CTRNet Standard Operating Procedure Snap Freezing of Tissue			
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Subject:	Snap Freezing of Tissue	Category	Material Handling and Documentation

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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary
LP 002.001	2005	JdSH	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.003	2008	JdSH	Revised to cover snap freezing of tissue only

1.0 PURPOSE

Tissue (surplus to the needs of Pathology) samples are collected from patients that have been through the informed consent process and agreed to participate in the tumour repository program. Fresh frozen tissue collections are a valuable resource for research purposes. Tumour tissues are only suitable for proteomic and genomic studies if frozen in a timely and appropriate manner. The purpose of this document is to outline standardized procedures for CTRNet repositories to follow during snap freezing tumour tissue.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how tissues are snap frozen. 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: 8.3.001 Tissue Collection and Transportation to Pathology
7. CTRNet SOP: 8.3.002 Tissue Harvesting
8. CTRNet SOP: 8.1.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITY

The policy applies to all personnel from CTRNet member repositories who are responsible for snap freezing of the harvested tissue.

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Lab Technician	Transportation of tumour tissue, harvesting processing, freezing and storage of tissue.	
Pathology Assistant	Assists with harvesting and transportation of tissue. May communicate with lab technician.	

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)	
Sufficient appropriate labels (see SOP # 8.1.001) for	

cryovials	
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 bench surface	
Tissue Collection/Processing Worksheets (see Appendix 1 for sample form)	

6.0 DEFINITIONS

Cryopreservation: A process for storing biological material at very low temperatures for lengthy periods of time.

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.

7.1 Snap freezing of Tumour Tissue

1. Treat all tissue as potentially infectious.
2. Freezing is performed by the laboratory technician or trained personnel designated by the tumour repository.
3. Have materials and equipment for ready. Have as many cryovials as needed labelled and ready.
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 from resection.
5. Do not freeze the tissue directly on ice.
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. Snap frozen tissue is suitable for preparation of DNA, RNA and protein. Do not place the sample in contact with formalin at any point in the process. Do not add serum to the sample.
8. Cool isopentane by suspending the container of isopentane in liquid nitrogen. Isopentane is sufficiently cooled when "pearls" form and the solution becomes hazy.
9. With clean forceps, place the specimen to be frozen into an empty screw capped cryovial.
10. Close the cryovial.
11. Place the cryovial with the specimen into the container of cooled isopentane.
12. The specimen should freeze within 30 seconds.
13. Alternatively, the isopentane freezing step can be the optional. Place the tissue specimen into an empty cryovial, close the cryovial, and immediately submerge the cryovial into liquid nitrogen. The specimen should freeze within 30-60 seconds. This is not recommended if the sample is large in size as longer freezing time will result in ruined morphology.
14. Once snap frozen, transfer the sample to liquid nitrogen storage container (preferred) or to a -80º C (or colder) freezer.
15. Samples should be placed on dry ice to be carried to the freezer or liquid nitrogen storage facility.
16. If storing the samples in liquid nitrogen, it is recommended that the samples be placed in the vapour phase of liquid nitrogen.
17. Record the storage location.
18. Record time of freezing on the Tissue Collection/Harvesting Work sheet (See Appendix 1). 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, 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. 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

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

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

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

Appendix A. Worksheets

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 Tumour id resected	
Time Tumour is resected	
Date Tumour Sample Received by Pathology Lab	
Time Sample is Received by Pathology Lab	
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: Technicians 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 (eg. In RNAlater®) Yes No
Date: Storage location: