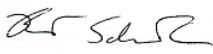


Freezing of Tissue in Optimal Cutting Temperature (OCT)

CTRNet Standard Operating Procedure Freezing of Tissue in Optimal Cutting Temperature (OCT)			
SOP Number:	08.03.004	Version:	e2.0
Supersedes:	8.3.004 e1.0	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

Tissue samples (surplus to the needs of pathology) are collected from patients that have been through the informed consent process and agreed to participate in the tumour biobank program. Fresh frozen tissue collections are a valuable resource for research purposes. Tumour tissues are only suitable for proteomic and genomic studies if cryopreserved in a timely and appropriate manner. Optimal cutting temperature (OCT) is a compound especially good for preserving cellular ultrastructure (histology) and likely nucleic acids as well. It is also used for specialized applications where formalin fixed tissue is not optimal.

2.0 SCOPE

This standard operating procedure (SOP) describes how tissues are frozen in OCT. 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 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 07 Material and Information Handling

3.5 CTRNet Standard Operating Procedure: SOP 08.03.001 Tissue Collection and Transportation

3.6 CTRNet Standard Operating Procedure: SOP 08.03.002 Tissue Harvesting

3.7 CTRNet Standard Operating Procedure: SOP 08.01.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from CTRNet member biobanks who are responsible for OCT freezing of the harvested tissue.

Tumour Biobank Personnel	Responsibility/Role
Pathology Assistant	Assists with resection, harvesting and transportation of tissue. May communicate with Laboratory technician/technologist.
Laboratory Technician/Technologist	Transportation of tumour tissue, harvesting processing and storage

Freezing of Tissue in Optimal Cutting Temperature (OCT)

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)
Container with dry ice (for transport of frozen tissue)	
Markers, ink and pens	
Clean forceps	
Clean scalpels for trimming tissue	
Liquid Nitrogen	
2-Methylbutane (isopentane)	
Container for isopentane	
Labeled cryovials for storage of frozen tissue (screw top)	
Plastic cryomolds (such as Tissue-Tek: #4557)	
Labeled small zip lock bags for storage of cryomolds	
Sufficient appropriate labels (see SOP # 8.01.001) for cryovials, molds and plastic bags	
Storage containers for cryomolds	
Dry shipper or Dewar for transportation of Liquid nitrogen	
Needle/sharps disposal unit	
Gloves worn to protect personnel handling tissue	
Safety glasses for personnel handling liquid nitrogen tank and storage container	
Insulated gloves suitable for handling liquid nitrogen tank and storage container	
Clean underpads for covering bench surface	
Tissue Collection/Harvesting worksheets (see Appendix A for sample form)	
Optimal Cutting Temperature Compound (OCT)	

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 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.

Freezing of Tissue in Optimal Cutting Temperature (OCT)

7.1 Freezing of Tissue in OCT

- 7.1.1 Treat all tissue as potentially infectious.
- 7.1.2 Freezing is performed by the laboratory technician/technologist or trained personnel designated by the tumour biobank.
- 7.1.3 Have materials and equipment ready. Have as many cryovials or cryomolds as needed labelled and ready.
- 7.1.4 Unless intended for another method of preservation fresh tumour tissue should be frozen as soon as possible. Optimally, tissue should be frozen within 30 minutes after resection.
- 7.1.5 Do not freeze the tissue directly on ice.
- 7.1.6 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.
- 7.1.7 OCT frozen tissue is suitable for preparation of DNA and RNA. It is especially useful for preserving fresh tissue intended for histopathology where morphological information is important. Do not place the sample in contact with formalin at any point in the process. Do not add serum to the sample.
- 7.1.8 Cool isopentane by suspending the container of isopentane in liquid nitrogen. Isopentane is sufficiently cooled when “pearls” form and the solution becomes hazy.
- 7.1.9 Place a few drops of the OCT compound into a pre-labeled plastic cryomold.
- 7.1.10 With clean forceps, place the specimen to be frozen onto the OCT in the cryomold. If relevant to your type of tumour specimen, orient the tissue in the cryomold.
- 7.1.11 Add more OCT to cover the tissue and fill the mould.
- 7.1.12 Use a forceps or transfer pipette to orient tissue and remove air bubbles.
- 7.1.13 Avoid introducing any air bubbles into the OCT. Release any bubbles that may become trapped around the tissue.
- 7.1.14 Place the cryomold in a small container containing pre-cooled isopentane.
- 7.1.15 Submerge the mould in the isopentane until the OCT is completely frozen (white and solid).
- 7.1.16 Alternatively, the cryomold or cryovial containing the tissue and OCT can be frozen directly in liquid nitrogen without the isopentane step. Hold the vial or cryomold with a forceps and gently immerse the mould in liquid nitrogen contained in a dry shipper allowing for freezing to proceed from the bottom of the cryomold or vial.
- 7.1.17 Remove the mould from the liquid nitrogen.
- 7.1.18 Place the mould into a small, labelled zip-lock bag and place the sealed bag on dry ice.
- 7.1.19 Transport the bags or vials on dry ice and for storage at -80°C or colder.
- 7.1.20 Record the storage location.
- 7.1.21 Record time of freezing on the Tissue Collection/Harvesting Worksheet (See Appendix A). 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.).

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 National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
<http://bioethics.georgetown.edu/nbac/hbm.pdf>
- 8.7 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.
- 8.8 SOP-PRO-Tissue-freezing.02, Oct 3, 2006. Freezing of Prostate Tissues. Procure Quebec Prostate Cancer Biobank.
- 8.9 Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups

9.0 APPENDICES

- 9.1 Appendix A – Sample Form - Tissue Collection/Harvesting Worksheet

Freezing of Tissue in Optimal Cutting Temperature (OCT)

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
LP 002.001	08-08-2005	JdSH	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.004	08-01-2008	JdSH	Revised to cover freezing of tissue in OCT only
8.3.004 e1.0	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 1.0: Inserted "Optimal cutting temperature" and deleted last sentence of paragraph.

SAMPLE FORM - TISSUE COLLECTION HARVESTING WORKSHEET

The Tissue Collection/Harvesting Worksheet can be customized by specific sites to capture information relevant to the site. The following may be used as a guide for relevant sets of information to record:

Tissue Collection and Transportation

Collection Site	
Date of Tumour is resected	
Time of Tumour is resected	
Date Tumour Sample Received by Pathology Laboratory	
Time Sample is Received by Pathology Laboratory	
Name of Person Transporting Tissue	
Was sample transported on ice?	YES NO
Pathologist (Name)	
Additional Collection Notes:	

Sample Information

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

Tissue Harvesting

Harvested by: Laboratory Technician/Technologist name

Time Frozen: Very Important to record this time

Indicate if Tissue was taken for:

1. Fresh Frozen Collection

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

2. Frozen in OCT

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

3. Formalin Fixed. Yes No
Date: Storage Location:

4. Stored in another form (e.g. In RNeasy®) Yes No
Date: Storage Location: