1.0 PURPOSE

The purpose of this procedure is to provide instructions biospecimen source sites (BSSs) for tissue collection. Specifically, this document and its associated **Work Instructions (PR-0004-W1** and **PR-0004-W4)** 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 Processing Procedure standard operating procedure (SOP) encompasses all GTEx-required activities to properly recover, process, and transfer tissue and organ aliquots for research purposes in the GTEx project.

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

- 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. If determined at the institutional level that research may be reviewed through an expedited process, is determined to be exempt or a waiver is granted, the tasked entity shall provide the documentation to support all such claims.

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

3.0 RESPONSIBILITY

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

All BSS staff responsible for biospecimen recovery are required to be trained to execute this procedure and all applicable **Work Instructions (PR-0004-W1** and **PR-0004-W4)**. 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 or tissue, for preservation by immersion in PAXgene® Tissue Fixative/Stabilizer or freezing on dry ice.

4.2 **BSS** – Biospecimen Source Site. Hospitals and/or research facilities tasked to collect, process, store, and ship clinically-annotated biospecimens and associated data for the in accordance with program-developed standard operating procedures and protocols.

4.3 **Case ID** – Unique alpha-numeric identification of a case. This coded ID is assigned by the BSS to all of the components of a collection kit at the time that a consented donor is identified. This ID subsequently identifies the genealogy of all specimens collected and/or aliquots derived from that case.

4.4 **CBR** – Comprehensive Biospecimen Resource. Centralized entity responsible for creating and managing the kit components shipped to the source sites. Also responsible for informatics support with the CDR, storage and processing all designated biospecimens (and associated inventory data), creation of slides and digital images, and maintenance of the image management system, as well as quality checks on collected and processed specimens.

4.5 **CRF** – Case Report Form. A paper or electronic form used to collect donor or case-related data. Most data for each patient is captured and/or documented on one or more CRFs. Each form captures all protocol-/project-related information and serves as the project's official data collection device.

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

4.7 **GTEx** – Genotype-Tissue Expression. An NIH Common Fund program initiated to determine how genetic variation controls gene expression and its relationship to disease. **GTEx aims to study human gene expression and regulation in multiple tissues, providing valuable insights into the mechanisms of gene regulation and, in the future, its disease-related perturbations. Genetic variation between individuals will be examined for correlation with differences in gene expression level to identify regions of the genome that influence whether and how much a gene is expressed. IATA** – The governing body that creates regulation for international air transport, including regulations controlling the transport of Dangerous Goods By Air. Persons handling transportation of biospecimens must be trained and certified to ship the appropriate hazard class according to International Air Transport Association (IATA)/International Civil Aviation Organization (ICAO) regulations. Packaging and shipping should conform to all governing regulations. Air shipments should conform to IATA standards. Ground shipments should conform to applicable federal standards. All personnel involved in shipping biological materials should be trained properly for both air and ground shipments.

4.8 **ID** – One or more characters used to identify, name, or characterize the nature, properties, or contents of a thing.

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

4.10 **LDACC** – Laboratory, Data Analysis, and Coordinating Center. Research institute responsible for the overall coordination of GTEx activities and molecular and statistical analysis laboratory.

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

4.12 **PAXgene® Tissue System** – Product manufactured by Qiagen for isolation and purification of intracellular RNA form that uses the 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. The fixation mode of the PAXgene Tissue Containers is based on an acidic, alcoholic fixation, without formalin and without cross-linking of biomolecules. The fixation reagent contains alcohols and an acid, in addition to other stabilization agents for rapid penetration and fixation. The stabilization reagents contain alcohol and other stabilization agents to stop fixation and to stabilize the specimen until processing.

4.13 **PPE** – Personal Protective Equipment. Safety equipment used in labs and all areas where potential contact with tissue or body fluids may occur. Equipment may include: gloves, eye protection, etc.

4.14 **PRC** - Pathology Resource Center. Centralized resource consisting primarily of expert pathologists whose function is to review biospecimens (either slides or images) collected for the program and assess their quality and fitness for use by researchers.

4.15 **Study Management Group** – A group whose mission is to provide integrated program management, operational, developmental, and analysis support for major programs requiring integration of biomedical science and informatics capabilities.

4.16 **SOP** – Standard operating procedure. An established procedure to be followed in carrying out a given operation or in a given situation.

4.17 **Specimen ID** – Identifies each biospecimen collected from a study subject and is used on all biospecimen containers (i.e., cryosettes, tissue cassettes, cryovials) and glass slides. Specimen identification consisting of a case ID (e.g., GTEX-123456) and a sequence number (e.g., 7890) that together form the final alpha-numeric ID; e.g., GTEX-123456-7890.

4.18 **TPM** – Technical Project Manager. The individual responsible for direct communications that concern submitted SOPs, forms or other documents, as well as ensuring that the tasked entity's relevant procedures and forms are assessed and controlled.

4.19 **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-1987) 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 Leidos Biomed team for 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 Personnel working with dry ice should protect their exposed skin by wearing cryo-gloves, lab coat, long pants that cover down to the top of footwear and non-porous closed-toed and closed-heeled footwear.

5.5 As defined by their institutional requirements, personnel should wear appropriate PPE at all times, such as a gown or lab coat, hair bonnet, mask, and shoe covers, latex or nitrile rubber gloves, etc.

5.6 For the safe transfer of blood, personnel collecting blood should 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 BSS TPM in conjunction with the PRC 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 according to institutional practices. Applicable documentation should be submitted to the BSS TPM as soon as possible for review by the Study Management Group Quality Management Team.

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** and **PR-0004-W4**, as applicable) for materials/equipment specifications.

7.0 PROCEDURES

7.1 Site Preparation

7.1.1 General

7.1.1.1 Provided that the guidance of the SOP is followed, all biospecimen collection procedures 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 – as based upon the size and layout of the facility - 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 initiating recovery procedures.

7.1.2.2.1 Upon receipt of collection kits, the BSS staff should use the appropriate discrepancy checklists (**GTEx Kit Receipt, Supplies, and Shipping Procedure, OP-0001** and associated forms), to review kit contents and upon detection of any discrepancy, should immediately report such findings to the 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 Each box of kit components contains a label on the outside of the box which identifies that kit type. This is referred to as the kit component ID (for example, GTEx collection, YELLOW KIT). **Note: The labels/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, which is applied at the donor level and is the same for all collected specimens within a case) and a sequence number (e.g., 7890, which is unique for each individual specimen/container collected)—that together form the final alpha-numeric Specimen ID; e.g., GTEX-123456-7890. Preprinted labels indicating these specimen IDs are provided within the overpack kit (see **GTEx Kit Receipt, Supplies, and Shipping Procedure, OP-0001** and its associated **Work Instructions** and **Checklists**) and are to be applied to the appropriate containers throughout case collection.

7.1.4.1.1 The recovery staff is responsible for collecting specimens and annotating their related information in the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**. This includes recording the time tissue is removed, the time aliquots are placed in solution #1 (fixative) and solution #2 (stabilizer) for the PAXgene® tissue preservation method, or the time placed on dry ice for frozen preservation (as applicable). **NOTE: The GTEx Organ Retrieval, Dissection, and Preservation Details Table, PR-0004-W1-G3 is a quick reference regarding specific aliquot locations and sizes.**

7.1.4.1.2 In addition to documentation within the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**, staff initials and the above timepoints are to be recorded on all PAXgene® tissue containers.

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:

- Two (2) 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 2 mL in each vacutainer.

7.1.5.1.3 Volume preference (if amount is limited): The blood should be collected in the following order: (1) one PAXgene®, (2) one yellow top, followed by (3) the second PAXgene® and (4) the second yellow top.

7.1.5.1.4 Collection preference: The collection preference is as follows: the femoral vein; subclavian vein, any peripheral vein and heart are other possible sites. Preference of location for the collection of blood will vary for organ donors [beating heart donors (usually arterial line) vs. non-beating heart tissue donors (venous route)]. The site of collection is to be recorded within the comments field in the **GTEx Tissue Recovery Form, PM-0003-F5.**

7.2 Tissue Procurement

7.2.1 Use the **GTEx Tissue Harvesting Work Instruction, PR-0004-W1** to collect the biospecimens.

7.2.2 If frozen tissues are to be collected per instruction by the BSS TPM, refer to **GTEx Work Instruction for Collection of Tissue and use of Dry Ice Prior to Storage, PR-0004-W4** for guidance on freezing aliquots from select organs/tissues (gastrocnemius muscle, esophageal mucosa, esophageal muscularis, skin, lung, heart, female breast tissue, prostate).

7.2.3 For non-brain donors, tissue collection must be started AND the first tissue must be placed into fixative within 8.0 hours of cardiac cessation or recorded time of death (observed or presumed). For brain donors, all tissues must be collected and placed into fixative within 24.0 hours of cardiac cessation (observed or presumed).

7.2.4 All designated GTEx tissues including all core tissues should be collected regardless of disease state**.** The core tissues are the tissues that are absolutely required from each collected GTEx case for the case to be considered complete, which means the sites must attempt to correctly procure and submit these core tissues from each GTEx donor. The five required GTEx core tissues are skin (leg), adipose tissue, skeletal muscle (gastrocnemius), tibial nerve and tibial artery. **Observed abnormalities should be noted in the comment section for each tissue retrieved.**

NOTE: In the event that the GTEx donor was a transplant recipient (either human or xenotransplant, as noted in question #15 of the Donor Eligibility Form), tissue should not be collected from the transplanted organ/tissue or the native organ/tissue of the same type.

7.2.5 The order of organ/tissue removal is left to the discretion of the BSS. However, when a brain is to be collected, it must be removed last per the **GTEx Tissue Harvesting Work Instruction, PR-0004-W1**. ***NOTE: The brain should NOT be collected if the donor was on a ventilator for ≥24.00 hrs.***

7.2.6 The tissue location for each aliquot is specified in the **Work Instruction(s)**. Any deviation from the preferred location must be documented on **GTEx Tissue Recovery Case Report Form, PM-0003-F5**.

Note: The 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.

7.2.7 To minimize contamination between tissues, supplies must not be re-used for multiple tissue dissections.

- As each organ/tissue is removed from the donor, place it on a new, clean cutting tray/surface.
- For EACH organ or tissue type:
  - Use a new disposable or reusable cutting board
  - Use new gloves
  - Use a new or normal saline–cleaned scalpel handle
  - Use a new blade
  - Use new forceps

7.2.8 **Special instructions for Hair and Brain removal**

7.2.8.1 **Hair**

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

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

7.2.8.2 **Brain and Brain Stem**

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

7.2.8.2.2 For brain removal, see **GTEx Tissue Harvesting Work Instruction, PR-0004-W1,** section 4.3.6.2.3.

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 in the appropriate comment field (except as noted above for transplant recipients).**

7.3.3 Determine if frozen tissue aliquots are to be collected, and if so, ensure preparations are in place as per the requirements in **GTEx Work Instruction for Collection of Tissue and use of Dry Ice Prior to Storage, PR-0004-W4**.

7.3.4 Aliquot size:

7.3.4.1 For aliquots to be preserved in PAXgene® Tissue fixative, aliquot size depends upon the tissue or organ and is specified in the organ-specific sections in the specific **Working Instructions, PR-0004-W1**. The aliquot thickness must be ≤ 4 mm. However, for the brain (cerebrum and cerebellum), 5 mm cubes are to be taken as aliquots.

