## 1.0 PURPOSE

The purpose of this Work Instruction is to provide step by step instructions to the biospecimen source sites (BSSs) for the collection of tissue from postmortem/organ procurement cases, as described in the **GTEx Tissue Processing Procedure, PR-0004**. Specifically, this document describes the proper removal, sectioning, and preservation of specified donor organs and tissues and associated blood draws for the Genotype-Tissue Expression (GTEx) project.

## 2.0 ENVIRONMENTAL HEALTH & SAFETY

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

2.2 See **GTEx Kit Receipt, Supplies, and Shipping Procedure, OP-0001** for specific shipping instructions.

2.3 Tissue transported in media, unprocessed blood, and brain tissue are shipped under IATA Class 6.2 regulations for UN3373, Biological Substances, Category B. Tissue transported in the PAXgene® Tissue Containers are transported under IATA Class 3 with subsidiary risks 6.1 and 8 for UN3286, Flammable, Liquid, Toxic, Corrosive, N.O.S (methanol, acetic acid, glacial), packing group II. Persons packaging the shipment must be trained in the use, handling, and shipping of Dry Ice (UN1845).

## 3.0 MATERIALS/EQUIPMENT

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

3.2 The BSS will be responsible for providing the following (non-kit) materials:

- Personal Protective Equipment (PPE)
- Surgical instruments (complete standard set), which may include the following: scalpels, rongeurs, rocker knife, and various types of scissors
- Needle, 18 gauge

## 4.0 PROCEDURE

### 4.1 OVERVIEW OF BIOSPECIMEN COLLECTION

The GTEx project requires each BSS, after appropriate training, to independently collect tissues and corresponding donor blood.

### 4.2 SITE PREPARATION

As the rapid recovery of organs/tissues is the goal of this procedure, efficient organization of the recovery team is essential. The blood collection, organ/tissue recovery, dissection, and aliquot preparation and preservation processes should be optimized at each BSS according to its capabilities. Consideration should be given to the optimal size of the recovery team, the dissection and aliquot recovery areas, and the space available.

## 4.3 TISSUE PROCUREMENT

### 4.3.1 General

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).

***NOTE: The brain should NOT be collected if the donor was on a ventilator for ≥24.00 hrs.***

***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*.**

### 4.3.2 Documentation

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

### 4.3.3 Organ Priority

The order of organ removal is left to the discretion of the individual BSSs, with TWO important distinctions:

- The brain **must** be **removed last**.
- If there is difficulty dissecting the coronary artery, it should be removed after the brain.

### 4.3.4 Aliquot Location

Any deviation from the preferred tissue location of collected aliquots 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 it into the "comment" field.

### 4.3.5 Aliquot Preparation

The aliquot size depends upon the organ and is specified in the organ-specific sections below.

A ruler or the cutting board marker should be used to measure the aliquot size. It is important to follow the required aliquote size for tissues to ensure that they are properly fixed. Any deviation to the aliquot size should be documented on the **GTEx Tissue Recovery Case Report Form, PM-0003-F5**. This should be done by noting the deviation in the "comment" field.

#### 4.3.5.1 Preferred Aliquot Size

In general, contiguous aliquots should be obtained per organ/tissue site.

4.3.5.1.1 For tissue to be preserved in the PAXgene® Tissue fixative, the preferred aliquot size is 10 mm x 10 mm x ≤4 mm; two aliquots per cassette; one cassette for histology (CBR) and one cassette for molecular studies (LDACC). The preferred thickness range is 3 to 4 mm.

4.3.5.1.2 To obtain these aliquots, one suggestion is that a 20 mm x 10 mm x ≤8 mm tissue slice be divided into two 10 mm x 10 mm x ≤8 mm adjacent portions, each of which will be further divided into 10 mm x 10 mm x ≤4 mm contiguous aliquots. At the discretion of the BSS, the aliquots may be prepared in serial slices from a larger portion of tissue and 'cut down' to the appropriate aliquot dimension if that is deemed more feasible when working with unfixed tissue. Alternate aliquot slices should be placed in cassettes for histology processing at the CBR and molecular analysis at the LDACC.

4.3.5.1.3 In order to have aliquots for histology and molecular studies be as comparable as possible, it is important that from each 10 mm x 10 mm x ≤8 mm specimen, one 10 mm x 10 mm x ≤4 mm aliquot be placed in the cassette for molecular studies (LDACC) and the other 10 mm x 10 mm x ≤4 mm contiguous aliquot placed in the cassette for histological examination. That is, in each cassette the two aliquots are adjacent but not contiguous with each other; however, they are contiguous with their "sister aliquot" in the other cassette.

4.3.5.1.4 The same approach would apply to aliquots from linear specimens, e.g., artery and nerve; contiguous aliquots would not be placed in the same cassette.

4.3.5.1.5 For mucosal tissues or skin, the 10 mm x 10 mm x thickness (≤ 4 mm) aliquots can be obtained by dividing a 20 mm x 20 mm x thickness tissue strip into four 10 mm x 10 mm squares and placing two adjacent squares into the "histology" cassette and the other two adjacent squares into the "molecular" cassette. Or two adjacent 20 mm x 10 mm x thickness slices (≤4 mm), can be divided to each yield two contiguous aliquots with dimensions of 10 mm x 10 mm x thickness. Each cassette should contain two 10 mm x 10 mm x thickness aliquots.

4.3.5.1.6 For most organs, the preferred aliquot size is 10 mm x 10 mm x ≤4 mm tissue slices. Equivalent alternative sizes such as two (2) 10 mm x 5 mm x ≤4 mm strips may be employed as warranted. Aliquots should be taken as close together as possible. The **4 mm thickness should not be exceeded** as it will lead to crushing of the aliquot in the tissue cassette. However, for the brain (cerebrum and cerebellum), 5 mm cubes are routinely to be taken as aliquots.

4.3.5.1.7 Trim unwanted adipose tissue. Adipose tissue that encases other tissues should be dissected off so that it does not "contaminate" the molecular characteristics of the designated aliquot, e.g., coronary artery, tibial artery, nerve, adrenal, and pancreas.

4.3.5.1.8 When placed into fixative, solution 1, shake the closed container to ensure air bubbles are removed from the cassette to prevent focal inadequate fixation.

**Note**: If a cassette is inadvertently placed first in stabilizer rather than in fixative, this 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. All specimens should be forwarded to the CBR regardless of an identified fixing/stabilizing error, even those over the 2 minute limit.

**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 for container returns.

**NOTE**: In the event of a compromised PAXgene® container (such as due to a spill), the BSS is directed to utilize a container left over 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. Please ensure that a label with appropriate identifier has been applied to any replacement container used.

4.3.5.1.9 If collecting frozen tissues follow **GTEx Work Instruction for Collection of Tissue and use of Dry Ice Prior to Storage, PR-0004-W4.**

4.3.6 **Recommended Dissection Process and Order**

4.3.6.1 **Hair**

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

4.3.6.1.1 Before the donor's head is packed in ice (see section 4.3.6.2.1) and while the hair is still attached to the head, tie a section of hair approximately 4 mm thick with a 5- to 6-in length of plain (nonwaxed, unflavored) dental floss near the scalp. Once the knot is tightened on the hair strand, securely grasp the hair strand and pull it from the head so that the root of the hair is still attached. If the hair does not separate from the head, use a rongeur to scoop it from the scalp.

4.3.6.1.2 Ensure that the hair is dry before it is placed in the glassine envelope. If needed, dry the hair by blotting with absorbent paper towels used in the lab or to avoid contamination, allow to air dry. Once hair is dry, place it in the glassine envelope.

4.3.6.1.3 Apply a label with a full Specimen ID from the Label Sheet to the glassine envelope, place the glassine envelope into the manila envelope and seal it shut.

4.3.6.1.4 Apply a second label with matching full Specimen ID on the manila envelope.

4.3.6.1.5 Place the manila envelope containing the hair on top of the Styrofoam™ inner lid before the brain kit (**Green Kit**) is closed. Do not place the hair inside the cooled chamber of the **Green Kit**.

4.3.6.2 **Brain and Brain Stem**

4.3.6.2.1 Preparation: Immediately after the hair is removed, pack the head with five (5) zip-locked bags of ice, filled to capacity. This is approximately 4 lbs of wet ice. The time the brain is placed on ice should be documented on the **GTEx Tissue Recovery Case Report Form, PM-0003-F5.** Place one bag under the head, the second and third bags on each side of the head, the fourth bag over the face, and the fifth bag on the dome of the head. Place a plastic-backed pad around the iced head and tape and/or clamp it to secure the ice bags and keep the head cold since the brain is removed last and the collection of other organs may take hours to complete.

4.3.6.2.2 Time of removal: The brain should be removed last during the autopsy. Once the brain is removed, sampling and packaging the brain takes priority over any other organs waiting for processing. Keep the sampling, handling, and packing time of the brain to a minimum.

4.3.6.2.3 Procedure: Retract the cerebellum superoanteriorly and, using the longest scalpel available with a relatively thin blade, go through the foramen magnum and cut to include the cervical spinal cord.

4.3.6.2.4 Packing: Pack and ship the brain, brain stem and hair according to the instructions in the **GTEx Kit Receipt, Supplies, and Shipping Procedure, OP-0001** and associated work instructions.

4.3.6.3 **Whole Blood Collection**

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

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

4.3.6.3.2 **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** and associated work instructions**)**. 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. (Two extra yellow top tubes are provided in the kit 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.

4.3.6.3.3 **Blood tube preference** (if blood is limited): Blood should be collected in the following order: (1) one PAXgene® RNA tube, (2) one yellow top, (3) the second PAXgene® RNA tube and (4) the second yellow top.

4.3.6.3.4 **Collection site preference**: The collection site 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).

4.3.6.4 **Blood Collection Transfer Device**

4.3.6.4.1 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.

4.3.6.4.2 Use a new Blood Transfer Device for the delivery of blood into each different type of vacutainer. Use one transfer device to fill the Yellow top vacutainers, and a second to fill the PAXgene® RNA blood vacutainers.

4.3.6.4.3 Ensure the full blood collection is transferred to blood tubes. If the vacutainer will not accept the full mL, fill an additional tube.

4.3.6.5 **Inverting Vacutainer Tubes**

Invert all blood collection tubes 10 times immediately after the tubes have been filled to ensure adequate mixing of the blood and additives within the tubes (See Diagram 1 below).

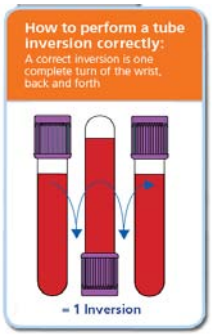



*Diagram 1: Illustration of how to perform a tube inversion (BD Blood and Urine Collection) http://www.bd.com/vacutainer/labnotes/Volume19Number1/tube_inversion1.asp)*

4.3.6.6 **Skin, Leg**

4.3.6.6.1 **Preferred Location:** Left or right leg (designate side) 2 cm below patella on medial side. If not available, an "other" location should be chosen.

4.3.6.6.2 **Procedure:** After cleaning with alcohol two times, remove a portion of skin and send to aliquot processing station. From the skin portion, prepare two 4 mm squared aliquots, place in fibroblast tube containing culture medium, and tightly seal with parafilm for shipment directly to the LDACC (Yellow Kit). Return to skin tissue and prepare the remaining aliquots of skin and subcutaneous adipose tissue.

4.3.6.6.3 **Skin for Fibroblast Culture**

4.3.6.6.3.1 **Skin, fibroblast culture:** Two 4 mm x 4 mm x thickness. *Thickness not to exceed **4 mm.*** **Underlying visible subcutaneous fat should be trimmed off**.

4.3.6.6.4 **Skin Tissue for Fixation in PAXgene®**

4.3.6.6.4.1 **Preferred Aliquot:** 10 mm x 10 mm x thickness slices; suggest a 20 mm x 10 mm x thickness (≤4 mm) slice, divided to yield two contiguous aliquots with dimensions of 10 mm x 10 mm x thickness (≤4 mm). Each cassette should contain two 10 mm x 10 mm x thickness aliquots. **Underlying visible subcutaneous fat should be trimmed off**.

4.3.6.7 **Skin, suprapubic (non-sun exposed) area**

4.3.6.7.1 **Preferred Location:** Extension of the abdominal incision to the suprapubic area avoiding pubic hair. Visible antiseptic should be gently removed with an alcohol swab.

4.3.6.7.2 **Preferred Aliquot:** 20 mm x 10 mm x thickness portion, divided into two adjacent 10 mm x 10 mm x ≤4 mm thickness aliquots. Each cassette should contain two 10 mm x 10 mm x thickness aliquots. **Underlying visible subcutaneous fat should be trimmed off**.

4.3.6.8 **Adipose Tissue (Subcutaneous)**

4.3.6.8.1 **Preferred Location:** Subcutaneous tissue beneath the leg's skin sample

4.3.6.8.2 **Preferred Aliquot:** The aliquot is 10 mm x 10 mm x ≤4 mm. Each cassette should contain two 10 mm x10 mm x ≤4 mm aliquots.

4.3.6.8.3 It may be easier to remove the skin first by dissecting it off subcutaneous fat. Then the fat can more easily be dissected from the underlying tissue.

4.3.6.8.4 Using the thumb against the dissecting tray's surface, attempt to remove as much of the fat globular component as possible by compressing the tissue to about a sheet of adipose tissue ≤4 mm. This removes fat/oil globules and retains adipose cellular tissue, which improves preservation and molecular recovery.

4.3.6.9 **Heart**

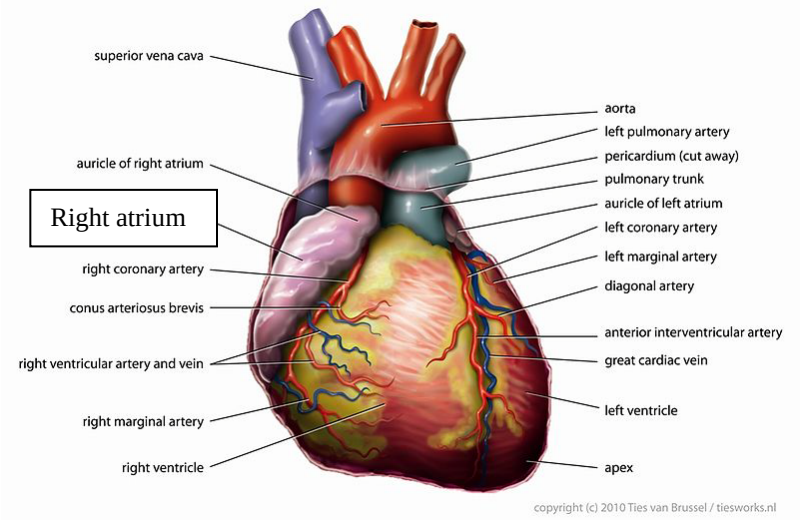
4.3.6.9.1 **Preferred Location: Anterior left ventricle**, 1 cm above apex and 1 cm from left anterior descending coronary artery if possible.

4.3.6.9.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤8 mm slice, divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.9.3 **Preferred Location: Right atrial appendage, tip.**

4.3.6.9.4 **Preferred Aliquot:** 10 mm x 10 mm x thickness slice, divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots. External fat should be trimmed off. **If the tip of the atrial appendage is fatty or discolored take specimens more proximally.**

**Right Atrial Appendage (Right atrium) Dissection Guide** (Diagram 2)




*Source: adapted from Wikimedia Commons:* Anatomy of the human heart, in English, by Ties van Brussel / http://www.tiesworks.nl; copyright holder releases this work into the public domain.

4.3.6.10 **Aorta**

4.3.6.10.1 **Preferred Location:** Ascending aorta or other thoracic regions (non-atherosclerotic).

4.3.6.10.2 **Preferred Aliquot**: 20 mm x 20 mm x thickness portions divided into four adjacent 10 mm x 10 mm x thickness (≤4 mm) aliquots. Each cassette should contain two 10 mm x 10 mm x thickness (≤4 mm) aliquots. Trim off any periaortic fat or fibrous tissue. If "thickness" exceeds 4 mm, the aliquot should be trimmed from the advential surface 'inward' to a thickness of ≤4mm.

4.3.6.11 **Mammary Tissue (Breast, female and male)**

4.3.6.11.1 **Preferred Location:** Incise the deep surface of the right (if present; if not, use left) breast, after the chest wall soft tissues have been reflected anterolaterally after the standard "Y" shaped incision (males) or superiorly after the sub-mammary "C" shaped incision (females). As closely as possible try to aim for the central breast, subareolar (this may be difficult due to distortion induced by position). Dissect to an estimated depth 1-2 cm deep to the overlying skin/nipple (i.e., 1-2 cm 'under' the skin surface of the nipple region).

**Note:** A precise depth of incision from the deep surface cannot be provided due to the wide variability in mammary size from patient to patient in females; in men, and some elderly women, this may be the general thickness of the reflected soft tissue. Clinical and professional judgment may be necessary.
**Note:** To avoid further incisions, an alternative route for mammary tissue is via the midline incision with lateral blunt dissection (for organ donors, beating heart donors).
**Note:** In many patients, the breasts have abundant fat: try to find areas with more fibrous (grey-white) streaks and include these in the aliquots.

4.3.6.11.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤4 mm. Obtain two 10 mm x 10 mm x ≤8 mm portions and divide along the 8mm axis: place one half of each (i.e., a 10 mm x 10 mm x ≤4 mm aliquot) in each of the 2 designated cassettes. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.11.3 **Note**: It is important that the thickness NOT exceed 4mm due to the high fat content in mammary tissue. If possible, strive for 3-4 mm thickness.

4.3.6.12 **Lung**

4.3.6.12.1 **Preferred Location:** Inferior segment of left upper lobe, 1 cm below the pleural surface. If this region is grossly abnormal, select the aliquots from a grossly normal region in any lobe, excluding the right middle lobe. **Avoid any large arteries, veins, and bronchi.**

4.3.6.12.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤8 mm slices divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.13 **Esophagus**

4.3.6.13.1 Cut a full-thickness circumferential section of the white squamous esophagus about 20 mm long.

4.3.6.13.2 Open the tubular structure and using scissors, sharply separate the white mucosa from the nonwhite muscularis.

4.3.6.13.3 Separate the mucosa from the muscularis. Trim any fat from these.

4.3.6.13.4 Divide both squamous mucosa and muscularis into aliquots.

4.3.6.13.5 Further dissect each portion into adjacent 10 mm x 10 mm x thickness (≤4 mm) aliquots for preservation. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.13.6 **Squamous Mucosa**

4.3.6.13.6.1 **Preferred Location:** Squamous region **(distal/lower third), at least 4 cm above gastroesophageal junction**. Getting well above the GE junction avoids the area of most gastric reflux and hiatal hernias.

4.3.6.13.6.2 **Preferred Aliquot**: 10 mm x 10 mm x thickness aliquots from mucosa (≤4 mm). Each cassette should contain two 10 mm x 10 mm x thickness aliquots.

4.3.6.13.7 **Muscularis**

4.3.6.13.7.1 **Preferred Location:** Squamous region **(distal/lower third), at least 4 cm above gastroesophageal junction**. **Avoid the mid-esophagus, where skeletal muscle is found.**

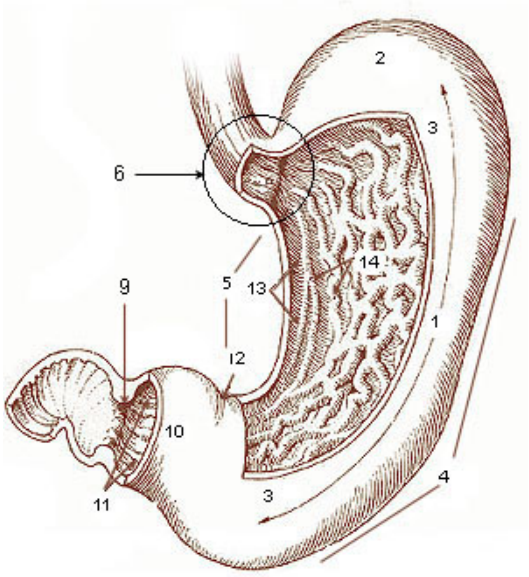
4.3.6.13.7.2 **Preferred Aliquot:** 10 mm x 10 mm x thickness aliquots (≤4 mm) from muscularis. **If "thickness" exceeds 4 mm, the aliquot should be trimmed from the advential surface 'inward' to a thickness of ≤4mm.** Each cassette should contain two 10 mm x 10 mm x thickness aliquots.

4.3.6.14 **Gastroesophageal junction**

4.3.6.14.1 **Preferred Location:** Gastroesophageal junction: the lowest portion of the esophagus just proximal to the stomach. **Obtain only muscularis propria**; discard mucosa; avoid stomach.

4.3.6.14.2 **Preferred Aliquot:** 20 mm x 10 mm x thickness portion, divided into two adjacent 10 mm x 10 mm x thickness (≤4 mm) aliquots. Each cassette should contain two 10 mm x 10 mm x thickness aliquots.

**Gastroesophageal junction "6" Dissection Guide** (Diagram 3)




*Source: adapted from:* **training.seer.cancer.gov:**
Work of the United States Government Gray's *subject #247 1161*

4.3.6.15 **Liver**

4.3.6.15.1 **Preferred Location:** Central right lobe, 1 cm below capsule. **Avoid large blood vessels.**

4.3.6.15.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤8 mm slice divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.16 **Spleen**

4.3.6.16.1 **Preferred Location:** Central region, 5 mm below capsule. **Avoid large blood vessels.**

4.3.6.16.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤8 mm slice divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.17 **Pancreas**

4.3.6.17.1 **Preferred Location:** Mid-portion (not tail). **Avoid large blood vessels and ducts.**

4.3.6.17.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤8 mm slice divided into two contiguous 10 mm x 10 mm ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots. **Trim adjacent fat from parenchyma.**

4.3.6.18 **Kidney**

4.3.6.18.1 **Preferred Location:** Left cortex

4.3.6.18.2 **Preferred Aliquot (Cortex):** 10 mm x 10 mm x ≤8 mm slice divided into two 10 mm x 10 mm x ≤4 mm contiguous aliquots. **If cortex is too thin to obtain an 8 mm thick slice, prepare aliquots from a 20 mm x 10 mm x ≤4 mm thick slice, divided evenly across the long (20 mm) axis.** Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.19 **Adrenal Gland**

4.3.6.19.1 **Preferred Location:** Left, followed by the right if necessary for sufficient aliquots.

4.3.6.19.2 **Preferred Aliquot:** Full cross-sections, ≤4-mm thick, contiguous if feasible. Each cassette should contain two cross-sections, if possible. **Trim adjacent fat from parenchyma**.

4.3.6.20 **Colon**

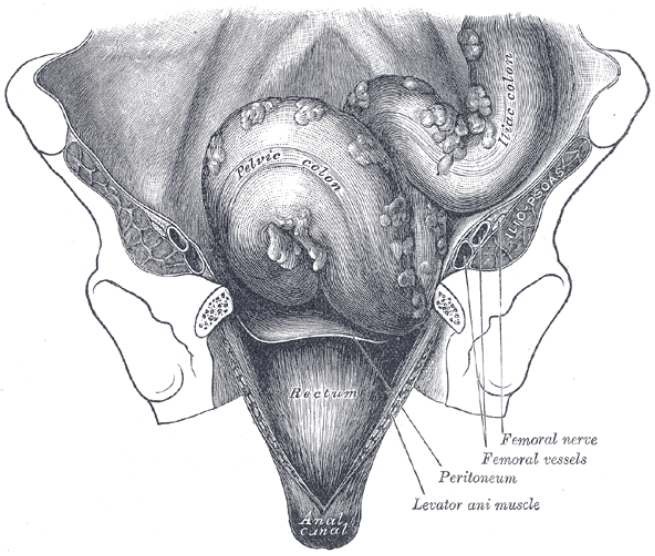
4.3.6.20.1 **Preferred Location: Transverse colon**. Gently rinse mucosa with normal saline before aliquot preparation. Aliquots should contain the full thickness of the colonic wall, i.e., **mucosa and muscularis propria**. **Trim adjacent adipose tissue.**

4.3.6.20.2 **Preferred Aliquot:** 20 mm x 10 mm x thickness (≤4 mm), divided into two adjacent 10 mm x 10 mm x thickness aliquots. Each cassette should contain two 10 mm x 10 mm x thickness aliquots.

4.3.6.20.3 **Preferred Location: Sigmoid colon.** Preferred Location: Sigmoid colon. Gently rinse mucosa with normal saline before aliquot preparation. **Obtain only muscularis propria**; discard mucosa and any serosal adipose tissue.

4.3.6.20.4 **Preferred Aliquot:** 20 mm x 10 mm x thickness (≤4 mm), divided into two adjacent 10 mm x 10 mm x thickness aliquots. Each cassette should contain two 10 mm x 10 mm x thickness aliquots.

**Sigmoid Colon ('pelvic colon') Dissection Guide** (Diagram 4)




*Source: Lewis (1918) Gray's Anatomy 20th ed (in public domain)*

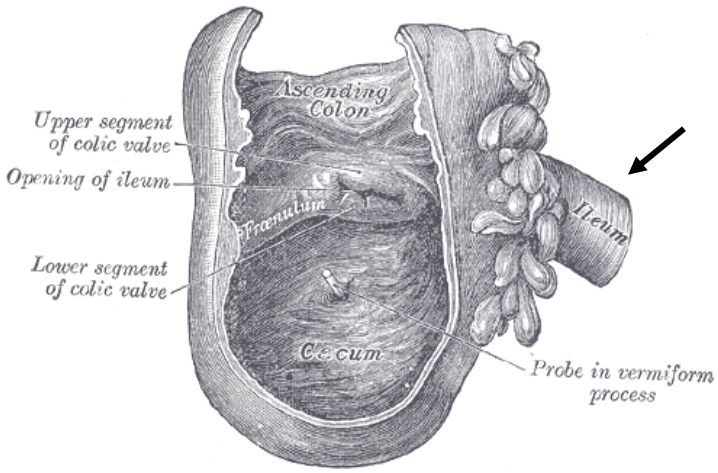
4.3.6.21 **Lymphoid tissue of terminal ileum (Peyer Patches)**

4.3.6.21.1 **Preferred Location:** The most distal part of the small intestine; thickened mucosa with a punctuate appearance corresponding to lymphoid nodules (Peyer Patches) just proximal to the ileocecal valve. Separate the mucosa with submucosa from the muscularis propria, discarding the latter. The area with the most lymphoid tissue has a nodular or even polypoid surface. Cleaving or scraping the mucosa/submucosa off the muscularis with a scalpel blade at a 45 degree angle may be helpful.

4.3.6.21.2 **Preferred Aliquot:** 20 mm x 20 mm x thickness portions divided into four adjacent 10 mm x 10 mm x thickness (≤4mm) aliquots (or equivalent; see note below). Each cassette should contain two 10 mm x 10 mm x thickness(≤4 mm) aliquots.

**Note: Identify the right colon and cecum before sampling the distal ileum. Choosing several separate mucosal nodular areas in the ileum is preferable to getting long pieces of non-nodular mucosa; this will result in gathering the most lymphoid tissue.** Given that this aliquot involves 'scraping' gelatinous-type mucosa off the underlying muscularis, it is recognized the aliquots may not maintain discrete integrity and some aggregating may be necessary.

**Terminal Ileum (Peyer Patches) Dissection Guide** (Diagram 5)




Terminal ileum at right; dilated cecum at left

*Source:* Wikimedia Commons. *Adapted from a reproduction of a lithograph plate from* Gray's Anatomy. *This image is in the public domain because its copyright has expired.*

4.3.6.22 **Stomach**

4.3.6.22.1 **Preferred Location:** Body. Rinse mucosa with normal saline before aliquot preparation.

4.3.6.22.2 **Preferred Aliquot:** 20 mm x 10 mm x thickness (≤4 mm), divided into two adjacent 10 mm x 10 mm x thickness (≤4mm) aliquots. Each cassette should contain two 10 mm x 10 mm x thickness (≤4mm) aliquots. If the wall is too thick, trim from the outer muscular portion.

4.3.6.23 **Greater omentum (visceral adipose tissue)**

4.3.6.23.1 **Preferred Location:** The greater omentum is a large fold of parietal peritoneum that hangs down from the greater curvature of the stomach, passing in front of the small intestines and reflects on itself to ascend to the transverse colon. It always contains some adipose tissue, which is the object of sampling**.**

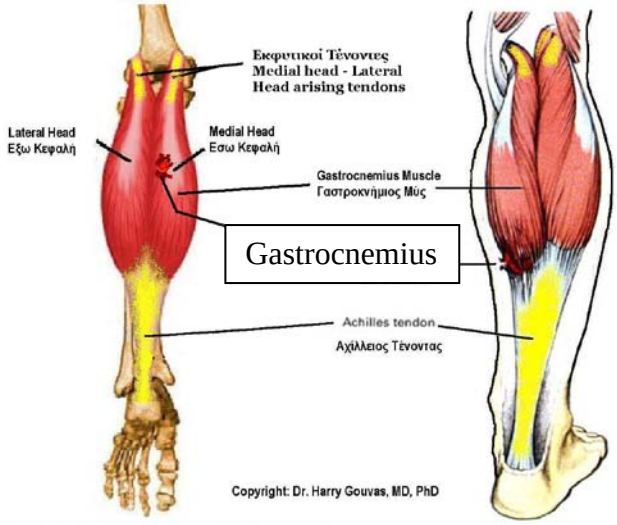
4.3.6.23.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤8 mm slice, divided into two contiguous 10 mm x 10 mm x ≤4 mm thick aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots. If the omentum is very fatty, attempt to remove as much of the fat globular component as possible by compressing the tissue, using the thumb, to a sheet of adipose tissue ≤4 mm in thickness.

4.3.6.24 **Muscle, Skeletal (Gastrocnemius)**

4.3.6.24.1 **Preferred Location:** The gastrocnemius muscle, 2 cm below the patella (seeDiagram 6).

4.3.6.24.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤8 mm slice, divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x thickness (≤4mm) aliquots. Trim adjacent fat.

**Gastrocnemius Muscle Dissection Diagram** (Diagram 6)



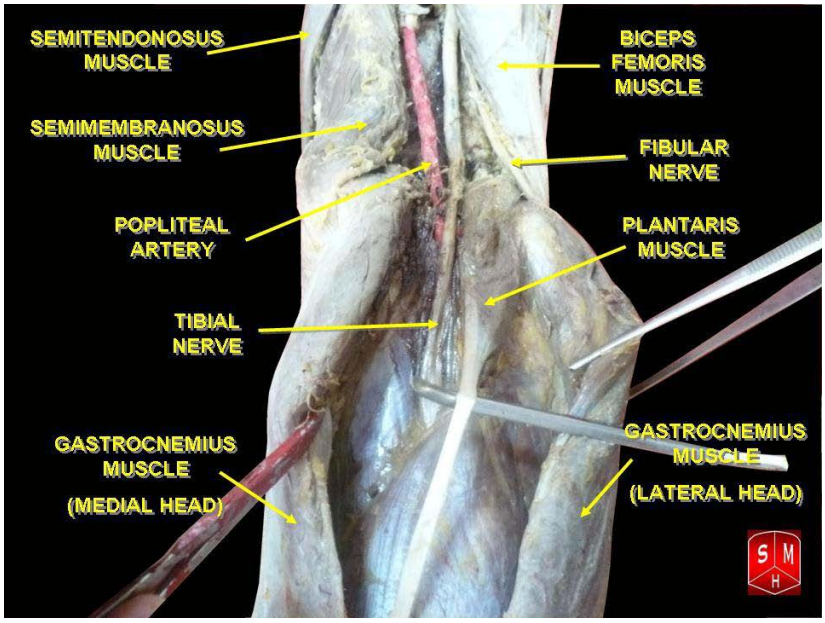

*Source: Adapted from Wikimedia Commons:* The copyright holder of this file, Harrygouvas, allows anyone to use it for any purpose, provided that the copyright holder is properly attributed.

4.3.6.25 **Peripheral Nerve (Tibial)**

4.3.6.25.1 **Preferred Location:** Left tibial

4.3.6.25.2 **Preferred Aliquot:** 10-mm length. Two aliquots per cassette. **Trim off adjacent fat**. Avoid tendons (which can simulate nerves).

**Peripheral Nerve (Tibial) Dissection Diagram** (Diagram 7)




*Source: Wikimedia Commons:* This file is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license. User is free to copy, distribute and transmit the work.

4.3.6.26 **Artery (Tibial)**

4.3.6.26.1 **Preferred Location:** Left tibial

4.3.6.26.2 **Preferred Aliquot:** 10 mm in length. Two aliquots per cassette. **Trim off adjacent fat.**

4.3.6.27 **Female organs**

4.3.6.27.1 **Uterus**

4.3.6.27.1.1 **Preferred Location:** Corpus. Bivalve uterus along endocervical canal to fundus.

4.3.6.27.1.2 **Preferred Aliquot:** 20 mm x 10 mm x ≤4 mm slice or equivalent including endometrium divided into two adjacent 10 mm x 10 mm x ≤4 mm aliquots including endometrium along one edge. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots. If the wall is too thick, trim from the outer portion.

4.3.6.27.2 **Ovary**

4.3.6.27.2.1 **Preferred Location:** Left (and right if necessary to obtain sufficient aliquots). Use most normal regions.

4.3.6.27.2.2 **Preferred Aliquot:** 10 mm x 10 mm x ≤4 mm adjacent aliquots, as feasible. Each cassette should contain two 10 mm x 10 mm x≤ 4 mm aliquots.

4.3.6.27.3 **Vagina**

4.3.6.27.3.1 **Preferred Location:** Anterior is preferred over posterior.

4.3.6.27.3.2 **Preferred Aliquot:** 20 mm x 10 mm x thickness (≤4 mm) portion divided into two adjacent 10 mm x 10 mm x thickness aliquots. Each cassette should contain two 10 mm x 10 mm x thickness aliquots. If the wall is too thick, trim from the outer portion.

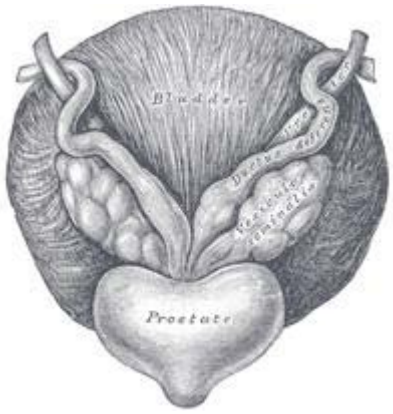
4.3.6.28 **Male organs**

4.3.6.28.1 **Prostate Gland**

4.3.6.28.1.1 **Preferred Location:** Representative region (non-nodular region, if possible).

4.3.6.28.1.2 **Preferred Aliquot**: 10 mm x 10 mm x ≤8 mm slice divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

**Prostate Dissection Guide (avoid seminal vesicles)**. (Diagram 8)




*Source: 20th U.S. edition of* Gray's Anatomy of the Human Body*, originally published in 1918.*

4.3.6.28.2 **Testis**

4.3.6.28.2.1 **Preferred Location:** Left testis (and right testis if necessary to obtain sufficient tissue for aliquots). Remove the capsule before sampling.

4.3.6.28.2.2 **Preferred Aliquot (exclude capsule and epididymis)**: 10 mm x 10 mm x ≤8 mm slice if sufficient tissue, divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.29 **Thyroid Gland**

4.3.6.29.1 **Preferred Location:** Most grossly non-nodular normal regions from either side (whichever side is observed to be more normal).

4.3.6.29.2 **Preferred Aliquot**: 10 mm x 10 mm x ≤8 mm slices divided into two contiguous 10 mm x 10 mm x ≤4 mm aliquots. Each cassette should contain two 10 mm x 10 mm x ≤4 mm aliquots.

4.3.6.30 **Brain**

4.3.6.30.1 **Preferred Location:** Right cerebral frontal pole cortex and cerebellum

4.3.6.30.2 **Preferred Aliquot**

4.3.6.30.2.1 **Cerebral Cortex:** 5 mm cubes or equivalent outer gray matter with minimal white matter. Four 5 mm cube aliquots per cassette. Strip off any loose membranous meninges from the aliquots.

4.3.6.30.2.2 **Cerebellum: 5 mm cubes or equivalent. Four 5 mm cube aliquots per cassette. Strip off any loose membranous meninges from the aliquots.**

4.3.6.30.3 **Remaining Brain**

Once the cortex and cerebellum aliquots have been obtained (**Aqua Kit**), place the remaining brain (including olfactory bulbs, brain stem and cervical spinal cord) into the brain kit (**Green kit**) for shipment to the Brain Bank. Refer to **GTEx Kit Receipt, Supplies, and Shipping Procedure, OP-0001** and associated work instructions for specific procedures.

4.3.6.31 **Pituitary Gland**

4.3.6.31.1 **Preferred Location:** Entire pituitary gland

4.3.6.31.2 **Preferred Aliquot:** Cross-section anterior-posterior (sagittally) and place one section in each cassette. Aliquot may exceed 10 mm x 10 mm surface area.

4.3.6.32 **Coronary Artery**

4.3.6.32.1 **Preferred Location**: Left and right, noncalcific regions only

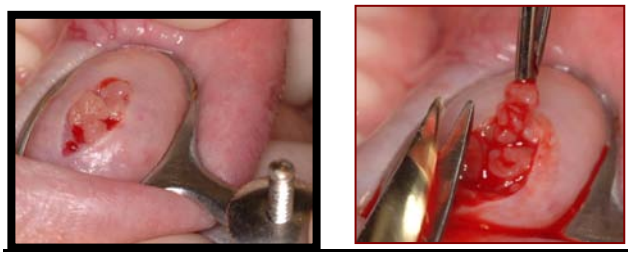
4.3.6.32.2 **Preferred Aliquot**: 10 mm length. Use both the left and right descending and circumflex coronary arteries if noncalcific to acquire sufficient aliquots. Two aliquots per cassette. **Trim adjacent fat.**

4.3.6.33 **Minor salivary glands**

4.3.6.33.1 **Preferred Location:** Inner surface of lower lip. Evert the lower lip and, using a chalazion clamp (if available), make a 10 mm long and 5 mm deep incision of the inner surface of the lip. **Trim off adjacent adipose tissue and lip surface. (Avoid incising full thickness, to the skin inferior to the lower lip resulting in a cosmetic defect.)**

4.3.6.33.2 **Preferred Aliquot:**. Two or more adjacent glands per cassette; not greater than 5 mm cubed.

**Minor Salivary Gland Dissection Diagram** (Diagram 9)



Chalazion Clamp Nodular Glands
*Source: Photos courtesy of Sjogren's Clinic, NIDCR/NIH*

4.4 **PAXgene® Fixative Transfer**

Follow the instructions in the **GTEX Tissue Processing Procedure, PR-0004** to ensure transfer of the aliquots from solution #1 (fixative) to solution #2 (stabilizer) after a minimum of 6 hours/maximum of 24 hours. For the determination of minimum fixation time of 6 hours, the time should be calculated from the time the last tissue was placed into fixative. For the determination of maximum fixation time of 24 hours, the time should be calculated from the time the first tissue was placed into fixative. When transferring the tissue from fixative to stabilizer, be sure to unscrew the PAXgene® lid and remove the lid **with the attached cassette**, place the cassette into stabilizer, and then screw the lid securely into place.

4.5 **Completion of Dissection**

Verify that the **GTEx Tissue Recovery Case Report Form**, **PM-0003-F5** has been populated with the time of the solution transfers.

## 5.0 REFERENCES

5.1 Centers for Disease Control and Prevention Web site: Universal Precautions for Preventing Transmission of Bloodborne Infections. Available at: http://www.cdc.gov/niosh/topics/bbp/universal.html

5.2 PAXgene® Tissue Container Product Circular. Available at: www.qiagen.com/literature/render.aspx?id=104361

5.3 BD Vacutainer Blood Transfer Device brochure. Available at: http://www.bd.com/vacutainer/pdfs/blood_transfer_device_brochure_VS7019.pdf

5.4 Diagram 1: Illustration of how to perform a tube inversion (BD Blood and Urine Collection) http://www.bd.com/vacutainer/labnotes/Volume19Number1/tube_inversion1.asp)

5.5 Diagram 2: Adapted from Wikimedia Commons: Anatomy of the human heart, in English, by Ties van Brussel / http://www.tiesworks.nl; copyright holder releases this work into the public domain.

5.6 Diagram 3: Adapted from: **training.seer.cancer.gov:** Work of the United States Government Gray's subject #247 1161.

5.7 Diagram 4: Lewis (1918) Gray's Anatomy 20th ed (in public domain).

5.8 Diagram 5: Wikimedia Commons. Adapted from a reproduction of a lithograph plate from Gray's Anatomy. This image is in the public domain because its copyright has expired.

5.9 Diagram 6: Adapted from Wikimedia Commons: The copyright holder of this file, Harrygouvas, allows anyone to use it for any purpose, provided that the copyright holder is properly attributed.

5.10 Diagram 7: Wikimedia Commons: This file is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license. User is free to copy, distribute and transmit the work.

5.11 Diagram 8: 20th U.S. edition of Gray's Anatomy of the Human Body, originally published in 1918 (in public domain).

5.12 Diagram 9: Chalazion Clamp Nodular Glands Source: Photos courtesy of Sjogren's Clinic, NIDCR/NIH.