1.0 PURPOSE

1.1 The purpose of this document is to provide step by step instructions to Biospecimen Source Sites (BSSs) for the collection and preservation of frozen tissue specimens for the Genotype-Tissue Expression (GTEx) project.

2.0 ENVIRONMENTAL HEALTH & SAFETY

2.1 Universal Precautions (CDC-1987) are to be used for all phases of organ/tissue procurement, dissection, processing, and handling.

2.2 Use appropriate precautions and Personal Protective Equipment (PPE) when working with dry ice.

2.3 The International Air Transport Association (IATA) Dangerous Goods Regulations require that dry ice be packaged in accordance with packing instruction 904, and be labeled with Dry Ice, UN 1845, Class 9 Miscellaneous label (see **GTEx Work Instruction for Dry Ice Preserved Tissue Collection Receipt and Shipping (Pink Kit), OP-0001-W6)**.

2.4 Frozen tissue is shipped under IATA Class 6.2 regulations for UN3373, Biological Substances, Category B.

3.0 MATERIALS/EQUIPMENT

3.1 Refer to **GTEx Tissue Processing Procedure, PR-0004** for specific materials.

3.2 Materials to be provided by the BSS:

- PPE including lab coat, gloves, and safety glasses
- Surgical instruments (complete standard set), which includes the following: scalpels and various types of scissors

  NOTE: The use of disposable equipment is preferred.
- Cryo-Apron and Cryo-Gloves to support handling or access to dry ice
- Transport containers (such as a Styrofoam™ cooler) for dry ice
- Dry ice pan (or Styrofoam™ container) and cover
- Adequate quantities of crushed or block dry ice in the Styrofoam™ container to maintain a one-inch thick bed under the entire bottom surface of the ThermalTray™ LP
- ThermalTray™ LP (Biocision)

4.0 PROCEDURE

4.1 OVERVIEW OF BIOSPECIMEN COLLECTION

The GTEx project requires each BSS, after appropriate training, to independently collect, process, store and ship tissues in accordance with project specific and approved Standard Operating Procedures (SOPs).

4.2 SITE PREPARATION

Efficient organization of the recovery team is essential. The organ/tissue recovery, dissection, and aliquot preparation and preservation processes should be optimized at each site according to its capabilities. Consideration should be given to the optimal size of the recovery team, the dissection and aliquot recovery areas, storage vessels, temperature requirements, transportation requirements, data collection and the space available.

4.3 TISSUE PROCUREMENT

4.3.1 General

The rapid recovery of organs/tissues is the goal of this procedure.

4.3.1.1 Tissue aliquots for preservation on dry ice should be collected in conjunction with those of the GTEx project preserved in PAXgene® Tissue fixative, i.e., from the same donors, locations, and using the same procedures for sequence of tissue removal. For each tissue type, the frozen aliquots should be preserved immediately after the corresponding tissue aliquots have been placed in PAXgene® Tissue fixative.

- For all tissues, preservation in PAXgene® Tissue fixative takes precedence over preservation on dry ice.

4.3.1.2 **The sites should collect these additional aliquots from at least five (no particular order or preference) and up to seven of the following tissues** from each consented (non-brain) donor:

- gastrocnemius muscle,
- esophagus, mucosa,
- esophagus, muscularis,
- skin (leg),
- lung,
- heart, left ventricle,
- sex-specific organs: breast tissue (female only) or prostate

4.3.1.3 Up to 6 additional aliquots per tissue type should be sampled for frozen preservation. Aliquots from sampled tissues should be placed in pre-labeled white cryosettes, frozen on dry ice, and subsequently stored in 2” labeled boxes pre-chilled on dry ice. These are to be maintained and locally transported on dry ice, and then stored within a designated -80^{o}C freezer until shipment. The frozen aliquots should be shipped on dry ice to the Comprehensive Biospecimen Resource (CBR) for subsequent storage within an LN2 vapor phase unit.

4.3.1.4 To qualify as a frozen case, a minimum of five tissues of the listed tissue types must be collected. If it is the team's initial assessment that there is an insufficient amount of tissue available for at least two contiguous aliquots (20mm x 10mm x ≤4mm each) from a minimum of five tissues to be collected, the GTEx procurement team should not move forward with the frozen collection. If there is an insufficient amount of tissue to allow for the collection of all requested frozen aliquots (six for each of the above tissues) after the PAXgene® Tissue fixative aliquots have been taken, collection of at least two aliquots from at least five of the specified tissues should be performed. If there is enough for four aliquots, do collect them, storing them in two cryosettes with two aliquots each.

4.3.1.5 To minimize contamination between tissues, <u>supplies must not be re-used</u> for multiple tissue dissections:

- Instruments used for tissues preserved in PAXgene® Tissue fixative may be used for the corresponding frozen tissues.
- As each organ/tissue is removed from the donor, place it on a new, clean, cutting tray/surface. The cutting tray may be reused for the preparation of corresponding frozen tissues.
- For <u>EACH</u> 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

4.3.1.6 Pre-chill cryobox that is to be used for sample storage in the transport container. Label this box with the assigned GTEx case ID.

4.3.2 **Documentation**

Capture biospecimen-related data on the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**, pink kit section.

4.3.3 **Organ Priority**

The order of organ removal is left to the discretion of the individual BSSs, and should follow the standard sequence used in GTEx procurement.

4.3.4 **Aliquot Location**

4.3.4.1 The preferred location is immediately adjacent to the region for PAXgene® Tissue fixative.

4.3.4.2 The two aliquots collected per tissue and stored within a single cryosette should be contiguous.

4.3.4.2.1 The only exception to the contiguous requirement is the collection of breast tissue. The only requirement for the breast

tissue is that all aliquots for frozen fixation be collected from the same breast.

