CTRNet Standard Operating Procedure Snap Freezing of Tissue			
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Supersedes:	8.3.003 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 frozen in a timely and appropriate manner. The purpose of this document is to outline standardized procedures for CTRNet biobanks to follow during snap freezing tumour tissue. Similar procedures may be adopted for other tissues of interest that may be harvested for the biobank during surgery (such as non-malignant tissue, adjacent non-malignant tissue, lymph nodes, muscle samples, etc.); document clearly if the procedures deviate for the non- malignant samples.

2.0 SCOPE

This 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 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 08.03.001 Tissue Collection and Transportation*
3.6 *CTRNet Standard Operating Procedure: SOP 08.03.002 Tissue Harvesting*
3.7 *CTRNet Standard Operating Procedure: SOP 08.03.003 Biohazardous Material Waste Management*

4.0 ROLES AND RESPONSIBILITIES

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

Tumour Biobank Personnel	Responsibility/Role
Laboratory Technician/Technologist	Transportation of tumour tissue, harvesting processing and storage.
Pathology Assistant	Assists with harvesting and transportation of tissue and performs tasks delegated by the pathologist. May communicate with laboratory technician/technologist.

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) (optional)	
Container for Isopentane (optional)	
Labeled cryovials for storage of frozen tissue (screw top)	
Sufficient appropriate labels (see *SOP # 8.01.001*) 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/Harvesting worksheets (see Appendix A for sample form) later in the protocol use harvesting worksheet	

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.

7.1 Snap Freezing of Tumour Tissue

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 for ready. Have as many cryovials 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 from 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 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.

Choose either Steps 7.1.8 to 7.1.10 (snap-freezing with isopentane), or Step 7.1.11 (snap-freezing with liquid nitrogen):

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 With clean forceps, place the specimen to be frozen into an empty screw capped cryovial. Close the cryovial.

7.1.10 Place the cryovial with the specimen into the container of cooled isopentane. The specimen should freeze within 30 seconds.

or

7.1.11 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.

Then

7.1.12 Once snap frozen, transfer the sample to liquid nitrogen storage container (preferred) or to an -80º C (or colder) freezer.

7.1.13 Samples should be placed on dry ice to be carried to the freezer or liquid nitrogen storage facility.

7.1.14 If storing the samples in liquid nitrogen, it is recommended that the samples be placed in the vapour phase of liquid nitrogen. Note: the glass transition temperature of water is -134^{o} C.

As such, samples intended for indefinite long-term storage should be kept at temperatures lower than -135 ^{o} C.

7.1.15 Record the storage location.

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

8.8 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

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
8.3.003	2008	JdSH	Revised to cover snap freezing of tissue only
8.3.003	2011	MMA	Section 5: Isopentane materials listed as optional Section 7: Procedure wording changed to clarify that user can choose either isopentane or liquid nitrogen for snap freezing. Step 14 text added to suggest lower temperature freezing for indefinite storage based on glass transition Section 8: TCP2 referenced
8.3.003	2012	SD	Mention program/committee and not an individual; apply this change to all SOPs for this section -Other minor edits shown using track tool
8.3.003 e1.0	June 2012	CMG	• Grammatical and formatting throughout • Definitions removed • Revision History moved to bottom • Reference links updates • Updated SOP references • Section 1.0 – Added last sentence in this section. • Section 5.0-Noted two items are “Optional” • Section 7.7-Inserted “Choose either Steps…” • Section 7.14 – Inserted Note in this item.

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 resection	
Time of Tumour resection	
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 RNAlater®) Yes No
Date: Storage Location: