1.0 PURPOSE

The purpose of this guidance document is to provide the steps and instructions for surgical tissue collection, as described in the GTEx Tissue Procurement Procedure (PR-0004). Specifically, this document describes the proper removal, sectioning, and preservation of specified donor tissues/ organs and associated blood draws during the Genotype-Tissue Expression (GTEx) project.

2.0 ENVIRONMENTAL HEALTH & SAFETY

2.1 Universal Precautions (CDC-1978) 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 are classified and shipped under IATA Class 6.2 regulations for UN3373, Biological Substances, Category B. Tissue transported in the PAXgene® Tissue Containers are classified and 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.

3.0 MATERIALS/EQUIPMENT

3.1 Refer to ***GTEx Kit Receipt, Supplies, and Shipping Procedure (OP-0001)*** for specific materials.

3.2 Materials to be provided by the BSS:

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

- PPE
- Surgical instruments (complete standard set), which may include the following: scalpels, rongeurs, rocker knife, and various types of scissors
- Needle, 18 gauge
- Latex or nitrile rubber gloves

4.0 PROCEDURE

4.1 OVERVIEW OF BIOSPECIMEN COLLECTION

4.1.1 The GTEx project allows each BSS, after appropriate training, an opportunity to independently collect tissues and associated blood draws

4.1.2 Above knee (AK) and below knee (BK) leg amputations are collected and processed according to BSS-specific and caHUB-specific instructions listed below; i.e., BSS guidance on the collection and transport of the leg portion and caHUB instructions on dissection, aliquot preparation and preservation, and shipping will be expected to be followed.

4.2 SITE PREPARATION

Efficient organization of the recovery team is essential. The blood collection, leg and tissue recovery, dissection, 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.

5.0 TISSUE PROCUREMENT

5.1 General

5.1.1 The rapid recovery of tissues from leg amputations is the goal of this procedure. All recovery, dissection, aliquot preparation, and preservation processes should be optimized at each BSS to reduce the postamputation interval to a minimum for each organ, section, and aliquot.

5.1.2 **Documentation: GTEx Tissue Recovery Form:** Capture biospecimen-related data on the ***GTEx Tissue Recovery Form (PM-0003-F5)*.**

5.1.3 **Aliquot Location:** Any deviation from the preferred organ/tissue location must be documented on the ***GTEx Tissue Recovery Form (PM-0003-F5)***.

5.1.4 **Aliquot Preparation**

5.1.4.1 The aliquot size depends upon the tissue and is specified in the tissue-specific sections below as well as on ***GTEx Tissue Recovery Form (PM-0003-F5).***

5.1.4.2 A ruler is used to measure the appropriate aliquot size.

5.1.4.3 Any deviation to the aliquot size (7.4) should be documented on the ***GTEx Tissue Recovery Form (PM-0003-F5).***

5.1.4.4 **Preferred Aliquot Size**

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

5.1.4.4.2 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).

5.1.4.4.3 To obtain these aliquots, it is suggested 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.

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

5.1.4.4.5 For skin, the 10 mm x 10 mm x thickness (<4mm) aliquots can be obtained by dividing a 20 mm x 20 mm by 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 (not greater than 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.

5.1.4.4.6 For most aliquots, the preferred aliquot is 10 mm x 10 mm x 4 mm tissues slices. Equivalent alternative size is two (2) 10 mm x 5 mm 4 mm strips. 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.

5.1.5 Tissue Dissection Technique

5.1.5.1 General

5.1.5.1.1 Whole blood collection should occur within **0 to 24 hours <u>PRIOR</u> to the administration of pre-anesthesia drugs, anesthesia, and leg amputation.**

5.1.5.1.2 Record all required recovery and processing information on the ***GTEx Tissue Recovery Form (PM-0003-F5)***.

5.1.5.1.3 Document any deviations related to the completion of the ***GTEx Tissue Recovery Form (PM-0003-F5)*** or deviations to recovery in the comments section.

5.1.5.2 Whole Blood Collection

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

5.1.5.2.1 <u>Collection timeline</u>**:** Blood collection should occur within 0 to 24 hours **<u>PRIOR</u> to the administration of pre-anesthesia drugs, anesthesia, and leg amputation.**

5.1.5.2.2 <u>Total volume: A tot</u>al of four (4) whole blood vacutainers will be collec<u>ted and ship</u>ped to the LDACC **(*GTEx Kit Receipt, Supplies, and Shipping Procedure (OP-0001)*.** This incl*udes:*

- *Two (2) 10 mL ACD (yellow top) vacutainers*
  - *A minimum of* 6 mL of blood **i**s requested in each yellow top collection, if available.
- Two (2) 2.5 mL PAXgene® RNA blood vacutainers

- A minimum of 2 mL of blood is requested in each PAXgene® blood vacutainer, if available.