4.3.4.3 Any deviation from the preferred tissue location, as designated in **GTEx Tissue Harvesting Work Instruction, PR-0004-W1**, must be documented on the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**. This should be done by noting the actual location either by checking one of the listed locations or manually entering the location into the "comment" field.

4.3.5 **Aliquot Amount**

4.3.5.1 A ruler or the cutting board marker should be used to measure aliquot size.

4.3.5.2 For each tissue type (minimum of 5), a minimum of one 20mm x 20mm x ≤4mm piece of tissue (bisected into two 20mm x 10mm x ≤4mm aliquots in advance of freezing) is required for frozen preservation. If less than this amount is collected, it does not count towards the "minimum five" target tissue aliquot collection.

4.3.5.3 A maximum of three 20mm x 20mm x≤4mm pieces (bisected into six 20mm x 10mm x ≤4mm aliquots) are to be collected.

4.3.5.4 Any deviation from the preferred aliquot size must be documented on the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**.

4.3.6 **Aliquot Preparation**

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

4.3.6.2 For specimens embedded in or adjacent to adipose tissue (skeletal muscle, skin), dissect/tease off peripheral fat as thoroughly as feasible without damaging the target tissue. The 4 mm thickness should not be exceeded as it will lead to crushing of the aliquot within the tissue cryosette.

4.3.6.3 Two aliquots of 20mm x 10mm x ≤4mm should be frozen in each labeled cryosette. Use up to three cryosettes as needed to accommodate all aliquots (up to six total) collected for dry ice preservation.

4.3.6.4 If the GTEx collection team suspends dry ice preservation after initiated (such as due to inadequate tissue), the aliquots collected up until that point for frozen preservation should be discarded according to the established local (BSS) procedures for the destruction of biological specimens.

4.3.6.5 Failure to achieve collection of at least two contiguous bisected aliquots of the requested size (20mm x 10mm x ≤4mm) for at least five target tissue sites represents a deviation from the SOP and the reason for this should be clearly recorded in the comment field of **GTEx Tissue Recovery Case Report Form, PM-0003-F5**.

NOTE: Fusion of adjacent aliquots within a single cryosette during dry ice preservation may be anticipated and does not constitute an SOP deviation.

4.3.7 **Recommended Dissection Process**

Refer to the **GTEx Tissue Processing Procedure, PR-0004, GTEx Tissue Harvesting Work Instruction, PR-0004-W1,** and **GTEX Organ Retrieval, Dissection, and Preservation Details Table, PR-0004-W1-G3** for guidance on preferred location of tissues to be sampled. As noted, corresponding tissue designated for frozen preservation is to be collected adjacent to specimens submitted in PAXgene® Tissue fixative.

4.4 **Freezing on Dry Ice Using ThermalTray™ LP**

4.4.1 **Transport of Trays**

4.4.1.1 The ThermalTray™ LP should be transported in an appropriate container (such as a Styrofoam™ cooler) with dry ice. This will expedite the cooling of the tray for use.

4.4.2 **Prior to Sample Acquisition –** Preparation of dry ice pan with the ThermalTray™ LP

4.4.2.1 Add enough crushed or block dry ice in the pan to create a one-inch thick bed to underlie the entire bottom surface of the ThermalTray™ LP.

4.4.2.2 Wearing appropriate gloves, remove the ThermalTray™ LP from transport container and transfer to the dry ice pan.

4.4.2.3 The dry ice in the pan should be replenished as needed throughout the collection to ensure the one-inch thick bed is maintained until the collection and preservation of all samples is done and the samples have been subsequently transferred to storage.

4.4.3 **Post-Recovery Dissection and Transport to Storage**

4.4.3.1 With clean forceps, place the specimen aliquots to be frozen into an empty labeled cryosette and secure lid.

4.4.3.2 Place the cryosettes containing the tissue aliquots onto the ThermalTray™ LP.

4.4.3.3 The cryosette containing the tissue aliquots should be maintained on the ThermalTray™ LP for a minimum of 10 minutes.

4.4.3.4 The frozen samples may be maintained on dry ice while the remaining samples are being processed and frozen. A cover (Styrofoam™ lid) should be placed over the dry ice pan between sampling to maintain temperature. The ThermalTray™ LP will maintain samples at approximately -78^{o}C while in direct contact with the dry ice.

4.4.3.5 After completion of the collection, place the cryosettes into a pre-chilled slotted 2” labeled cryobox and maintained within the transport container on dry ice until storage.

4.4.3.6 The remaining dry ice in the pan should be safely poured into the transport container.

4.4.3.7 Transfer the container with specimens to the laboratory for storage.

4.4.4 **Post-Recovery Transfer to Long-term -80° Freezer**

4.4.4.1 Upon returning to the laboratory, place the transport container with cryoboxes near the long-term storage -80°C freezer.

4.4.4.2 Annotate on the box the number of specimens within each box and ensure that a box inventory has been recorded.

4.4.4.3 While wearing proper PPE, carefully remove the cryobox from the transport container.

4.4.4.4 Immediately place the cryobox into the designated GTEx long-term storage -80°C freezer.

4.4.4.5 Document the location of the frozen samples according to standard BSS institutional practices.

4.4.4.6 Follow the institutional handling and disposal procedures for dry ice removal and disposal.

NOTE: Do Not Use the dry ice used during specimen collection for packing the shipment as it may evaporate at a quicker rate after being exposed to the air during the collection.

4.5 For shipping of samples, please refer to **GTEx Work Instruction for Dry Ice Preserved Tissue Collection Receipt and Shipping (Pink Kit), OP-0001-W6.**