CTRNet Standard Operating Procedure Preservation of Tissue: Paraffin Embedding			
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Subject:	Preservation of Tissue: Paraffin Embedding	Category	Material Handling and Documentation

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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary of Revisions
LP 002.001	2005	JdSH	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.005	09-01-2008	JdSH	Revised to cover paraffin embedding of tissue only

1.0 PURPOSE

Tumour tissues are only suitable for specific research studies if preserved appropriately. To date, formaldehyde is the most widely used universal fixative because it preserves a wide range of tissues and tissue components. Formaldehyde fixed and paraffin embedded (FFPE) tissue can easily be stored under normal laboratory conditions for a long period. The method is effective for preserving histological morphology of the tissue specimen. The purpose of this document is to outline standardized procedures for CTRNet repositories to follow when preserving tissue by the FFPE method.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how tissues should be preserved by FFPE. 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 FFPE treatment of the harvested tissue.

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Lab Technician	Transportation of tumour tissue, harvesting processing and storage	
Histology Lab Technician	May be specifically responsible for processing FFPE tissues	

5.0 MATERIALS, REAGENTS, 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)**
Markers, ink and pens	
Clean Forceps	
Clean Scalpels for trimming tissue	
Containers for fixing tissue	
Sufficient appropriate labels (see SOP # 8.1.001) for tubes and histology cassettes	
Histology casettes	
Needle/sharps disposal unit	
Gloves worn to protect personnel handling tissue	
Clean underpads for covering bench surface	
Tissue Collection/Processing Worksheets (see Appendix	

1 for sample form)	
Neutral pH Phosphate buffered Formalin	
Alcohol (ethanol)	
Xylene	
Paraffin	

6.0 DEFINITIONS

Preservation: Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of a specimen.

Dehydration: Removal of water from tissue.

7.0 PROCEDURES

This procedure is intended to ensure that tissue samples collected from consented participants will be preserved in a safe and efficient manner while eliminating the risks of contamination and loss of molecular and structural integrity. Banked tissue that has been adequately preserved is useful for a greater variety of studies. Consistency in procedure is important for obtaining comparable and reliable test results. Formalin fixation is standard practice in most routine histopathology laboratories. The following guidelines address specific issues related to preservation of formalin-fixed specimens.

1. Tissue specimens should not be bigger than 1.5 x 1 x 0.5 cm.
2. Under-fixation is a greater risk but also avoid over fixation as it can create problems for immunohistochemical methods.
3. Fixatives such as Bouin's containing Picric acid must be avoided as this compound interferes with subsequent PCR analysis of extracted nucleic acids.

The site may have an automated paraffin-embedding processor which have standardized processing times. However, use the following steps as a guide.

7.1 Fixation in Formalin

1. Treat all tissue as potentially infectious.
2. Fixation is performed by the laboratory technician or trained personnel designated by the tumour repository.
3. Have materials and equipment ready. Have as many containers, cassettes or vials as needed labelled and ready.
4. Fixation of tissue should be undertaken as soon as possible. Optimally, tissue should be fixed within 4 hours from resection.

5. Record time from resection to fixation.
6. Use 10% neutral pH phosphate buffered Formalin as a fixative. It is important that the fixative is buffered to avoid the formation of formaldehyde pigment on blood rich tissues.
7. Perform fixation at room temperature (25º C).
8. The volume of the fixative should be at least 10-15 times greater than the volume of the tissue (i.e., 10-15 ml for every gram of tissue).
9. If needed, dissect the tissue before fixation to ensure adequate penetration of the fixative.
10. It is recommended that specimen thickness should be 2.5 mm or thinner to be adequately fixed. If this is not possible, do not use specimens that are over 8 mm in thickness.
11. Optimally, duration of fixation should be overnight to 24 hours but no more than 48 hours.

7.2 Processing for Embedding

1. Dehydrate tissues through series of alcohols.
2. Clear tissue by treatment with xylene
3. The following steps for dehydration and clearing can be can be used as a guide.

STEP	TIME	SOLUTION
2	30 min	ALCOHOL 70%
3	30 min	ALCOHOL 95%
4	30 min	ALCOHOL 100%
5	60 min	ALCOHOL 100%
6	60 min	ALCOHOL 100%
7	60 min	ALCOHOL 100%
8	60 min	XYLENE
9	60 min	XYLENE
10	60 min	XYLENE

4. After step 10 in the table continue to embedding in paraffin.

7.3 Embedding in paraffin

1. Preferably use low melt paraffin as it will improve quality of nucleic acids.
2. Use the following steps as a guide to follow after step #10 in the table above

STEPS	TIME	Temperature ℃	SOLUTION
11	60 min	58℃	PARAFFIN
12	60 min	58℃	PARAFFIN
13	60 min	58℃	PARAFFIN

3. After completion of processing, the labeled cassettes are opened at the processing center.
4. Remove the tissue and place it in an appropriate sized heated mould.
5. Hold the tissue specimen down with a dissecting needle while partially filling the mould with molten paraffin. Secure the tissue by quickly cooling the base of the mould.
6. Place the labels as appropriate and fill the mould to the top with paraffin.
7. Cool the blocks in a cooling area to set the paraffin for 30 minutes
8. Remove blocks from the mould.
9. The blocks are now ready to be sectioned or stored.
10. Store paraffin blocks at or below room temperature. Prevent exposure to sun or extreme temperature variance. Store blocks in moisture resistant cardboard boxes or plastic storage boxes
11. Record storage location.

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

9. SOP-PRO-Tissue-Paraffin-02, Oct 3, 2006, Paraffin. Procure Quebec Prostate Cancer Biobank.

10. Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups. http://ctep.cancer.gov/forms/draft_ffpe_tissue.pdf

11. SOP# TB306.001, 18th Sep. 2006. Paraffin Block Generation, Ontario Institute for Cancer Research Tumour Bank.

12. Snell L. and P. H. Watson. 2006, Breast Tissue Banking: Collection, Handling, Storage and Release of Tissue for Breast Cancer Research. Methods Mol Med. 120:3-24.

APPENDIX 1.

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**

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