the study, we will tell you why.

On-Study

The tests and exams you will have on this study help us find out if being in this study causes any effects that are important to know about. We use them to check on the safety of people in the study. We also use them to learn if the experimental treatment is helping or not.

Entry Visit(s)

NOTE – these are examples of possible studies that may be done. If there is a separate informed consent for the research study then this consent may only need to contain the details of the sigmoidoscopy procedure and biopsies.

The entry visits are a group of **[two to four visits that take place over one week]**. If you are one of the first **XX people** to continue in the study who are taking a specific group of antiretroviral drugs, you will need to have two visits to have blood samples collected for antiretroviral drug levels (PK visits). One of the PK visits can be combined with the entry visit if you prefer, or it can be done on a separate day. If you

enter the study after at least **XX people** who are taking the same antiretroviral regimen as you have entered the study, you will only need to have two visits during the first week.

The first one to three visits will be at **XXXXXXXXXXXX**. The first visit will last about **XX minutes.** At this visit, the following will happen:

- Review of the study and confirmation that you are willing to participate.
- Confirm that you are going to continue to take your antiretroviral medicines
- Review of any medical issues, new medications, or new symptoms that you have had since your screening visit
- Brief physical exam, including measurement of your waist
- Blood draw (**X tablespoons** for routine blood tests, CD4, viral load, levels of inflammation, and blood for storage for later study tests)
- If you are a female, you would have a pregnancy test.
- You will have secretions from your rectum collected. This involves having a small plastic tube about the diameter of a finger put into your rectum and secretions collected with thin strips of flexible filter paper.
- We will ask you to complete a questionnaire about taking medicines and whether you ever miss your antiretroviral drugs

We will educate you about **[insert drug name]** and how to take it. **Your dose of [insert drug name] will be two XXX mg tablets (total of XXXX mg) taken three times a day**. We will not give you **[insert drug name]** to take home at this visit, but we will give it to you at the end of the first sigmoidoscopy visit.

Pharmacokinetic (PK) Visits

NOTE: Add specific information if needed for the planned study

There will be **XX PK** visits. The first of these visits will last about **XX hours** and the second of these visits will last about **XX minutes**. If you wish, the **XX hour** visit can be combined with the entry visit described above.

The purpose of the PK visits is to measure the levels of antiretroviral drugs in your blood. We will ask you not to eat or drink anything except water after midnight the night before the first of these visits. We will ask you not to take your antiretroviral drugs in the evening before and in the morning of the day of the first PK visit. Please bring your antiretroviral drugs with you to this visit. We will insert a small plastic tube (catheter) into a vein in your arm. The tube will stay in your arm for the entire visit. This tube will allow us to draw serial blood samples without having to insert a needle for each sample. We will draw your blood from the tube in your arm before you take a dose of your antiretroviral drugs and **[insert drug name]** and **XX minutes**, **[XX]** hours after that time. The total amount of blood drawn will be XX tablespoons. If you need to take your antiretroviral drugs with food, we will provide you food to take. We will also provide breakfast, lunch, snacks, and dinner or meal vouchers during this visit. We will remove the tube from your arm before the end of this visit. We will remind you not to take any doses of your antiretroviral drugs the evening of this visit.

Next Day PK Visit

The purpose of this visit is to collect a blood specimen **[XX]** hours after you took the dose of antiretrovirals and **[insert drug name]** in the clinic on the first PK day. This visit will last about **XX minutes**. We will draw a blood sample from a vein in your arm to measure the levels of antiretroviral drugs in your blood.

Flexible Sigmoidoscopy Visit

This visit will occur at the **[Insert location]**. This visit will last **about [XX-XX minutes]**. The purpose of this visit is to look inside part of your large intestine (sigmoid colon) and your rectum (the part of your intestine just inside the anus). We will also take samples (biopsies) from these two areas to study in the laboratory. The gastroenterologist (gut specialist) will use a sigmoidoscope to do these tasks. A sigmoidoscope is a long thin flexible tube that will be put inside your colon through your anus.

Note: Sigmoidoscopy for the purpose of HIV Research is usually done without preparation other than what an enema prior to the procedure. Other pre-test preparations should be listed here. Whether the participant should be fasting at the time of the visit would also be listed here.

During the first part of this visit, the staff will give you an enema to clean out the right side of your colon. An enema is done by putting liquid into your colon through a small tube that is put into your anus. You will then empty the liquid into the toilet. After the enema, the gastroenterologist will insert the sigmoidoscope. Biopsies of your sigmoid colon (**n=XX**) and rectum (**n=XX**) will be collected. You will be observed after the procedure to make sure you are stable before you will be allowed to leave the office. Before you leave, we will give you **[insert drug name]** to start taking the day after this visit.

