Title	Sectioning of Paraffin and OCT Embedded Tissue
SOP Code	SOP117_01
Effective Date	01-Sep-2012

Site Approvals

Name and Title (typed or printed)	Signature	Date dd/Mon/yyyy

1.0 PURPOSE

This Standard Operating Procedure (SOP) outlines standardized procedures for biorepositories to follow when sectioning tissue preserved in paraffin or Optimal Cutting Temperature (OCT) medium. This SOP also outlines minimum assessment that should be in place to evaluate the quality and integrity of paraffin and frozen tissue sections. This SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

2.0 SCOPE

This procedure applies to all biorepository personnel that are responsible for sectioning tissue preserved in paraffin or OCT blocks.

3.0 RESPONSIBILITIES

Sectioning is performed by the laboratory or histology technician, or personnel trained to use a microtome and cut histological sections. Frozen sections are cut by personnel specifically trained to perform the task of sectioning OCT embedded tissue in a cryotome.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

5.1 Sectioning Formalin Fixed Paraffin Embedded Tissue

5.1.1 Have materials and equipment ready. Label a sufficient number of slides. Clean all instruments and equipment, as required, before and between each specimen.

5.1.2 Pre-cool paraffin blocks, tissue side down, on a tray of ice-water. Use molecular grade water for floating sections for nucleic acid extraction. This will facilitate sectioning especially fatty, bloody, or tissue. 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.

5.1.3 Label slides serially for histological sections.

5.1.4 Dry paraffin sections at 55°C for 1 hour.

5.1.5 Remove the sections from the oven and allow cooling at room temperature.

5.1.6 Store the sections for shipping in slide mailers or in slide holder boxes at 4°C. Avoid extended storage of unstained FFPE slides, as this may result in the loss of antigens. While not established, vacuum sealing and refrigeration may help preserve some unstable antigens.

5.1.7 For nucleic acid extraction from blocks with sufficiently high tumour content, allow the individual sections to roll up naturally and place them directly into sterile microfuge tubes ready for nucleic acid extraction. Add the extraction buffer directly to the microfuge tube, in order to preserve the molecular integrity of the sample.

5.2 Sectioning OCT Embedded Tissue

5.2.1 Transfer the frozen tissue cryomolds or vials to the cryotome on dry ice.

5.2.2 Have materials and equipment ready. Label a sufficient number of slides. Clean all instruments and equipment, as required, before and between each specimen.

5.2.3 Set the section thickness at 4-5 microns for IHC, ISH, or H&E and 10-20 microns for nucleic acid extraction samples. Use charged slides for IHC sections.

5.2.4 Mount sections on ambient 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. Place the slide at -20°C after 30minutes at room temperature.

Alternatively, fix the section immediately in cold 95% EtOH, directly after electrostatic adherence to the slide and process immediately.

5.2.5 For nucleic acid extraction from blocks with sufficiently high tumour content, allow the tissue sections to roll naturally and place them into pre-labelled, pre-cooled microfuge tubes. Store samples at -80°C, or alternatively add the appropriate extraction buffer immediately and process or store at -80°C..

5.2.6 When sectioning is done, remove the block carefully from the specimen disc. Reseal the block in foil and immediately place on dry ice for return to cryostorage.

5.2.6. 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.

5.3 Quality Assessment

5.3.1 At a minimum, perform a morphologic review of tissue sections.

5.3.2 Ensure that representative tissue remains in the block after sections are cut for an assay. Do not completely deplete paraffin or frozen blocks.

5.3.3 Ensure that there is sufficient material on a histological section for the intended assay without compromising representative material in tissue block.

5.3.4 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).

5.3.5 If sections are intended for PCR-based molecular studies, ensure that all attempts are made to eliminate or minimize nucleic acid contamination from equipment or other samples.

5.3.6 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.

5.3.7 Ensure that section thickness is consistent and appropriate for intended use.

5.3.8 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.

5.3.9 Ensure that thin sections are placed on electrostatically charged slides to avoid loss of section during the assay.

5.3.10 Ensure that paraffin and frozen sections are stored and shipped under appropriate conditions and temperatures.

5.4 Sectioning for Quality Assessment

5.4.1 Use this schema to ensure that representative sections from a sectioned block are kept for quality assurance purposes.

5.4.2 Obtain H&E sections at different depths to ensure that representative tissue is present.

5.4.3 If no H&E is available from the last sectioning of the block, retain a “top” section for H&E review.

5.4.4 If many sections are taken from a block, retain “intermediate” sections from the tissue block for H&E review. Every 30 sections is recommended.

5.4.5 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.

5.4.6 Label sections serially starting at 1. Also indicate the date the section is cut.

5.4.7 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.

6.0 REFERENCES

Health Canada, Food and Drug Regulations, Part C, Division 5, Drugs for Clinical Trials Involving Human Subjects, (Schedule 1024), June 20, 2001.

Health Canada, Guidance for Industry, Good Clinical Practice: Consolidated Guideline, ICH Topic E6, 1997.

2011 NCI Best Practices for Specimen Resources. Office of Biorepositories and Biospecimen Research, National Cancer Institute, Bethesda, MD. http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf

ISBER Best Practices for repositories: Collection, storage, retrieval and distribution of biological materials for research. Cell Preservation Technology 6(1), 3-58, 2008 http://www.isber.org/Pubs/BestPractices2008.pdf

CTRNET Standard Operating Procedures, Canadian Tumour Repository Network, http://www.ctrnet.ca/operating-procedures

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP117_01	01-Sep-2012	Original version