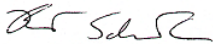


Sectioning of Tissue – Paraffin and OCT Embedded Tissue

CTRNet Standard Operating Procedure Sectioning of Tissue – Paraffin and Optimal Cutting Temperature (OCT) Embedded Tissue			
SOP Number:	08.03.006	Version:	e2.0
Supersedes:	8.3.006 e1.1	Category:	Material Handling and Documentation – Solid Tissue
Approved By:	CTRNet Management Group (CMG)	01-June-2012	
	Per: Brent Schacter 	28-June-2012	

1.0 PURPOSE

Preserved tumour tissues collected through the informed consent process are valuable for specific research studies. Formalin fixed and paraffin embedded (FFPE) tissue and tissue frozen in Optimal Cutting Temperature (OCT) compound can be sectioned for studies needing preservation of histomorphology of the specimen. For studies involving immunohistochemistry or *in situ* hybridization, 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 biobanks to follow when sectioning tissue preserved in paraffin or OCT.

In addition, quality control is fundamental to the successful operation of a tumour biobank offering tissue specimens for research purposes. CTRNet biobanks 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

This standard operating procedure (SOP) describes how tissues preserved in paraffin and OCT should be sectioned. The SOP also outlines minimum assessment that should be in place to evaluate the quality and integrity of paraffin and frozen tissue sections.

3.0 REFERENCE TO OTHER CTRNET SOPS OR POLICIES

Note: When adopting this SOP for local use please reference CTRNet.

- 3.1 CTRNet Policy: POL 5 Records and Documentation
- 3.2 CTRNet Policy: POL 2 Ethics
- 3.3 CTRNet Policy: POL 4 Privacy and Security
- 3.4 CTRNet Policy: POL 7 Material and Information Handling
- 3.5 CTRNet Standard Operating Procedure: SOP 08.03.003 Snap Freezing of Tissue
- 3.6 CTRNet Standard Operating Procedure: SOP 08.03.005 Preservation of Tissue: Paraffin Embedding
- 3.7 CTRNet Standard Operating Procedure: SOP 05.001 Assessing Quality of Tissue Specimens
- 3.8 CTRNet Standard Operating Procedure: SOP 08.01.002 Biohazardous Material Waste Management

Sectioning of Tissue – Paraffin and OCT Embedded Tissue

4.0 ROLES AND RESPONSIBILITIES

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

Tumour Biobank Personnel	Responsibility/Role
Pathologist	Conduct histopathological characterization
Laboratory or Histology Technician/Technologist	May be specifically responsible for processing FFPE tissues and sectioning paraffin and OCT blocks. Conducts and assists with quality control. Records and documents outcomes.

5.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed below 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)
Solvent resistant markers, ink, pencils, and pens	
Microscope	
Microtome	
Hot water bath	
Microtome blades	
Fine tipped paint brush	
Fine tipped tissue separator	
Appropriate labels for slides	
Labelled glass slides	
Tray to hold slides	
Ice tray	
Oven	
Cryostat	
Labeled 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	
Optimal Cutting Temperature Compound (OCT)	
Harris Haematoxylin (filtered)	
Eosin	

6.0 DEFINITIONS

See the CTRNet Program Glossary: <http://www.ctrnet.ca/glossary>

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 Tumour Bank and NCIC-CTG.

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

- 7.1.1 Treat all tissue as potentially infectious.
- 7.1.2 Sectioning is performed by the laboratory or histology technician/technologist or personnel trained to use a microtome and cut histological sections.
- 7.1.3 Have materials and equipment ready. Have as many slides as needed labelled and ready.
- 7.1.4 Pre-cool paraffin blocks, tissue side down, on a tray of ice. In some cases this may facilitate sectioning. Using a steel microtome knife or disposable blade cut sections that are 4-5 microns for histological sections, and 5-10 microns for nucleic acid extraction and up to 20 microns for protein extraction purposes.
- 7.1.5 For histological sections label slides serially.
- 7.1.6 Dry paraffin sections at 37° C overnight, although this depends on purpose.
- 7.1.7 Remove the sections from the oven and allow cooling at room temperature.
- 7.1.8 The sections are stored for shipping in slide mailers or stored in slide holder boxes most often at room temperature. Extended storage (usually more than 3 days) 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.
- 7.1.9 For nucleic acid extraction sections, allow the individual sections to roll up naturally and place them directly into sterile 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. When cutting sections for DNA or RNA extraction, all instruments and equipment must be pre-cleaned and wiped down with RNase-away before and between each specimen. Gloves must be worn. Molecular grade water must be used for floating sections for RNA extraction. If sections require floating for RNA analysis, molecular grade water must be used.

7.2 Sectioning OCT Embedded Tissue

Sectioning tissue can be dangerous and carries a biohazard risk. Personnel sectioning tissue should receive adequate training in operating sectioning equipment and using safety precautions

Sectioning of Tissue – Paraffin and OCT Embedded Tissue

- 7.2.1 Frozen sections are cut by personnel specifically trained to perform the task of sectioning OCT embedded tissue in a cryostat. The frozen tissue cryomolds or vials are transferred to the cryostat on dry ice.
- 7.2.2 Set the section thickness at 4-5 microns for immunohistochemistry, *in situ* hybridization or Haematoxylin and Eosin and 5-10 microns for nucleic acid extraction and up to 20 microns for protein extraction samples. Since OCT may interfere with the further manipulation of nucleic acids, it is recommended that if the sections are to be extracted for nucleic acids, care should be taken to avoid OCT contamination of the sample.
- 7.2.3 Sections are mounted on room temperature slides by inverting the slide on a slight angle over the section as it lies on the knife back. The section will be attracted to the slide electrostatically. However, the slide should be placed at -20° C after 30 minutes at room temperature. Alternatively, the section can be fixed immediately in cold 95% ethanol immediately after electrostatic adherence to the slide and processed immediately.
- 7.2.4 For nucleic acid or protein 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° C or alternatively the appropriate extraction buffer can be added immediately and samples processed or stored at -80° C.
- 7.2.5 When sectioning is done, remove the block carefully from the specimen disc. Then reseal the block with foil and immediately place it on dry ice for return to cryostorage. When cutting sections for DNA or RNA extraction, all instruments and equipment must be pre-cleaned and wiped down with RNase-away. Clean the cryostat mix with 70% ethanol on sterile gauze (to prevent freezing) before and between each specimen. Gloves must be worn. Molecular grade water must be used for floating sections for RNA extraction.
- 7.2.6 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

- 7.3.1 At a minimum, assessment must consist of morphologic review of tissue sections.
- 7.3.2 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

- 7.4.1 Make sure that representative tissue remains in the block after sections are cut for an assay. Do not completely deplete paraffin or frozen blocks.
- 7.4.2 Make sure there is sufficient material on a histological section for the intended assay without compromising representative material in the tissue block.
- 7.4.3 Ensure that the block used for tissue sectioning is appropriate for the purpose of the intended assay. (e.g., for a study of invasive cancer, representative invasive cancer cells need to be present in sufficient quantity on all sections provided for the study).
- 7.4.4 If sections are intended for Polymerase Chain Reaction (PCR)-based molecular studies make sure that all attempts are made to eliminate or minimize nucleic acid contamination from equipment or other samples.

Sectioning of Tissue – Paraffin and OCT Embedded Tissue

- 7.4.5 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.
- 7.4.6 Ensure that section thickness is consistent and appropriate for intended use.
- 7.4.7 Ensure that sections are not scored or torn by the microtome knife as this will obscure microscopic observation and may cause uneven staining or bias assay results.
- 7.4.8 Ensure that thin sections are placed on electrostatically charged slides to avoid loss of the section during the assay.
- 7.4.9 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 for quality assessment purposes. Perform these steps at the time the block is being sectioned for a research application.

- 7.5.1 Ensure that a representative Hematoxylin and Eosin (H&E) section is retained from the block within the biobank.
- 7.5.2 If no H&E is available from the last sectioning of the block retain a “top” section for H&E review.
- 7.5.3 If many sections are taken from a block, it may be useful to retain “intermediate” sections from the tissue block for H&E review.
- 7.5.4 Label sections serially. Also record the date the section is cut.

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

- 8.1 Declaration of Helsinki
<http://www.wma.net/en/30publications/10policies/b3/index.html>
- 8.2 Tri-Council Policy Statement 2; 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, December 2010.
<http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/>
- 8.3 Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
<http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420>
- 8.4 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/Search/search.asp?zoom_query=best+practices+for+repositories
- 8.5 US National Biospecimen Network Blueprint
<http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp>
- 8.6 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.
- 8.7 Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups
- 8.8 SOP No.3 (Draft 1). November 15, 2005. Standard Tissue Sectioning. NCIC CTG. Ontario.

Sectioning of Tissue – Paraffin and OCT Embedded Tissue

- 8.9 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.
- 8.10 Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups.
- 8.11 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.

9.0 APPENDICES

None

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
LP 002.001	2005	JdSH	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.006 e1.0	09-01-2008	JdSH	Revised to deal specifically with sectioning of tissue preserved in paraffin and OCT (and related QA issues) Updated format.
8.3.006 e.1.1	25-04-2009	JdSH	Step 7.2 Added safety notice for adequate training and biohazard before 7.2.1.
8.3.006 e1.1	June 2012	CMG	<ul style="list-style-type: none"> • Grammatical and formatting throughout • Definitions removed • Revision History moved to bottom • Reference links updates • Updated SOP references • Section 2.0: Deleted second paragraph. • Section 7.1 -7.5: Sections revised detailing procedures.