If you have difficulty relaxing or have pain during the sigmoidoscopy, we can give you one dose of a medicine to help you relax and make you sleepy. The name of the medicine is **[insert drug name]**, and it would be given to you in a vein in your arm. If you get a dose **of [insert drug name]** during the procedure, you would need to have someone escort you from the clinic to your home. The reason for the escort is that you may be sleepy and not alert enough to get home safely.

We will ask you not to have anal sex for 5 days after the sigmoidoscopy to decrease the risk of infection or bleeding.

Follow-up Phone Call

We will call you the day after the sigmoidoscopy to check how you are doing.

Follow-Up Visit Schedule

[Week X] PK Visits:

If you are in the group of participants who have PK visits, you would have a second set of PK visits during the next week of the study. The procedures would be identical to those described above except that in addition to your antiretroviral drugs, we would ask you to bring **[insert drug name]** to the first of this set of PK visits and to take one dose in the morning **[XX hours]** after you take your antiretroviral medications.

[Week X] Phone Call:

If you are not in the group of participants who have PK visits, we will call you during the **[week X]** to check how you are doing.

Other Visits:

The rest of the study visits will be fairly similar to the visits described. Most visits will take **XX-XX** minutes except for the sigmoidoscopy visits. Follow-up visits would occur at weeks **[Insert times].** At [**insert times]**, we would ask you to complete a questionnaire about how you are taking **[insert drug name]** and your antiretroviral drugs and whether you are missing any doses. The blood clotting blood tests and sigmoidoscopy would be repeated at [**insert times**]. Please see the chart at the end of this consent form for a chart that summarizes the visits for this study.

Note: Add any additional visit information here.

7. How long would I be in the study?

If you choose to take part in all the study visits, you would be in the study for [insert duration].

If you join the study, you can decide to stop **at anytime for any reason**. If you decided to stop, you would need to talk with a member of the study team so you leave the study in a safe way.

The research study staff could also decide to stop your **[insert drug name]** and/or take you out of this study. This might happen:

- if we find out that it is not safe for you to stay in the study
- if you stop taking **[insert drug name]** for more than **XX weeks**
- if you stop taking your antiretroviral drugs for more than **XX weeks**
- if you need to take a medication that is not allowed by the study
- if you cannot take anything by mouth for more than two weeks
- if you cannot come to enough of the study visits
- if you are female and become pregnant
- if the level of HIV in your blood increases in two consecutive tests to a detectable level above **1000 copies/mL** (this is what the study would call virologic failure)
- if the sponsor, local or federal oversight groups or Data Safety Monitoring Committee (a independent group of experts that will review the study) stop the study

If you stop the study or study medication before completing the study, we may ask you to return for a final visit that would involve an interim medical history, medication review and blood draw (**X tablespoons**).

If we ask you to leave the study or the study is stopped early, we would explain why.

8. What are the potential harms or risks if I join this study?

There are potential harms or risks if you take part in this study.

[insert drug name] Side effects are very rare. **[insert drug name]** might cause **constipation**. In addition, there have been a few reports of people taking **[insert drug name]** getting a **blockage** of their bowel (bowel obstruction).

The main risk of taking **[insert drug name]**

Because this research study involves an experimental use of **[insert drug name]**, we do not know all of the possible harms or risks. For your safety, you should tell the study staff about all medications you are taking before you start the study and before starting any new medications while on the study. Also, you should tell the study staff before enrolling in any other clinical trials while on this study.

<u>Blood Draws or IV Catheter</u>

Drawing blood may cause some discomfort, lightheadedness, bleeding, swelling, or bruising where the needle enters the body, and in rare cases, fainting or infection. Rarely, a blood clot can form when a catheter is used.

Rectal Biopsy

Discomfort with insertion of anoscope

Sigmoidoscopy
Discomfort with enema or insertion of sigmoidoscope

- Pain with biopsy
- Bleeding from the site of a biopsy
- Infection at the site of a biopsy
- Intestinal perforation

Breach of Confidentiality

Although the study staff will make every effort to protect your privacy and confidentiality, it is possible that others could find out that you are participating in this study. Social harms may result (because others might think or discover that you have HIV). For example, you could be treated unfairly, discriminated against by family members, friends, and/or the community.

Stress

You might find some of the questions about your health or medication-taking behavior are stressful to answer.

Pregnancy

If you are female and having sex that could lead to pregnancy, you must agree not to become pregnant. If you become pregnant, the study drug will be stopped and you will be followed until after the end of the pregnancy.

