


CTRNet Standard Operating Procedure Preservation of Tissue: Paraffin Embedding			
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Supersedes:	8.3.005 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

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 biobanks to follow when preserving tissue by the FFPE method.

2.0 SCOPE

This 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 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: 08.01.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITIES

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

Tumour Biobank Personnel	Responsibility/Role
Laboratory Technician/Technologist	Transportation of tumour tissue, harvesting processing and storage
Histology Laboratory Technician/Technologist	May be specifically responsible for processing FFPE tissues

5.0 MATERIALS, REAGENTS, EQUIPMENT AND FORMS

The materials, reagents, 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 # 8.01.001) for tubes and histology cassettes	
Histology cassettes	
Needle/sharps disposal unit	
Gloves worn to protect personnel handling tissue	
Clean underpads for covering bench surface	
Tissue Collection/Harvesting Worksheets (see Appendix A for sample form)	
Neutral pH phosphate buffered formalin	
Alcohol (absolute, 100% Anhydrous)	
Xylene	
Paraffin	

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 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.

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

The site may have a tissue processor, which has standardized processing times. However, use the following steps as a guide.

7.1 Fixation in Formalin

- 7.1.1 Treat all tissue as potentially infectious.
- 7.1.2 Fixation 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 containers, cassettes or vials as needed labelled and ready.
- 7.1.4 Fixation of tissue should be undertaken as soon as possible. Optimally, tissue should be fixed within 4 hours from resection.
- 7.1.5 Record time from resection to fixation.
- 7.1.6 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.
- 7.1.7 Perform fixation at room temperature (25° C).
- 7.1.8 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).
- 7.1.9 If needed, dissect the tissue before fixation to ensure adequate penetration of the fixative.
- 7.1.10 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 4-5 mm in thickness.
- 7.1.11 Optimally, duration of fixation should be overnight to 24 hours but no more than 48 hours. The fixation time should be documented

7.2 Processing for Embedding

- 7.2.1 Dehydrate tissues through series of alcohols.
- 7.2.2 Clear tissue by treatment with xylene.
- 7.2.3 The following steps for dehydration and clearing 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

- 7.2.4 After step 10 in the table continue with embedding in paraffin.

7.3 Embedding in Paraffin

Preservation of Tissue: Paraffin Embedding

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

STEPS	TIME	Temperature °C	SOLUTION
11	60 min	58° C	PARAFFIN
12	60 min	58° C	PARAFFIN
13	60 min	58° C	PARAFFIN

- 7.3.3 After completion of processing, the labeled cassettes are opened at the embedding center.
- 7.3.4 Remove the tissue and place it in an appropriate sized heated mould.
- 7.3.5 Hold the tissue specimen down with a dissecting forceps while partially filling the mould with molten paraffin. Secure the tissue by quickly cooling the base of the mould.
- 7.3.6 Place the labels as appropriate and fill the mould to the top with paraffin.
- 7.3.7 Cool the blocks in a cooling area to set the paraffin for 30 minutes
- 7.3.8 Remove blocks from the mould.
- 7.3.9 The blocks are now ready to be sectioned or stored.
- 7.3.10 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.
- 7.3.11 Record storage location.

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-epct2/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 Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups
- 8.9 SOP-PRO-Tissue-Paraffin-02, Oct 3, 2006, Paraffin. Procure Quebec Prostate Cancer Biobank.
- 8.10 Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups.
- 8.11 SOP# TB306.001, 18th Sep. 2006. Paraffin Block Generation, Ontario Institute for Cancer Research Tumour Bank.
- 8.12 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.

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
8.3.005	09-01-2008	JdSH	Revised to cover paraffin embedding of tissue only
8.3.005 e1.1	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 7.0-deleted “an automated paraffin-embedding” and inserted “tissue processor”. • Section 7.3.3 and 7.3.5 – revised sentence • Inserted alcohol grade in the section 5.0 table.

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 Tumour is resected	
Time 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: 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

Date:

No

Storage Location:

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

Date:

Storage Location: