1.0 PURPOSE

The purpose of this procedure is to provide instructions to NCI cancer Human Biobank subcontracted biospecimen source sites (BSSs) for tissue collection. Specifically, this document and its associated ***Work Instructions (PR-0004-W1 and PR-0004-W2)*** attachments describe the proper removal, sectioning, and preservation of specified normal donor organs during tissue procurement for the Genotype-Tissue Expression (GTEx) project.

2.0 SCOPE

The Tissue Procurement Procedure standard operating procedure (SOP) encompasses all caHUB-required activities to properly recover, process, and transfer tissue and organ aliquots for research purposes in the GTEx project.

All GTEx project research involving human tissues or deceased donor tissues will be reviewed by the local institutional review board (IRB), including the following types of samples:

- Research samples from living donors
- Research samples from autopsy donors
- Research samples from organ or tissue donors

IRB approval must be received prior to tissue procurement. This approval must be maintained for any protocol amendments as well as at the intervals deemed appropriate by the IRB for continuing review if continuing review is required.

Samples and associated donor data transferred to or from the Study Management Group or a subcontracting facility are governed by signed agreements between the Study Management Group and the sending and/or receiving institution/entity/investigator. Agreements are made through a contract, Material Transfer Agreement, and/or Data Use Agreement.

3.0 RESPONSIBILITY

The caHUB Pathology Resource Center is responsible for maintaining this SOP and ensuring it is updated and controlled and that users receive proper training prior to implementation.

All BSS staff responsible for biospecimen recovery are required to be trained to execute this procedure and all appropriate ***Work Instructions (PR-0004-W1 and PR-0004-W2)***. The BSS site investigator is responsible for training local staff on adherence to this SOP.

4.0 DEFINITIONS & ABBREVIATIONS

4.1 **Aliquot, tissue** – The final tissue component that is dissected from the organ, for immersing in PAXgene® Tissue Fixative/Stabilizer

4.2 **BSS** – Biospecimen source site

4.3 **caHUB** – cancer Human Biobank

4.4 **Case ID –** Donor ("case") identification. The Case ID is an 11-character identification (e.g., GTEX-000000) which is obtained from the kits and assigned by the BSS at the time of donor procurement.

4.5 **CBR** – Comprehensive Biospecimen Resource.

4.6 **CRF** – Case report form. A paper or electronic form used to capture requested data elements.

4.7 **Cross section –** Cutting a piece of an organ at right angles to an axis.

4.8 **GTEx** – Genotype-Tissue Expression

4.9 **IATA** – International Air Transport Association. The governing body that creates regulation for international air transport, including regulations controlling the transport of Dangerous Goods By Air.

4.10 **ID –** Identification. A series of alpha or numeric digits used to provide a unique identifier.

4.11 **Kit Component ID –** Identification label on the outside of any box of kit components.

4.12 **LDACC –** Laboratory Data Analysis Coordinating Center.

4.13 **Organ Site** – One of multiple types of tissue within a complex organ or portion of the body.

4.14 **PAXgene® Tissue System** - A PreAnalytiX method of tissue fixation and stabilization that is comprised of a dual-chamber container, fixation reagent, stabilization reagent, and a screw cap lid that has a tissue cassette holder. Chamber 1 of the container holds the fixation reagent, referred to as solution 1, while chamber 2 holds the stabilization reagent, referred to as solution 2.

4.15 **SOP** – Standard operating procedure. A written procedure for repetitive use as a practice, in accordance with agreed upon specifications aimed at obtaining a desired outcome.

4.16 **Specimen ID** – The Specimen identification is used to refer to the identification label included on each specimen cassette.

4.17 **TRF –** Tissue Recovery Form. A paper or electronic form used to capture data during specimen procurement.

5.0 ENVIRONMENTAL HEALTH & SAFETY

5.1 Universal Precautions (CDC-1978) are used for all phases of blood collection and handling and organ/tissue procurement, dissection, processing, and handling.

5.2 Persons packaging and/or signing transport documents must be trained and/or certified to ship the appropriate hazard class according to International Air Transport Association (IATA)/ International Civil Aviation Organization (ICAO) regulations.

5.2.1 Training may be conducted through the BSS or offered through the Study Management Group.

5.2.2 Verification of training materials, testing and certification must be provided to the Study Management Group team to certify all staff involved with shipping procedures.