If you are a woman, you should use a reliable method of birth control. You should choose one of the birth control methods listed below:

- Condoms (male or female) with a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based contraceptive
- Tubal ligation

If you can become pregnant, you must have a pregnancy test within **72 hours prior** to starting the study drugs. The test must show that you are not pregnant.

Some of the methods listed above may not prevent the spread of HIV to other people. You should discuss your contraceptive choices with your health care provider to choose the best way for you to both prevent pregnancy as required by this study and to prevent the spread of HIV to your partner.

If you think you may be pregnant at any time during the study, please tell your study staff right away. If you become pregnant, you will have to stop taking the study drug and would no longer have study procedures. We would like to keep in contact with you and check with you about the outcome of your pregnancy.

Study Oversight

A Data Safety Monitoring Board will review the information from this research study. This board is made of a group of experts. They are responsible for looking at how people in the research study are doing. If

you take part in this study, we would tell you about any new information we learn that might affect your health or your willingness to stay in the study.

9. What are the potential benefits if I join this study?

Potential Benefits for You:

Being in this study might benefit you in the following ways:

- Close personal attention and support
- Closer monitoring of your HIV infection than is standard which may make you feel better

Potential Benefits for Others:

We hope to use information we get from this study to benefit others who have HIV infection.

10. What other options do I have?

If you choose not to be in this study, you can have:

- standard treatment for HIV
- treatment with experimental drugs, if you qualify
- no treatment

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

11. How would you keep my information confidential?

If you take part, we will make every effort to keep your information confidential. However, no system for protecting your confidentiality can be completely secure. It is possible that unauthorized persons might discover that you are in this study, or might obtain information about you. **XXXXXXXXXXX**, University and government oversight offices, National Institutes of Health (NIH), the Federal Office for Human Research Protections (OHRP), the FDA, study staff, and their designees sometimes review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

We will not release any information that identifies you without your written permission, except as required by law. We will ask you to sign a medical release of records form. This will allow us to obtain information from your primary care provider and to send study information, like your test results, to that provider using our hospital's electronic medical records system. We will need to have access to your medical records for this study. For example, we need to look at your past records of blood tests or might need to look at records about illnesses that occur during the study. If you are not eligible for the study after completion of all screening tests, your results will be kept indefinitely in a confidential file at the ACTU.

To help us protect your privacy, we will obtain a Certificate of Confidentiality from the Federal Government. With this Certificate, we cannot be forced to disclose information in our research records that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal,

administrative, legislative, or other proceedings. However, there are some limits to the Certificate. It cannot be used to resist a demand for information from authorized federal employees, when they want the information to (1) audit or evaluate a federally-funded research project, or (2) meet the requirements of the federal Food and Drug Administration.

The Certificate does not prevent us from voluntarily disclosing identifiable information. We will voluntary disclosure identifiable information, which may include your name as follows. Any of the information that is disclosed will not be protected by the Certificate of Confidentiality.

- To the study sponsor
- To state or federal public health authorities to whom certain contagious diseases (such as tuberculosis, syphilis) are reported (if we see such diseases in any participant). In the State of **XXXXXX**, researchers are not required to report HIV infection to the health department, but we are required to remind your personal physician that HIV is a reportable disease.
- To law enforcement authorities: information that suggests the occurrence of child abuse, elder abuse, or your intent to immediately and substantially harm yourself or others.
- To your **XXXX** Medical Center medical record: specific information as described elsewhere in this form.
- To state, federal, and institutional offices involved in auditing or compliance of research, risk management, patient safety, financial controls.

In addition, any study test or procedure results or other study information or documents (including this consent form) that are included in your **XXXXXXXXX** medical record will not be covered by the Certificate of Confidentiality and may be released if requested by a lawful subpoena or other lawful and appropriate request for the information. The Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If you give your written consent to an insurer, employer, or other person to receive research information about you, then we cannot use the Certificate to withhold that information.

The Certificate of Confidentiality is not an endorsement from the Federal Government for our research.

Persons who have access to your medical record will have access to any research-related information or documents that are in your record. Access to your medical records is governed by **XXXXX** State law (**12345**) and by the federal HIPAA law. Study data that are sent to a database at **YYYYYYY** will be identified by a code number. The database contains all study information, such as subject characteristics, study drug assignments, and lab test results. We will use the database to analyze the study results. The code number will only be linked to your name on a master list that will be kept in our clinic indefinitely in a locked file cabinet and a double password protected file on a computer. Any publication of this study will not use your name or identify you personally.

