CTRNet Standard Operating Procedure Sectioning of Paraffin and OCT Embedded Tissue			
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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary of Revisions
LP 002.001	2005	JdSH	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.006	09-01-2008	JdSH	Revised to deal specifically with sectioning of tissue preserved in paraffin and OCT (and related QA issues)

1.0 PURPOSE

Preserved tumour tissues collected through the informed consent process are valuable for specific research studies. Formaldehyde fixed and paraffin embedded (FFPE) tissue and tissue frozen in OCT can be sectioned for studies needing preservation of histo-morphology of the specimen. For studies involving immunohistochemistry (IHC) or *in situ* hybridization (ISH), sections of unfixed tissue frozen in OCT may be more appropriate. Some research studies also use sections to extract nucleic acids from specimens. The purpose of this document is to outline standardized procedures for CTRNet repositories to follow when sectioning tissue preserved in paraffin or OCT.

In addition, quality control is fundamental to the successful operation to a tissue bank offering tissue specimens for research purposes. CTRNet repositories should be confident that they are providing tissue sections with high quality to appropriately meet the research needs of the investigators. Testing procedures should be in place to monitor and assess the quality and integrity of the sections released for prospective research studies.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how tissues preserved in paraffin and OCT should be sectioned. The SOP also outlines minimum assessent that should be in place to evaluate the quality and integrity of paraffin and frozen tissue sections.

The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 REFERENCE TO OTHER POLICIES AND SOPS

1. CTRNet Policy: POL 005.001 Records and Documentation
2. CTRNet Policy: POL 002.001 Ethics
3. CTRNet Policy: POL 004.001 Privacy and Security
4. CTRNet Policy: POL 007.001 Material and Information Handling Policy
5. CTRNet Generic Procedure: FS 002.001 CTRNet Generic SOP for Collection and Processing of Tumour Tissue
6. CTRNet SOP: 8.3.003 Preservation of tissue: Freezing in OCT
7. CTRNet SOP: 8.3.005 Preservation of Tissue: Paraffin embedding
8. CTRNet SOP: 5.1.001 Assessing Quality of Tissue Samples
9. CTRNet SOP: 9.1.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITY

The policy applies to all personnel from CTRNet member repositories that are responsible for sectioning tissue preserved in paraffin or OCT blocks.

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Pathologist	Conduct Histopathological characterization	
Lab technician /Histology Lab Technician	May be specifically responsible for processing FFPE tissues and sectioning paraffin and OCT blocks. Conducts and Assists with quality assurance procedures. Records and documents outcomes.	

5.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	**Materials and Equipment (Site- Specific)**
Markers, ink and pens	
Microscope	
Microtome	
Hot Water Bath (set at 40-45°C)	
Microtome blades	
Fine tipped paint brush	
Fine tipped tissue separator	
Appropriate labels for slides	
Labelled glass Slides	
Tray to hold slides	
Ice Tray	
Oven (set at 50-52°C)	
Cryotome	
Labelled electrostatically charged slides (such as Superfrost Plus)	
Container with dry ice for OCT blocks	
Film for sealing slide boxes such as Parafilm	
Slide storage boxes and/or slide shippers	
Mounting medium such as Cryomount to attach OCT block to cryotome chuck	

6.0 DEFINITIONS

BSA: Bovine serum albumin

Chuck: Part of Microtome used to hold the block.

Cryotome: A device that consists of a Microtome placed inside a freezer and used for sectioning frozen tissue.

Formalin Fixed Paraffin-embedded (FFPE) Tissue: Tissue that has been fixed Formalin and set in paraffin.

H&E: Hematoxylin and Eosin

IHC: Immunohistochemistry

ISH: *In situ* Hybridization

FISH: Fluorescent *In situ* Hybridization

Microtome: Device used to cut sections from a block to place on slides.

OCT: "Optimal Cutting Temperature" compound is the name used for polyethylene glycol/sucrose-based freezing medium. OCT preserves ultrastructure and prevents tissue from desiccation, degradation, acts as an insulator from thermal variation and minimizes crystal formation. It is especially useful for preserving fresh frozen that may need to be sectioned.

Electrostatically Charged Slides: Microscope slides formulated to permit electrostatic bonding of negatively charged tissue sections to the positively charged slide surface.

Preservation: Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of a specimen.

Quality: Conformance of a specimen or process with pre-established specifications or standards.

Quality Assurance (QA): All those planned and systematic actions that are established to ensure that the Tumour Repository Program is performed and the data are generated, documented (recorded), and reported in compliance with applicable regulatory requirement(s).

Quality Control(QC): Quality control is the system of technical activities that measures the attributes and performance of a process, or item, against defined standards, to verify that the stated requirements are fully met.

QMS: Same as QA above

7.0 PROCEDURES

This procedure is intended to ensure that tissue samples preserved for research studies are sectioned in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. It also ensures rationing of the tissue blocks associated for each case for multiple assays and projects and maintenance of the block orientation. Consistency in procedure is important for obtaining comparable and reliable test results. The following steps are based on procedures followed at the Manitoba Breast Tumor Bank and NCIC CTG Ontario.

These procedures also outline minimum steps that should be followed to ensure that tissue samples collected stored and distributed are of sufficient morphological and molecular caliber to meet the research needs of the investigators.

7.1 Sectioning Formalin Fixed Paraffin Embedded Tissue

1. Treat all tissue as potentially infectious.
2. Sectioning is performed by the laboratory or histology technician or personnel trained to use a microtome and cut histological sections.
3. Have materials and equipment ready. Have as many slides as needed labelled and ready.
4. Pre-cool paraffin blocks, tissue side down, on a tray of ice-water. This will facilitate sectioning especially fatty tissue like breast. Using a steel microtome knife or disposable blade cut sections that are 4-5 microns for histological sections, and 10-20 microns for nucleic acid extraction purposes.
5. For histological sections label slides serially.
6. Dry paraffin sections at 55°C for 1 hour.
7. Remove the sections from the oven and allow cooling at room temperature.
8. The sections are stored for shipping in slide mailers or stored in slide holder boxes at 4° C. Extended storage of unstained FFPE slides should be avoided as this may result in the loss of antigens. While not established, vacuum sealing and refrigeration may help preserve some unstable antigens.
9. For nucleic acid extraction sections allow the individual sections to roll up naturally and place them directly into microfuge tubes ready for nucleic acid extraction. The extraction buffer can be added directly to the microfuge tube in order to preserve the molecular integrity of the sample.

7.2 Sectioning OCT Embedded Tissue

1. Frozen sections are cut by personnel trained to perform the task of sectioning OCT embedded tissue in a cryotome. The frozen tissue cryomolds or vials are transferred to the cryotome on dry ice.
2. Set the section thickness at 4-5 microns for IHC or ISH and 10-20 microns for nucleic acid extraction samples.
3. Sections are mounted on pre-cooled slides by inverting the slide on an slight angle over the section as it lies on the knife back. The section will be attracted to the slide electrostatically. The slide may be briefly warmed on the back of a gloved hand after picking up the section (to facilitate adhesion of the section to the slide). However, the slide should be cooled immediately against the cooled metal surface of the cryostat to refreeze tissue. Alternatively, the section can fixed immediately in cold 95% EtOH directly after electrostatic adherence to the slide and processed immediately.
4. For nucleic acid extraction, simply allow the tissue sections to roll naturally and place them into pre-labelled, pre-cooled microfuge tubes. Samples can be stored at -80 or

alternatively the appropriate extraction buffer can be added immediately and samples processed or stored at -80. See SOP # 8.3.011.

5. When sectioning is done, seal the exposed tissue is with a drop of OCT and remove the block carefully from the specimen disc. Then reseal the block in plastic wrap and immediately placed on dry ice for return to in cryostorage.
6. Frozen sections on slides not requiring a fixation step can go directly into pre-cooled plastic slide boxes or slide mailers sealed with Parafilm for storage in a -80° C freezer.

NOTE: During the sectioning procedure avoid allowing the OCT blocks to warm up. In particular, avoid cycles of heating and cooling.

7.3 Quality Assessment – General Considerations for section review.

1. At a minimum, assessment must consist of morphologic review of tissue sections.
2. Use researcher feedback about section quality to refine practices and guide evolution of Quality Control procedures.

7.4 Quality Assessment - Issues concerning quality of sections.

1. Make sure that representative tissue remains in the block after sections are cut for an assay. Do not completely deplete paraffin or frozen blocks.
2. Make sure there is sufficient material on a histological section for the intended assay without compromising representative material in tissue block.
3. Ensure that tissue on each section is appropriate for the purpose of the intended assay. (e.g., for a study of invasive cancer, representative invasive cancer cells need to be presenting sufficient quantity on all sections provided for the study).
4. If sections are intended for PCR-based molecular studies make sure that all attempts are made to eliminate or minimize nucleic acid contamination from equipment or other samples.
5. Ensure that type of fixation, processing duration and temperatures used during the fixation and sectioning procedures minimize the antigen masking or deterioration of molecular components. This is important for certain proteins in assays such as immunohistochemistry.
6. Ensure that section thickness is consistent and appropriate for intended use.
7. Ensure that sections are not scored or torn by the mictrotome knife as this will obscure microscopic observation and may cause uneven staining or bias assay results.
8. Ensure that thin sections are placed on electrostatically charged slides to avoid loss of section during the assay.
9. Ensure that paraffin and frozen sections are stored and shipped under appropriate conditions and temperatures.

7.5 Quality Assessment – general sectioning regimen for QA safeguards.

The use of this schema is recommended to ensure that representative sections from a sectioned block are kept quality assurance purposes. Perform these steps at the time the block is being sectioned for a research applications.

1. Obtain H&E sections at different depths to ensure that representative tissue is present.
2. If no H&E is available from the last sectioning of the block retain a "top" section for H&E review.
3. If many sections are taken from a block retain "intermediate" sections from the tissue block for H&E review. Every 30 sections is recommended (see #6 below).
4. Cut and retain a "bottom" section from the tissue block for H&E review. This section becomes the "top" for the subsequent use of the block.
5. Label sections serially starting at 1. Also indicate the date the section is cut.
6. If the tissue block is large, sections for quality assurance may be taken more frequently to ensure that they are representative of the material supplied for research studies. The frequency is up to the discretion of the technician and should be judged according to the size and nature of the tissue block or the specific needs of the research studies.

7.6 Quality Assessment – Review of 3, H&E slides.

These steps ensure that sufficient and representative tumour tissue appears on slides and that tissue appears adequately fixed.

1. Use the following coding system to score the H&E stained slides from the 3 levels:
    - 01: contains insufficient representative cancer
    - 02: no representative cancer present
    - 03: insufficient tissue for assay
    - 04: no primary tumor present
    - 05: contains sufficient representative cancer cell and normal epithelium for controls.
2. Record all scores.
3. Do not release the corresponding sections if they do not meet the criteria of the researcher or study.

8.0 APPLICABLE REFERENCES, REGULATONS AND GUIDELINES

1. Declaration of Helsinki. http://ohsr.od.nih.gov/helsinki.php3 http://www.wma.net/e/policy/b3.htm

2. Tri-Council Policy Statement; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, August 1998. http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm

3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series. http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf

4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). http://www.isber.org

5. US National Biospecimen Network Blueprint http://www.ndoc.org/about_ndc/reports/NBN_comment.asp

6. Jewell, S. et al. 2002, Analysis of the Molecular Quality of Human Tissues, an experience from the Cooperative Human Tissue Network. Am. J. Clin. Pathol. 118:733-741.

7. Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups http://ctep.cancer.gov/forms/guidelines_fresh_tissue.pdf

8. SOP No.3 (Draft 1). November 15, 2005. Standard Tissue Sectioning. NCIC CTG. Ontario.

9. Snell L. and P. H. Watson. 2006, Breast Tissue Banking: Collection, Handling, Storage and Release of Tissue for Breast Cancer Research. Methods Mol Med. 120:3-24.

10. Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups. http://ctep.cancer.gov/forms/draft_ffpe_tissue.pdf

11. Dressler, L.G. et al. 1999, Policy guidelines for the utilization of formalin-fixed, paraffin-embedded tissue sections: the UNC SPORE experience. Breast Cancer Research and Treatment, 58: 31-39.

