1.0 PURPOSE

1.1 The purpose of this procedure is to provide a protocol for the uniform histologic analysis of GTEx tissue specimens sent to the Pathology Resource Center (PRC) from the Comprehensive Biospecimen Resource (CBR) and for generating a PRC Case Summary Report (CSR) and Procurement Feedback Form (PFF) for the tissues received and evaluated.

2.0 SCOPE

2.1 This procedure is applicable to all PAXgene®-fixed paraffin-embedded (PFPE) GTEx tissues and select frozen GTEx tissues that are procured at Biospecimen Source Sites (BSS) and processed at the CBR, whereby digital images, and in selected cases, glass slides are provided by the CBR to the PRC for review.

2.2 The procedure applies to all PRC staff involved in the handling of materials and microscopic interpretation of the tissues for project use. This includes receipt and review of slides and images, resolution of issues, management of collected data in the Comprehensive Data Resource (CDR), production of a CSR and PFF, final storage, and inventory of slides and images.

2.3 This Standard Operating Procedure (SOP) refers to the specimens collected in postmortem and organ donor cases.

3.0 RESPONSIBILITY

3.1 Pathologist – American Board of Pathology-certified anatomic pathologist responsible for histological analysis and annotation of specimens and subsequent notification of results to all relevant parties

3.2 Comprehensive Biospecimen Resource (CBR) – Responsible for creating the slides and shipping them to the PRC (upon request), creating digital images from slides, and maintaining the image management system

3.3 Director, Pathology – American Board of Pathology-certified anatomic pathologist responsible for oversight of histologic analysis of specimens and assignment of incoming cases to the pathologists

3.4 Pathology Resource Center (PRC) – Staff or representatives involved in activities for case reviews for the project

3.5 Program Manager – Assigned to lead overall management of program and ensure the procedures and operational connections between parties with responsibilities in this procedure are met.

3.6 QC Pathologist – Pathologist responsible for performing a Quality Control check of the images/slides and verifying the findings documented in the corresponding PRC Case Summary Report

3.7 Technical Project Manager (TPM) – Responsible for overall interface and supporting operational issues between the PRC and BSSs

4.0 DEFINITIONS & ACRONYMS

4.1 **BRIMS -** The CBR's Biorepository Information Management System used for tracking shipments to and from the CBR and BSS, as well as inventory.

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

4.3 **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) collected for GTEx, creation of slides and digital images, and maintenance of the image management system, as well as quality checks on collected and processed specimens.

4.4 **CDR** – Comprehensive Data Resource. Centralized custom-made informatics system that stores and reports all collection, handling, and processing data for biospecimens and annotations collected for use by this program. The system provides secure, role-based access for BSSs to input data related to each case collected that is associated with a Limited Data Set related to the donor. Interfaces are provided to other systems that contain related case data (e.g., inventory data at the CBR, molecular data at the molecular analysis facility, research data in dbGaP, etc.).

4.5 **CoC** – Chain of Custody. Documentation of the chronological movement and operational steps for each sample and annotations throughout their receipt, handling, and processing lifecycle from the time of collection through inventory, processing, distribution, analysis, and final storage.

4.6 **CRF -** Case Report Form. A paper or electronic form used to collect donor or case-related data. Most data for each patient participating in the GTEx are 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.

4.7 **CSR**– Case Summary Report. Compilation of findings from the examination of histologic images provided by the CBR from GTEx cases.

4.8 **Donor Eligibility Form** - Paper or electronic data collection form within the CDR used for documentation of donor screening and eligibility for the GTEx project.

4.9 **IMS**– Imaging Management System. The information system that stores the digital images made from specimen/sample slides. Images are added and maintained in this system by the CBR.

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

4.11 **LCM**– Laser Capture Microdissection. Process for isolating specific cells or structures of interest from tissue sections.

4.12 **OCT**– Optimum Cutting Temperature Compound. A formulation of glycols and resins used to embed tissue for cryosectioning.

4.13 **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.

4.14 **PDF**– Portable Document File. Invented by Adobe Systems and perfected over 20 years, Portable Document Format (PDF) is now an open standard for electronic document exchange maintained by the International Organization for Standardization (ISO). When you convert documents, forms, graphics, and web pages to PDF, they look just like they would if printed. But unlike printed documents, PDF files can contain clickable links and buttons, form fields, video, and audio — as well as logic to help automate routine business processes. When you share a PDF file, virtually anyone can read it using an Adobe Reader app.

4.15 **PFF** - Procurement Feedback Form. An assessment from the examination of histologic images by PRC sent to the BSS to provide technical feedback on the suitability of the tissues for the GTEx project.

4.16 **PFPE**–PAXgene®-Fixed Paraffin-Embedded

4.17 **PM**- Project Manager. Each GTEx Project Manager is responsible for the planning and implementation of all activities that will deliver the systems, documents, and resources to meet the requirements of their project using all appropriate processes and procedures.

4.18 **PMT**– Project Management Team.

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

4.20 **QC** – Quality Control. Specific tests to be performed within the Quality Management System to monitor procurement, processing, preservation and storage; biospecimen quality; and test accuracy. These may include but are not limited to performance evaluations, testing, and controls used to determine accuracy and reliability of the biospecimen resource's equipment and operational procedures as well as monitoring of the supplies, reagents, equipment, and facilities.

4.21 **SOP** – Standard Operating Procedure. An established procedure to be followed in carrying out a given operation or in a given situation.

4.22 **TMA** – Tissue Microarray. A block containing numerous tissue cores which is used to enable concurrent histologic analysis such as by immunohistochemistry.

4.23 **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.

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

4.25 **WSI** – Whole Slide Image

5.0 ENVIRONMENTAL HEALTH & SAFETY

Not Applicable

6.0 MATERIALS/EQUIPMENT

6.1 Materials provided by GTEx:
- Dual headed microscope
- Microscope camera
- Computer with Internet access
- Slide storage cabinets
- Secured storage area for slides and associated paperwork

6.2 Materials/other items provided by CBR:
- Glass microscope slides
- Slide storage cabinets
- Secured storage area
- Digital images in the CBR IMS
- Web access to digital images from CBR using their image viewer (Aperio)

6.3 Materials provided by PRC:
- PRC Tissue Review SOP, **Pathology Resource Center (PRC) GTEx Tissue Review, PR-0002**
- GTEx Pathology Resource Center Case Summary Report
- GTEx Pathology Resource Center Procurement Feedback

7.0 PROCEDURE

All PAXgene® -fixed tissues submitted to the CBR and representative frozen tissues are processed by the CBR for interpretation by the PRC. The CBR receives and processes these tissue specimens from the BSSs and generates digital images for such specimens. Images (and/or slides) of PFPE specimens will be ready for PRC review approximately within five (5) working days of tissue receipt at the CBR. Any change in this timeframe should be approved by the TPM. The CBR assigns a status of "Quarantine/In process" in the inventory management system to indicate that the slides have been processed and their images have been made available for viewing in the CBR Imaging Management System (CBR IMS).

When the images are ready for review by the PRC, an automatic alert is sent from the CDR to the PRC team stating that the images are ready. If the reviewing pathologist needs glass slides to complete the case assessment, a request will be made to the CBR, and the CBR will send the slides and corresponding CoC to the requestor. The PRC case assessment is documented in a PRC CSR Form and a PFF.

7.1 GTEx Case Review/Whole Slide Image Review

NOTE: Cases may be reviewed solely based on the WSIs in the CBR IMS once made available to the PRC by the CBR. Glass slides may be used when WSIs are insufficient to interpret the case findings completely (see Operations Manual for Nikon Microscope).

7.1.1 A CDR Alert to appropriate GTEx staff and the CBR will indicate that images have been made available.

7.1.2 A PRC Pathologist is assigned to review the case by the Director or his delegate.

7.1.3 In advance of case review, the pathologist will review clinical and case collection data as provided within the CRF, TRF, and Donor Eligibility Form.

7.1.3.1 In the event that the donor is identified as a transplant recipient (either human or xenotransplant, as noted in question #15 of the Donor Eligibility Form), tissue should not have been collected from the transplanted organ/tissue or the native organ/tissue of the same type.

7.1.3.2 If such tissues have been submitted, the pathologist is to mark these as "unacceptable" during case review, as noted in the following sections.

7.1.4 The assigned PRC Pathologist will review the images.

7.1.5 The pathologist reviews and annotates the images in the CBR IMS, as appropriate.

NOTE: Pathologists review all tissues primarily to confirm the following but not limited to:
a) the presence of the intended morphology,
b) the presence and degree of autolysis (PFPE tissues only),
c) the presence of pathologic findings (e.g., inflammation, hemorrhage, neoplasms), and
d) verification of aliquot numbers/tissue pieces as specified in the applicable GTEx Work Instructions.

7.1.6 Upon identifying any issues, the PRC will work with the relevant parties to ensure their complete resolution prior to assigning a final specimen status. Issues may include:

- requests for recuts for verification or special stains,
- mislabeled images or slides,
- broken slides,
- extraneous tissues,
- sampling errors, and
- unexpected findings.

7.1.6.1 In the event that specimens appear to be mislabeled based on image review, the following will be done:

7.1.6.1.1 The CBR will be advised of the apparent sample switch via email from the CBR manager and requested to compare applicable specimen blocks, slides, and images to ascertain the source of the discrepancy. The findings of this assessment are to be relayed to the PRC.

7.1.6.1.2 In the event that the labeling issues cannot be reconciled based upon this review, **Pathology Resource Center (PRC) GTEx Request for Clarification Form, PR-0002-F2** will be generated to request that additional slides be prepared from the relevant blocks. Images from these tissues will be reviewed by the PRC and findings shall be documented in the CDR. Based upon this evaluation, CBR specimens (blocks, slides, images) are to be re-labeled as warranted. The data management team will further annotate issue resolution as per **OP-0016 GTEx Erroneous Data Correction Procedure**.

7.1.7 The pathologist can, on request, also assess the suitability of the tissue for further analyses by the CBR, project analysis facilities, and other end users.

7.2 PRC Receipt and Inventory of Slides and Images

7.2.1 If slides are required to supplement the WSI review, the applicable pathologist will ensure that a request is sent to the CBR.

7.2.2 If slides are requested by the PRC, upon receipt the materials are inventoried into BRIMS by logging into the system and receiving the slides into inventory. This process also includes verifying the manifest shipped with the slides against what is listed in BRIMS in conjunction with performing a visual check for any damaged slides. A PRC representative completes the CoC form (paper or electronic) that accompanies the shipment and files the information into the GTEx Cases Folder in the designated Shared Drive. The external case ID and barcode ID for each tissue are on the shipping manifest that accompanies the CoC.

7.2.3 The slides are then provided to the requesting PRC Pathologist for review.

7.2.4 Slides are to be returned to the CBR for storage upon case completion.

7.3 Pathology Case Summary Report

7.3.1 All findings and issues identified from the WSI review are documented in a timely fashion in the electronic PRC CSR, **Pathology Resource Center (PRC) GTEx Case Summary Report Form, PR-0002-F1**.

7.3.2 The Comment section for each tissue in the CSR should include:

- the number of tissue pieces,
- the presence of extraneous tissue, e.g., fat, its location (internal or external),
- relative (%) or actual size of extraneous tissue,
- pathologic findings, e.g., congestion, hemorrhage, inflammation, atrophy
- specific entities such as Hashimoto's thyroiditis, cirrhosis, diabetic glomerulosclerosis, should be specified to facilitate data base searches.

7.3.3 Each tissue reviewed per case is assigned an inventory status in the PRC CSR based on the review. These statuses are implemented at the CBR in the inventory management system as follows:

7.3.3.1 **Acceptable:** Biospecimens that have been processed by the CBR and reviewed by the PRC and deemed acceptable for quality and for use in the GTEx Project.

7.3.3.2 **Unacceptable:** Biospecimens that have been processed by the CBR, reviewed by the PRC and are deemed unacceptable for GTEx project use. Reasons for unacceptability include incorrect target tissue, inadequate target tissue, or collection of a tissue from a GTEx donor with a documented history of trasplant of that particular organ/tissue.

NOTE: 'Unacceptable' means not for use in the current GTEx project. However, tissues labeled 'Unacceptable' are typically retained as they may be of use in future studies. If 'Unacceptable', the reason should be specified in the Comment section to facilitate data base searches, e.g., 'seminal vesicle sampled, no prostate present'. Also, such tissues may be tagged for use in TMA or LCM evaluations by including a statement in the PRC CSR indicating the potential utility for TMAs or LCM.

7.3.3.3 **Quarantine/Issues Pending:** Biospecimens that have been processed by the CBR, reviewed by the PRC and have been identified as having issues that require resolution.

7.3.3.4 Invalidated:

a) Biospecimens that have not been consented for by the donor or donor family as verified by the data management team (samples may either be destroyed or returned to the BSS per the request of the donor or donor family) OR

b) Biospecimens that have been withdrawn by the donor, donor family or BSS (samples may either be destroyed or returned to the BSS per the requester)- see **Biospecimen and Data Withdrawal and Recall Procedure, ER-0005.**

7.3.4 Issues identified by the PRC that require response or further action at the BSS or CBR (excluding those noted above in section 7.1.6.1.1) are documented on the **Pathology Resource Center (PRC) GTEx Request for Clarification, PR-0002-F2,** as warranted, and subsequently managed as per the **GTEx Erroneous Data Correction Procedure, OP-0016.**

7.3.5 Once the PRC CSR has been completed, the reviewing pathologist approves and submits the PRC CSR. The CSR is reviewed by Data Management and then made available within the CDR to all approved parties.

Note: The PRC CSR is generated for research purposes only and is not intended for clinical use. It is not provided to the BSS.

7.3.6 In the event that a CSR requires modification, a new instance of the report will be generated and assigned an incremental serial version number.

7.4 Procurement Feedback Report

7.4.1 The **GTEx Procurement Feedback Form, PR-0002-F5**, has been implemented in the CDR to enable automated reporting of procurement issues to the BSSs. This is to be completed for each GTEx case.

7.4.2 After the PRC CSR has been completed by the PRC pathologist, a separate feedback form should be formulated within the CDR to provide the BSS with feedback on procurement issues and observations. The contents are based on the enumerated issues and pathologist's comments in the PRC CSR. This form should address the acceptability of aliquots and adequacy of tissue sampling, including deficiencies, such as mixed-up samples and excessive adipose tissue content.

7.4.3 The prepared PFF should be submitted to Data Management. Following Data Management review, this report shall be made available to the respective BSS within the CDR.

7.4.4 Revised versions of the PRC CSR will not prompt the provision of additional Procurement Feedback to the BSSs.

7.5 Case Quality Control

7.5.1 At a minimum, images from 10% of randomly selected GTEx cases will receive an additional review by a second GTEx Pathologist (QC Pathologist) as a quality control measure. This review takes place after the primary review, and is performed after the release of the PRC CSR.

7.5.2 The QC pathologist provides concurrence or reports any discrepancies on the images reviewed.

7.5.3 The QC pathologist provides signature to the primary PRC CSR, if no issues are reported.

7.5.4 All discrepancies are resolved by the PRC pathology team by reviewing and coming to a mutual agreement on case findings. The PRC Director has final responsibility for the resolution of the problem.

7.5.5 For issues identified and resolved, a revised version of the PRC CSR will be generated, as warranted, to reflect any changes to the original PRC CSR.

7.5.6 If a new PRC CSR is generated and submitted by the PRC (typically by the original pathologist responsible for the case), the revised report will be made available to approved parties within the CDR. Subsequent reports are assigned incremental serial version numbers.

7.6 Review of Frozen Tissues

7.6.1 As per approved GTEx Scale Up requirements, collected frozen tissue is to be stored by the CBR and will not undergo regular pathology review.

7.6.2 For a minimum of 10% of cases in which frozen tissues have been collected, however, the PRC will manually designate cases for frozen tissue review. A summary report of applicable cases will be reviewed which includes the sub-site/collection facility, the total number of frozen aliquots submitted, the number of tissue types with frozen tissue submitted, and the number of issues reported in the PAXgene® component of the case. Based upon this review, the PRC will endeavor to distribute the frozen specimen review over all sub-sites and to ensure that reviewed cases have an adequate number of sampled tissue types and specimens. In addition, cases in which issues have been identified may be prioritized for review of frozen tissue.

7.6.3 For these selected cases, a portion of one aliquot of each tissue type with frozen tissue collected will be embedded in OCT and frozen sections reviewed by the PRC to confirm the tissue type present.

7.6.3.1 The residual OCT-embedded tissue block will be banked-in a traceable manner that retains the parent-child relationship-at the CBR.

7.6.3.2 For cases that have not been designated for concurrent molecular analysis, the remaining aliquot (not embedded in OCT) will be stored in the original cryosette at the CBR.

7.6.3.3 For a subset of these cases, the remaining portion of the aliquot divided to yield tissue for review by the PRC will be submitted to the LDACC for concurrent molecular analysis. The CBR will place this residual tissue in a labeled cryovial and store at -190°C until shipment on dry ice to the LDACC. Assigned child specimen IDs will be traceable back to the parent GTEx case ID.

7.6.4 PRC findings are to be documented in **Pathology Resource Center (PRC) GTEx Case Summary Report Form for Frozen Tissues, PR-0002-F4**. In addition, as warranted, a Procurement Feedback Form may be provided to the BSS after the review of frozen tissues.

7.7 Archival

7.7.1 All WSIs will be stored in the CBR IMS and made accessible to the PRC for review.

7.7.2 All glass slides will be stored at the CBR.

7.7.3 PRC Case Summary Reports Forms and Procurement Feedback Forms will be stored within the CDR.