12. Would it cost me money to be in the study?

You will not pay for study-related visits, the study drug, physical examinations, laboratory tests, or other procedures. You, your insurance company, or health care system will need to pay the cost of your anti-HIV drugs, which are not provided by the study, and any routine care that you get from your regular medical care provider.

13. What if I were injured because I joined the study?

If you were injured as the direct result of this research study, we would provide treatment. We would refer you for treatment if needed

You would NOT need to pay for this treatment and neither would your insurance company. This is the only compensation offered for study-related injuries.

It is important that you tell the Principal Researcher **XXXXXXXX**, if you think that you have been injured because of taking part in this study. You can call **XXX** at **XXX-XXX-XXXX**.

14. Would I be paid if I join this study?

Compensation for participating in the study is as follows:

- You will receive **$XX** per clinic visit, beginning at entry (other than the PK and sigmoidoscopy visits)
- You will receive an additional **$XX** each time rectal secretions are collected
- You will receive **$XX** for each sigmoidoscopy visit
- You will receive **$XX** for each group of PK visits (13 hour day and next day blood draw)
- If you complete all of the study procedures, you will receive a **$XX** bonus
-

The IRS has certain rules about paying people who take part in research studies. If you took part in this study, we would ask you to provide your name, mailing address, and social security number so we could pay you. **XXXXXXXXXXXXXXXXX** is required to report to the IRS study payments that total more than **$XX** per year made to anyone.

You can be in this study even if you do not give us this information. If you decide not to give us this information, you could receive no payment.

15. Who do I contact if I have problems, questions or want more information?

If I have questions or would like to know about …	You can call …	At …
• Emergencies • General study questions • Research-related injuries • Any research concerns or complaints	**XXXXXXXXXX**, MD	Phone: **XXX-XXX-XXXX** Pager: **XXX-XXX-XXXX**
• Emergencies • General study questions • Research-related injuries • Any research concerns or complaints	AIDS Research/ ACTU Research Team Member on Call	Phone: **XXX-XXX-XXXX**
• Your rights as a research participant	Institutional Review Board This is a group of scientists and community members who make sure research meet legal and ethical standards.	Phone: **XXX-XXX-XXXX**
• Assistance with figuring out what questions to ask the research team • Help understanding the research process	Research and Family Liaison A person who works with families to ensure they receive the information they need to make an informed decision about taking part in a research study.	Phone: **XXX-XXX-XXXX** Pager: **XXX-XXX-XXXX**

More Information:

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

16. If I join the study, can I stop?

Yes. Taking part in research is always a choice. If you decide to be in the study, you can change your mind at any time.
We ask that you tell the study team member (**XXXXXXXXXXX**). You can contact her by phone at **XXX-XXX-XXXX**.

If you choose to leave the study, it will not affect your routine medical care. You will not lose any benefits or be penalized if you choose to leave the study.

17. What else do I need to know?

You must register at **XXXXXXXXXXXXXXXXX** to get a hospital number if you don't already have one. A hospital medical record number is needed when visits occur at the **XXXXXX** ACTU. This means that you will have a hospital chart with the registration information. Records of study provided medication(s) are stored on the pharmacy computer system; this system is linked to the **XXXXXX** Pharmacy and the **XXXXXXXXXX** Alliance Pharmacy. Access to information on the pharmacy and medical records computer system is restricted to authorized staff with passwords for the system. It is possible that unauthorized persons might discover that you are in this study or might obtain information about you. When you come to a **XXXXXXXX** study location, like **XXXXXXXX**, you will be asked to sign a consent for care form. This allows **XXXX** Medicine to provide care for you in emergency situations. If you want to be in this study, you must sign the consent for care form. You will be asked for information such as your social security number when you register. This medical record will be permanent. It will be stored with all other **XXXX** medical records. We will send a copy of this consent form to the hospital chart if you get your medical care at **XXXXXXXXXXXXXX**.

18. What would my signature on this form mean?

Your signature on this form would mean:

- The research study was explained to you.
- You had a chance to ask all the questions you have at this time. All your questions have been answered in a way that is clear.
- You understand that the persons listed on this form will answer any other questions you may have about the study or your rights as a research study participant.
- **You have rights as a research participant. We will tell you about new information or changes to the study that may affect your health or your willingness to stay in the study.**
- By signing this consent form, you do not give up any of your legal rights. The researcher(s) or sponsor(s) are not relieved of any liability they may have.
- You agree to take part in the research study.

_____

Printed Name of Research Participant

_____

Signature of Research Participant

_____ _____

Date *Time*

18. Researcher's Signature

I have fully explained the research study described by this form. I have answered the participant's questions and will answer any future questions to the best of my ability. I will tell the person taking part in this research of any changes in the procedures or in the possible harms/possible benefits of the study that may affect their health or their willingness to stay in the study.