5.3 Persons handling the blood collection tubes or PAXgene® tissue containers should be aware of the hazards associated with the chemicals and how to handle an accidental spill or exposure by reviewing the Material Safety Data Sheets. Appropriate gloves (Latex or Nitrile rubber) are required for persons directly handling the PAXgene® Tissue Containers.

5.4 Appropriate personal protective equipment should be worn at all times to include but not limited to:

- Gown or lab coat;
- Hair bonnet, mask, and shoe covers, per institutional requirements;
- Latex or nitrile rubber gloves.

5.5 Blood Transfer Device:

- For the safe transfer of blood use a BD Vacutainer® Blood Transfer Device. Instructions for appropriate transfer are available at BD's Web site: http://www.bd.com/vacutainer/pdfs/blood_transfer_device_brochure_VS7019.pdf.

6.0 MATERIALS/EQUIPMENT

6.1 The Comprehensive Biospecimen Resource (CBR) will provide collection kits and kit materials. Refer to ***GTEx Kit Receipt, Supplies, and Shipping Procedure (OP-0001)*** for specific instruction.

6.1.1 It is a requirement to use CBR issued kits and kit materials for collection.

6.1.2 Any deviation or change from this SOP, known prior to a collection, should be approved by the Study Management Group and well documented by the site.

6.1.3 Any deviation or change that is unexpected or identified during or after a collection, should be well documented by the site. This deviation should be submitted to the Study Management Group along with a corrective action description for the documentation and comment.

6.2 The BSS will be responsible for any additional materials/equipment to be utilized during a case collection that are not provided by the CBR or the Study Management Group.

6.2.1 Refer to appropriate ***Work Instruction (PR-0004-W1 or PR-0004-W2)*** for materials/equipment specifications.

7.0 PROCEDURES

7.1 Site Preparation

7.1.1 General

7.1.1.1 All biospecimen processes should be optimized at each BSS according to its capabilities and routine procedures to ensure efficient organization of the recovery team.

7.1.1.2 It is up to the site investigator to determine the optimal size of the recovery team, the dissection and aliquot preparation tables, and the recovery/dissection space.

7.1.2 Recovery Areas and Supplies

7.1.2.1 Recovery area(s) should be set up with the necessary reagents and supplies.

7.1.2.2 The BSS is responsible for ensuring all necessary supplies and materials are on-hand prior to recovery procedures.

7.1.2.2.1 Upon receipt of collection kits, BSS staff should review contents and immediately report any missing pieces to Study Management Group/CBR.

7.1.2.2.2 The BSS can utilize additional clean or sterile supplies or materials as needed to ensure a successful collection.

7.1.3 Kit Labeling

7.1.3.1 The label on the outside of any box of kit components is the kit component ID used to identify that kit type. **Note: The IDs contained in the overpack kit dictate the Case ID for a given collection event, and are not to be confused with the kit component IDs.**

7.1.4 Biospecimen Labeling

7.1.4.1 Each biospecimen will be identified using a unique specimen ID. The complete specimen ID is composed of two elements—a Case ID (e.g., GTEX 123456) and a sequence number (e.g., 7890)—that together form the final alpha-numeric Specimen ID; e.g., GTEX-123456-7890.

7.1.4.1.1 The recovery staff is responsible for recording each field in the ***GTEx Tissue Recovery Form (PM-0003-F5)***. This includes recording the time tissue is removed, and the time aliquots are placed in solution #1 (fixative) and solution #2 (stabilizer) for the PAXgene® tissue preservation method. Also see ***GTEx Organ Retrieval Dissection Table (PR-0004-W1-G3)*** for quick reference regarding specific aliquot locations and sizes.

7.1.4.1.2 All information documented on the PAXgene® tissue containers must include the staff's initials and date of recorded information.

7.1.5 **Whole Blood Collection**

7.1.5.1 Whole blood will be collected and shipped directly to the LDACC (***Yellow Kit***).

7.1.5.1.1 Collection timeline**:** Blood collection should occur as close as possible to the donor collection start time of procedure.

7.1.5.1.2 Total volume: A total of four (4) whole blood vacutainers will be collected and shipped to the LDACC **(*GTEx Kit Receipt, Supplies, and Shipping Procedure (OP-0001)*.** This includes:

- Four (4) 10 mL ACD (yellow top) vacutainers
  - A minimum of 6 mL of blood is requested in each of 2 yellow top vacutainers, if available. (4 are provided in case they are required due to logistical collection issues.)
- Two (2) 2.5 mL PAXgene® RNA blood vacutainers.
  - Minimum volume requested is 2mL in each vacutainer.

7.1.5.1.3 Volume preference (if amount is limited): The blood collection content preference, if amount is insufficient to collect all 4 containers: (1) one yellow top, (2) one PAXgene®, followed by (3) the second PAXgene® and (4) the second yellow top. If blood overall is limited for a given donor, fully fill at least ONE of the two PAXgene® blood tubes and then fill at least one of the YELLOW top tubes for DNA.

7.1.5.1.4 Collection preference: The collection preference is the femoral vein; subclavian vein and heart are other possible sites. Preference of location will vary for organ donors [beating heart donors (usually arterial line)] vs. non-beating heart tissue donors (venous route)

7.2 **Tissue Procurement**

7.2.1 Use the appropriate ***Work Instructions*** for Postmortem ***(PR-0004-W1)*** or Surgical Cases ***(PR-0004-W2)*** to collect the biospecimen.

7.2.2 All designated GTEx tissues should be collected regardless of disease state**.**

7.2.3 The order of organ/tissue removal is left to the discretion of the BSS. However, when a brain is collected it must be removed last per the ***Work Instruction (PR-0004-W1).***

7.2.4 The tissue location for each aliquot is specified in the ***Work Instruction.*** Any deviation to the preferred location must be documented on ***GTEx Tissue Recovery Form (PM-0003-F5).***

7.2.5 Removal of any organs as single organs/tissues or in groups as well as removing only sections of organs/tissues *in situ* is acceptable, where applicable.

7.2.6 Supplies must not be re-used for multiple tissue dissections. Minimize contamination between tissues by not re-using supplies:

- Use a new disposable or reusable cutting board and new gloves for EACH organ or tissue type.
- Use a new or normal saline–cleaned scalpel handle and NEW blade for EACH organ or tissue site.
- Use a new plastic toothpick, if needed, for EACH aliquot.
- As each organ/tissue is removed from the donor, place it on a new, clean, cutting tray/surface.

7.2.7 **Special instructions for Hair and Brain removal (NA for Surgical collections)**

7.2.7.1 **Hair**

Note: Hair is only collected when the brain and brain stem are procured.

7.2.7.1.1 Hair must be removed prior to the donor's head being packed in ice (see ***Work Instruction (PR-0004-W1)*** section 4.3.6.2.1), per the ***Work Instruction (PR-0004-W1).***

7.2.7.2 **Brain and Brain Stem**

7.2.7.2.1 Immediately after the hair is removed, the head is packed with bags of ice, to keep the head cold since the brain is removed last and the collection of other organs may take hours to complete.

7.2.7.2.2 For brain removal, retract the cerebellum superoanteriorly and, using the longest scalpel available with a relatively thin blade, go through the foramen magnum and cut the cervical cord.

7.3 Aliquot Collection

7.3.1 Dissection and preservation activities may be conducted concurrently as long as the order of procurement is properly maintained and recorded on the appropriate CRF.

7.3.2 **During procurement, GTEx aliquots should be taken from tissue that appears grossly normal. However, even if no normal tissue is apparent, the organ should still be sampled and abnormalities documented on the CRF.**

7.3.3 Aliquot size:

7.3.3.1 The aliquot size depends upon the tissue or organ and is specified in the organ-specific sections in the specific ***Working Instructions (PR-0004-W2).*** The aliquot thickness must not exceed 4 mm. However, for the brain (cerebrum and cerebellum), 5mm cubes are to be taken as aliquots.

7.3.3.2 For epithelial lined tissues (e.g., stomach, bladder, skin, colon, vagina,) if thickness is an issue, trimming should be from the outer surface not the mucosa.

7.3.3.3 For specimens embedded in adipose tissue (e.g., arteries, nerve, adrenal, pancreas, skeletal muscle) dissect/ tease off peripheral fat as thoroughly as feasible without damaging the target tissue using 'blunt' technique and following tissue planes.

7.3.3.4 Each aliquot should be measured by the BSS.

7.3.3.4.1 Any measurement that differs from the recommended size must be documented in the ***GTEx Tissue Recovery Form (PM-0003-F5).***

7.4 PAXgene® Tissue Preservation

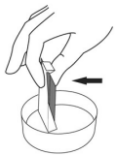
For tissues using the PAXgene® fixation method, the following instructions must be followed. Any deviation to this procedure must be adequately documented by the site staff.

