Title	Preservation of Tissue: Paraffin Embedding
SOP Code	SOP116_01
Effective Date	01-Sep-2012

Site Approvals

Name and Title (typed or printed)	Signature	Date dd/Mon/yyyy

1.0 PURPOSE

This Standard Operating Procedure (SOP) outlines standardized procedures for biorepositories to follow when preserving tissue by the FFPE method. The SOP does not describe detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

2.0 SCOPE

This procedure applies to all biorepository personnel responsible for FFPE treatment of the harvested tissue.

3.0 RESPONSIBILITIES

Fixation is performed by the laboratory technician or trained personnel designated by the biorepository.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

5.1 General Considerations

5.1.1 Tissue specimens should not be bigger than 1.5 x 1 x 0.5 cm.

5.1.2 Under-fixation is a greater risk, but also avoid over fixation as it can create problems for immunohistochemical methods.

5.1.3 Avoid fixatives such as Bouin's containing Picric acid, as this compound interferes with subsequent PCR analysis of extracted nucleic acids.

5.1.4 Use the following steps as a guide, if using an automated paraffin-embedding processor with standardized processing times .

5.2 Fixation in Formalin

5.2.1 Have materials and equipment ready. Have as many containers, cassettes or vials as needed labelled and ready.

5.2.2 Undertake fixation of tissue as soon as possible. Optimally, fix tissue within 4 hours of resection.

5.2.3 Record time from resection to fixation.

5.2.4 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.

5.2.5 Perform fixation at room temperature (20-25º C).

5.2.6 Ensure that the volume of the fixative is at least 10-15 times greater than the volume of the tissue (i.e., 10-15 ml for every gram of tissue).

5.2.7 If needed, Dissect the tissue before fixation to ensure adequate penetration of the fixative, if needed.

5.2.8 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.

5.2.9 Optimally, duration of fixation should be overnight to 24 hours, but no more than 48 hours.

5.2 Processing for Embedding

5.2.1 Dehydrate tissues through series of alcohols.

5.2.2 Clear tissue by treatment with xylene.

5.2.3 Use the following steps for dehydration and clearing 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

5.2.4 After step 10 in the table, continue to embedding in paraffin.

5.3 Embedding in Paraffin

5.3.1 Use low melt paraffin preferably, as it will improve quality of nucleic acids.

5.3.2 Use the following steps as a guide, 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

5.3.3 After completion of processing, open the labelled cassettes at the processing centre.

5.3.4 Remove the tissue and place it in an appropriate sized heated mould.

5.3.5 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.

5.3.6 Place the labels, as required, and fill the mould to the top with paraffin.

5.3.7 Cool the blocks in a cooling area for 30 minutes to set the paraffin.

5.3.8 Remove blocks from the mould.

5.3.9 Proceed to section or store the blocks.

5.3.10 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.

5.3.10 Record storage location.

6.0 REFERENCES

Health Canada, Food and Drug Regulations, Part C, Division 5, Drugs for Clinical Trials Involving Human Subjects, (Schedule 1024), June 20, 2001.

Health Canada, Guidance for Industry, Good Clinical Practice: Consolidated Guideline, ICH Topic E6, 1997.

2011 NCI Best Practices for Specimen Resources. Office of Biorepositories and Biospecimen Research, National Cancer Institute, Bethesda, MD. http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf

ISBER Best Practices for repositories: Collection, storage, retrieval and distribution of biological materials for research. Cell Preservation Technology 6(1), 3-58, 2008 http://www.isber.org/Pubs/BestPractices2008.pdf

CTRNET Standard Operating Procedures, Canadian Tumour Repository Network, http://www.ctrnet.ca/operating-procedures

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP116_01	01-Sep-2012	Original version