_____

Printed Name of Researcher Obtaining Consent

_____

Signature of Researcher Obtaining Consent

_____ _____

Date *Time*

cc: Research Team File

Participant
Participant's Physician

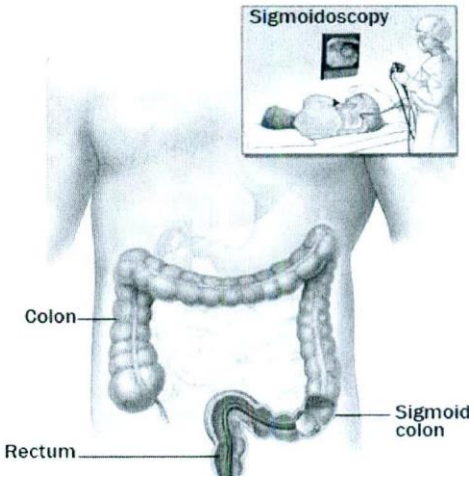
Schedule of Events Example

	N=150	N=90	N=30									
	Phone Screen	Screen*	Entry	Wk **X**	Wk **X**	Wk **X**	Wk **X**	Wk **X**	Wk **X**	Wk **X**	Wk **X**	Wk **X**
	X min	**X** hr	**X** visits		**X** min	**X** visits		**X** visits		**X** min	**X** min	**X** min
Verbal consent	X											
Written informed consent		X										
Obtain Documentation of HIV		X										
Medical records releases		X										
History, PE, Meds, System Review		X	X		X	X		X		X	X	X
Smoking history/Karnofsky Score		X										
Alcohol Questionnaire		X										
Rectal secretions (9 TearFlo)			X			X		X				
CBC		X	X		X	X		X				X
CHEM+Phos+Lipids		X	X		X	X		X				X
PT+PTT		X				X		X				
CD4		X	X		X	X		X		X	X	X
HIV RNA		X	X		X	X		X		X	X	X
PBMC Flow		X										
PBMC Flow/Plasma & PBMC Store			X	X	X	X		X		X	X	X
24hr PK: 1st 8 subjects/ARV regimen			X	X								
U/A		X										
Flex Sigmoidoscopy			X			X		X				
Phone Call***			X	X**		X	X					
Pregnancy Test (females)		X	X	If pregnancy is suspected								
Study Meds Dispensed			X		X							
Adherence Questionnaires			X		X	X						

*Within 30 days of 1st entry visit **If not in the PK subset ***All phone calls are 5 minutes except for the initial screening phone call

Appendix D: Example Sigmoidoscopy Patient Information

During the exam, a sigmoidoscope, a thin, flexible tube about the thickness of an adult finger, is inserted into the rectum. A tiny video camera at its tip allows your doctor to view the inside of your sigmoid colon and rectum, which is about the last 2 feet of the intestine.



During a flexible sigmoidoscopy exam, you lie on your left side, usually with your knees drawn toward your chest. Unlike colonoscopy, sedative and pain medication usually aren't given. The doctor inserts a sigmoidoscope into your rectum. Sigmoidoscopes are disinfected between procedures, so the risk of transmission of infection is extremely low.

The sigmoidoscope contains a fiber-optic light and a channel that allows your doctor to pump air into your bowel, inflating it to better view the interior lining. When the scope is moved or air is introduced, you may feel the urge to have a bowel movement or experience lower abdominal (belly) cramping. The sigmoidoscope also contains a tiny video camera at its tip. The camera transmits images to an external monitor so both you (if you chose) and your doctor can look closely at the inner lining of the rectum. Your doctor can also insert instruments through the channel to painlessly take tissue samples (biopsies). A flexible sigmoidoscopy exam takes about 15-20 minutes including the collection of up to 30 biopsies.

Please see below for information regarding **After Your Endoscopy Procedure**

<u>After Your Endoscopy Procedure</u>

You may experience mild abdominal discomfort after the exam. It's normal to feel bloated, have a few gas pains and pass gas until all air is released from your colon. Walking may help relieve this discomfort. You may also notice a small amount of blood with your first bowel movement following the exam. This usually isn't serious. You should contact the study staff or seek medical help if you:

- <u>Continue to pass blood or blood clots</u>, or
- <u>Experience persistent abdominal pain</u>, or
- <u>Run a fever of 100.5°F or higher</u>

These signs and symptoms may result from bleeding from the site where a biopsy was taken or, in rare circumstances, from perforation of the colon wall.