7.4.1 All aqua-colored tissue cassettes and containers will arrive pre-labeled with a riveted Specimen ID.

7.4.2 Recover appropriate tissue, prepare aliquot(s), and place aliquot(s) into aqua-colored tissue cassette(s). If the cassette has a central knob-like elevation on the base and/or the lid, avoid placing the tissue on it, as it may prevent adequate fixation.

7.4.3 Proper insertion of tissue cassette:

Insert the lower edge of the tissue cassette into the bottom edge of the rack. <u>The label should be outfacing and downward</u> (images from PAXgene® MSDS package insert).



Attach the tissue cassette to the rack.

7.4.4 Close the cassette and snap it into the rack in solution #1 (fixative) attached to the inside of the PAXgene® Tissue Fixative/Stabilizer screw cap lid.

7.4.5 Fixing the tissue:

7.4.5.1 Verify which chamber contains solution #1 (fixative) and submerge the tissue cassette.

7.4.5.2 Screw the lid securely into place and shake the container to displace air bubbles in the tissue cassette.

7.4.5.3 Leave the cassette in solution #1 (fixative) for a minimum of 6 hours and a maximum of 24 hours at room temperature.

7.4.5.4 Record the time that the last aliquot from each specimen site is placed into fixative on the ***GTEx Tissue Recovery Form (PM-0003-F5).***

7.4.5.5 Any deviation from the specified fixation time must be documented on the ***GTEx Tissue Recovery Form (PM-0003-F5).***

7.4.5.6 After fixation is complete (6 to 24 hours), unscrew the PAXgene® lid and remove the lid with the attached fixed cassette.

7.4.6 Stabilizing the tissue:

7.4.6.1 Verify which chamber contains solution #2 (stabilizer) and submerge the tissue cassette that is attached to the lid.

7.4.6.2 Record the times on the ***GTEx Tissue Recovery Form (PM-0003-F5).***

7.4.6.3 Screw the lid securely into place and shake the container to displace air bubbles in the tissue cassette.

7.4.6.4 The tissue cassette is ready for transport. Refer to ***GTEx Kit Receipt, Supplies, and Shipping Procedure (OP-0001)*** for instructions of packing and shipping specimens.

7.4.6.5 Note: **If a cassette is inadvertently placed first in stabilizer rather than in fixative, it should be corrected immediately.** PAXgene® data shows that there is no impact on RNA quality if the error is corrected and the tissue is placed in the fixative chamber within 2 minutes. In any case, ALL specimens should be forwarded to the CBR regardless of an identified fixing/stabilizing error, even those over the 2 minute limit. Any error must be recorded in the comment field noting, as accurately as possible, the total minutes the tissue spent in the erroneous chamber.

NOTE: **If the PAXgene® container's embossed number does not agree with its sticker number DO NOT USE. Mark the mis-matched container with an X . Contact the CBR regarding return of these mis-labled containers.**

NOTE: **In the event of a comprised PAXgene® container (such as due to a spill), the BSS is directed to utilize a container leftover from a previous Aqua kit. The lot numbers of the PAXgene® containers are retained by the CBR and can be verified for expiration date if needed.**

7.5 Shipping Preparation

7.5.1 After completion of transfer of aliquots into PAXgene® solution #2, the aliquots are ready for packing and shipment.

7.5.2 Shipping preparation and instructions for each individual kit type can be found in the ***GTEx Kit Receipt, Supplies, and Shipping Procedure (OP-0001).***

7.6 **Documentation**

7.6.1 Ensure all paper documentation is completed as required per ***GTEx CRF Completion Procedure (PM-0003).***

7.6.2 Ensure all electronic data entry and verification is completed as required per the ***caHUB Data Entry Procedure (IT-0001).***

8.0 REFERENCES

8.1 GTEx Kit Receipt, Supplies, and Shipping Procedure, OP-0001

8.2 GTEx CRF Completion Procedure, PM-0003

8.3 caHUB Data Entry Procedure, IT-0001

9.0 ATTACHMENTS

9.1 GTEx Work Instruction For Postmortem Collection of Normal Tissues, PR-0004-W1 including dissection guides, the organ retrieval dissection table G3, and brain G4.

9.2 GTEx Work Instruction For Surgical Collection of Normal Tissues, PR-0004-W2

APPROVALS	
NAME / TITLE	

INITIATION/REVISION HISTORY			
Version #	DESCRIPTION OF CHANGE	AUTHOR	EFFECTIVE DATE