James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

STANDARD OPERATING PROCEDURE

Title:	**Preservation of Tissue: Freezing in OCT**		
Procedure:	BB_HIST.003.01	**Supercedes:**	none
Originator and Date:	Crystal Leung 29OCT2008	**Effective Date:**	29OCT2008
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Revision History		
Date	**Reviewer**	**Summary of revision**
20Apr2009	Crystal Leung	Reformatted to iCAPTURE format

Purpose

The purpose of this document is to outline standardized procedures for BC Biobank personnel to follow when freezing fresh tissue in OCT embedding medium. Cryopreservation should occur in a timely and appropriate manner. OCT (optimal cutting temperature) compound is especially good for preserving cellular ultrastructure (histology). OCT allows for sectioning of frozen tissue and staining with certain stains, antibodies, or gene probes.

Responsibilities

This procedure is applicable to the following:

- Biobank personnel who are responsible for freezing tissue in OCT

Safety

Universal precautions are a method of infection control in which all human tissue, blood and body fluids are treated as if they are infectious. The SOP does not cover detailed

safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals. Refer to BB.001.01 (Biohazardous Material Handling).

Definitions

Cryopreservation	A process for storing biological material at very low temperatures for lengthy periods of time.
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 allows repeated production of frozens sections from one specimen without freeze-thaw cycles.
SOP	Standard Operating Procedure. Document used to control the method and requirements by which personnel will perform their activities.
Cryostat	A chamber, containing a microtome for sectioning frozen tissue, which can maintain temperatures at very low levels.

Materials and Equipments

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products.

Container with dry ice (for transport of frozen tissue)	Labeled small freezer bags (Ziploc) for storage of cryomolds
Markers, ink and pens	Labels for cryovials, cryomolds and plastic bags
Clean Forceps	Storage containers for cryomolds
Clean Scalpels for trimming tissue	Dry shipper for transportation of tissues at <150°C
Liquid Nitrogen	Needle/sharps disposal unit
2-Methylbutane (isopentane)	Insulated Gloves suitable for handling liquid nitrogen tank and storage container
Container for Isopentane (eg. small metal cup)	Clean blue underpads for covering bench surface
Labelled Cryovials for storage of frozen tissue (screw top)	Heavy Duty Aluminum foil

Plastic Cryomolds (such as Tissue-Tek :#4557)	Tissue Collection/Processing Worksheets (see Appendix 1 for sample form)
Parafilm	

Procedures

This procedure is intended to ensure that tissue samples collected from consented participants will be frozen in a safe and efficient manner while eliminating the risks of contamination and loss of molecular integrity. To facilitate the use of genomic and proteomic techniques, banked tissue that has been adequately frozen is vital to obtaining products with high integrity and quality.

CAUTION: Treat all tissue as potentially infectious.

1) Have materials and equipment ready. Have as many cryovials or cryomolds as needed labeled and ready.
2) Optimally, tissue should be frozen within 30 minutes from resection.
3) Do not freeze the tissue directly on ice.
4) Ensure that the resected tissue never desiccates or is contaminated by surrounding tissue or other samples. Use clean scalpels and forceps between samples to avoid cross contamination between samples or between tumour and normal tissue.
5) Do not place the sample in contact with formalin at any point in the process. Do not add serum to the sample.
6) If tissues were already formalin fixed for preservation prior to OCT freezing, use a clean forcep and rinses the tissue in a beaker of water. Dry the tissue by gently pressing it on a paper towel.
7) Fill a wide mouthed steel thermos or insulated container with liquid nitrogen. Cool isopentane by suspending the container of isopentane in liquid nitrogen. Isopentane is sufficiently cooled (super-cooled) when “pearls” form on the sides and bottom of the container and the solution becomes hazy.
8) Place a few drops of the OCT compound into a pre-labeled plastic cryomold.
9) With clean forceps, place the specimen to be frozen onto the OCT in the cryomold. If required, orient the tissue in the cryomold.
10) Add more OCT to cover the tissue and fill the cryomold.
11) Avoid introducing any air bubbles into the OCT. Release any bubbles that may become trapped around the tissue.
12) If using isopentane, submerge the cryomold in the super-cooled isopentane until the OCT is completely frozen (white and solid). This takes 10 seconds or less depending on size.

13) Alternatively, the cryomold or cryovial containing the tissue and OCT can be frozen directly in liquid nitrogen or in a liquid nitrogen vapour shipper without the isopentane step. To freeze directly in liquid nitrogen, hold the vial or cryomold with forceps and gently immerse the mold in liquid nitrogen allowing for freezing to proceed from the bottom of the cryomold or vial. To freeze directly in a liquid nitrogen vapour shipper, place the cryomold onto a shelf within the shipper.
14) Alternatively, the cryomold or cryovial containing the tissue and OCT can be frozen directly on dry ice. To freeze directly on dry ice, place the cryomold on or immerse the cryovial in the dry ice.
15) Alternatively, the cryomold containing the tussue and OCT can be frozen in a cryostat. To freeze in a cryostat, place the cryomold directly in the cryostat until OCT turns white and solid.
16) Once OCT is completely frozen, remove the cryomold from the isopentane, liquid nitrogen, liquid nitrogen vapour shipper, dry ice, or cryostat.
17) Place the cryomold into a small labeled freezer/ziploc bag and place the sealed bag on dry ice. Alternatively, extract the frozen tissue OCT block from the cryomold, wrap tightly in parafilm to prevent dessication, then wrap it in heavy duty aluminum foil and label. Then place the wrapped blocks in a freezer/ziploc bag or cryovial.
18) Transport the bags or vials on dry ice and for storage at -80º C or colder.
19) Record the storage location.
20) Record time of freezing on the Biospecimen Collection Work sheet (See Appendix 1). If possible, determine time elapsed between resection and freezing and record this as well. At the very least, record the approximate time (using 15 minute increments) after resection that the tissue was frozen (i.e. Within 30 minutes or between 30-45 minutes etc.)

References

i. *Canadian Tumor Repository Network*; SOP #8.3.004, OCT Freezing of Tissue.
ii. *The University of British Columbia, Department of Health, Safety and Environment*; Laboratory Biosafety Reference Manual, 4th Edition 2001

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Appendix I

The Biospecimen Collection Worksheet can be customized by users to capture relevant information. The following may be used as a guide for relevant sets of information to record.

Biospecimen Collection and Transportation

Label (Unique identifier)	
Biospecimen Collection Site	
Date of surgery/Collection	
Time Specimen is resected	
Time Specimen is Received by Pathology Lab	
Was sample transported on ice?	YES NO
Type of specimen	
Surgical case number	
Patient Name	
Patient Birthdate:	
PHN/MRN	

Biospecimen Information

Label (Unique identifier)	**Tissue type**	**Was matching normal available and taken ?**	**Tumour size**	**Tissue Observations**

Biospecimen Harvesting

Harvested by: Biobank Biospecimen Coordinators

Time Frozen: Very Important to record this time (estimate time elapsed from resection)

1. Tissue collection

a. Fresh Frozen

Label (identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

b. Frozen in OCT

Label (identifier)	Snap Frozen by	Date Frozen	Time Frozen	Sample Size	Storage location

c. Formalin Fixed

Label (identifier)	Fixed by	Date/Time in Fixative	Date/Time out of Fixative	No. of tissue cassettes	Storage location

d. Stored in another form (eg. In RNAlater®) Yes No

2. Blood Components:

Serum: Yes/No Plasma: Yes/No Buffy Coat: Yes/No