5.1.5.2.3 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.

Collection preference: The blood is usually drawn from the antecubital fossa with blood from the cephalic, basilic, or cubital veins.

5.1.5.2.4 Inverting Vacutainer Tubes

Invert all blood collection tubes 10 times immediately following filling of the tube to ensure adequate mixing of the blood and additives within the yellow top and PAXgene® RNA blood vacutainers (SEE Diagram 1 below).

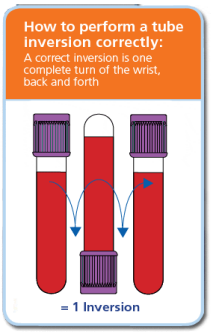


Diagram 1: Illustration of how to perform a tube inversion (BD Blood and Urine Collection) http://www.bd.com/vacutainer/labnotes/Volume19Number1/tube_inversion1.asp)

5.1.6 **Skin**

5.1.6.1 **Preferred Location:** Left or right leg (designate side) 1 cm below resection line on medial side for amputations. Gently remove surgical antiseptic with alcohol wipe.

5.1.6.2 **Procedure:** After EtOH preparation (x2), 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, and tightly seal for shipment directly to the LDACC (***Yellow Kit***). Return to skin tissue and prepare the remaining aliquots of skin and subcutaneous adipose tissue.

5.1.6.3 **Skin for Fibroblast Culture**

5.1.6.3.1 **Skin, fibroblast culture:** Two 4 mm x 4 mm x thickness

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

5.1.6.4.1 **Preferred Aliquot Skin**: 10 mm x 10 mm x thickness slices; suggest a 20 mm x 10 mm x thickness (not greater than 4 mm) slice, divided to yield two contiguous aliquots with dimensions of 10 mm x 10 mm x thickness (not greater than 4 mm). Each cassette should contain two 10 mm x 10 mm x thickness aliquots. **Trim off adherent subcutaneous adipose tissue.**

5.1.7 **Adipose Tissue**

5.1.7.1 **The aliqu**ot is 10 mm x 10 mm x <4 mm (not greater than 4 mm). Each cassette should contain two 10 mm x 10 mm x <4 mm aliquots.

5.1.7.2 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.

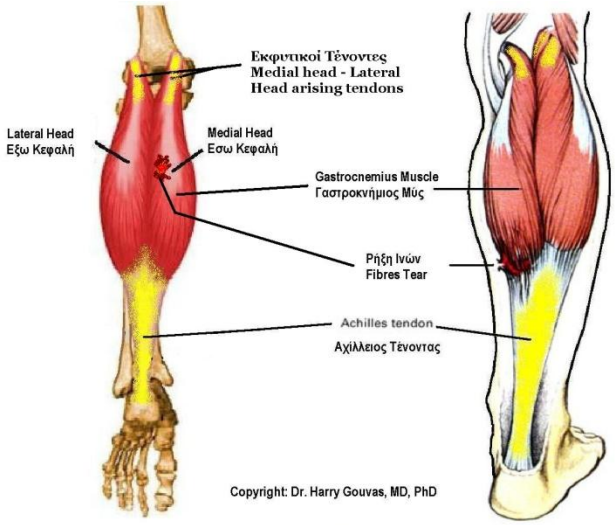
5.1.7.3 Using the thumb against the dissecting tray's surface, attempt to remove as much of the fat globular component as possible by compressing the tissue to about a sheet of adipose tissue no thicker than 4 mm. This removes fat/oil globules and retains adipose cellular tissue, which improves preservation and molecular recovery.

5.1.7.4 **Muscle, Skeletal (Gastrocnemius – Below the Knee (BK); Vastus Lateralis – Above the Knee (AK)**

5.1.7.4.1 **Preferred Location:** 1 cm below resection line of the lateral gastrocnemius in BK amputations and 1 cm below resection line of the vastus lateralis muscle in AK amputations (see Diagrams 2 and 3).

5.1.7.4.2 **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 aliquots**. Trim off adjacent adipose tissue.**

5.1.7.4.3 **BK - Gastrocnemius Muscle Dissection Diagram** (Diagram 2)



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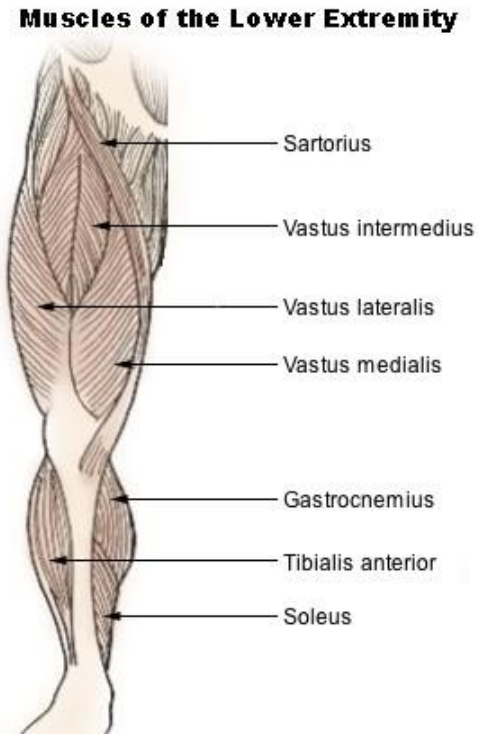
5.1.7.4.4 **AK - Vastus Lateralis Muscle Diagram** (Diagram 3)



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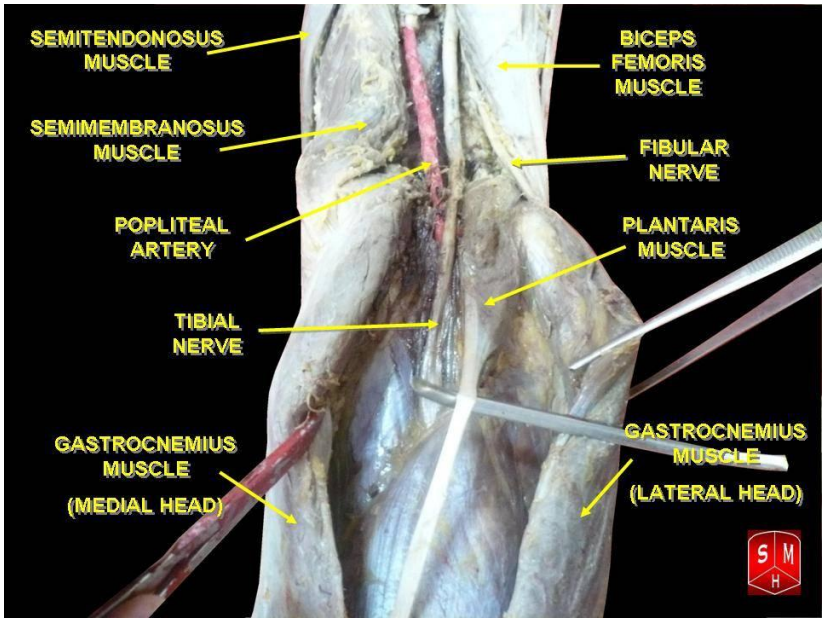
5.1.7.5 **Tibial Nerve, Peripheral**

5.1.7.5.1 **Preferred Location:** Tibial nerve (BK) or sciatic nerve (AK) beginning 1 cm below resection line. For above knee amputations the sciatic nerve (SEE Diagram 4) may be used (label accordingly).

5.1.7.5.2 **Preferred Aliquot:** 10-mm length. Two aliquots per cassette. **Trim off adherent adipose tissue.**

5.1.7.5.3 **Peripheral Nerve (Tibial) Dissection Diagram** (Diagram 4)



5.1.7.6 **Tibial Artery, Peripheral**

5.1.7.6.1 **Preferred Location:** Posterior tibial artery for BK amputations and popliteal or femoral artery for AK amputations, both beginning 1 cm below resection line (SEE Diagram 4).

5.1.7.6.2 **Preferred Aliquot:** 10-mm length. Two aliquots per cassette. **Trim off adjacent adipose tissue.**

5.1.8 **PAXgene® Fixative Transfer**

5.1.8.1 Follow the instructions in the ***GTEx Tissue Procurement SOP (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. The time should be calculated from the time the last tissue was placed into fixative. Be sure to unscrew the PAXgene® lid and remove the lid with the attached cassette when transferring from fixative into stabilizer.

5.1.8.2 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.

5.1.8.3 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.**

5.1.9 **Completion of Dissection**

5.1.9.1 Follow the instructions on the ***GTEx Tissue Recovery Form (PM-0003-F5)*** to record the time of the solution transfers.

6.0 REFERENCES

6.1 GTEx Kit Receipt, Supplies, and Shipping Procedure (OP-0001)

6.2 caHUB Data Entry Procedure (IT-0001)

6.3 GTEx Tissue Recovery Form (PM-0003-F5)

6.4 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

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

6.6 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)

6.7 Diagram 2: 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.

6.8 Diagram 3: Wikimedia Commons: This work is in the **public domain** in the United States because it is a work prepared by an officer or employee of the United States Government as part of that person's official duties under the terms of Title 17, Chapter 1, Section 105 of the US Code.

6.9 Diagram 4: 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.

7.0 ATTACHMENTS

None

APPROVALS	
NAME / TITLE	

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