7.3.4.2 For aliquots to be preserved on dry ice, aliquot size is as noted in **Work Instruction, PR-0004-W4.**

7.3.4.3 The dimensions of each aliquot should be measured by the BSS.

7.3.4.3.1 Any measurement that differs from the recommended size must be documented in the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**.

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

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

7.4 PAXgene® Tissue Preservation

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

Note: All aqua-colored tissue cassettes and containers will arrive pre-labeled with a riveted Specimen ID. This ID is to be matched to the appropriate pre-printed label to be applied to the container lid.

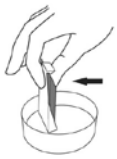
7.4.1 Recover appropriate tissue; prepare aliquot(s), and place aliquot(s) into aqua-colored tissue cassette(s). If the cassette has a central knob-like elevation on the

base and/or the lid, avoid placing the tissue on it, as it may prevent adequate fixation.

7.4.2 Fixing the tissue:

7.4.2.1 Properly insert the tissue cassette into the screw cap/rack assembly:

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



7.4.2.1.2 Attach the tissue cassette to the rack.

7.4.2.2 Verify which chamber contains solution #1 (fixative) and submerge the rack holding the tissue cassette into this chamber.

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

7.4.2.4 **Leave each cassette in solution #1 (fixative) for a minimum of 6.0 hours and a maximum of 24.0 hours at room temperature.**

7.4.2.5 Record the time that the last aliquot from each specimen site is placed into fixative on the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**.

NOTE: **Any deviation from the specified fixation time must be documented on the GTEx Tissue Recovery Case Report Form, PM-0003-F5.**

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. Any error must be recorded in the comment field noting, as accurately as possible, the total minutes the tissue spent in the erroneous chamber.

7.4.3 Stabilizing the tissue:

7.4.3.1 After fixation is complete (6.0 to 24.0 hours), unscrew the PAXgene® lid and remove the lid with the attached tissue cassette.

Note: It is acceptable for all cassettes to be transferred into stabilizer at the same time, provided that the 6.0 hour minimum fixation time is calculated from the time the last specimen was placed into fixative and the 24.0 hour maximum fixation time is calculated from the time the first specimen was placed into fixative.

7.4.3.2 Verify which chamber contains solution #2 (STABILIZER) and submerge the tissue cassette that is attached to the lid in the chamber containing solution #2.

7.4.3.3 Record the times of placement into solution #2 on the **GTEx Tissue Recovery Case Report Form, PM-0003-F5** and container lid(s).

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

7.4.3.5 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.

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

NOTE: In the event of a compromised PAXgene® container (such as due to a spill), the BSS is directed to utilize an unused container leftover from a previous Aqua kit. Check the expiration date before using the container. The lot numbers of the PAXgene® containers are retained by the CBR and can be verified for expiration date if needed. Please ensure that a label has been applied to the replacement container.

7.5 **Frozen Tissue Preservation**

7.5.1 As applicable, refer to **GTEx Work Instruction for Collection of Tissue and use of Dry Ice Prior to Storage, PR-0004-W4** for guidance on freezing aliquots from select organs.

7.5.1.1 Tissue aliquots for preservation on dry ice should be collected in conjunction with those preserved in PAXgene® Tissue fixative, using the same procedures for sequence of tissue removal.

7.5.1.2 Tissue to be frozen on dry ice should be collected immediately after the corresponding standard aliquots for PAXgene® fixation have been collected.

7.6 **Shipping Preparation**

7.6.1 After completion of transfer of applicable aliquots into PAXgene® solution #2 and the preservation of frozen aliquots on dry ice (as applicable), the aliquots are ready for packing and shipment.

7.6.2 Shipping preparation and instructions for each individual kit type can be found in the **GTEx Kit Receipt, Supplies, and Shipping Procedure, OP-0001** and its associated **Work Instructions**.

7.7 **Documentation**

7.7.1 All paper documentation should be completed as required per **GTEx Case Report Form Completion Procedure, PM-0003**.

7.7.2 All electronic data entry and verification should be completed as required.