James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

STANDARD OPERATING PROCEDURE

Title:	**Preservation of Tissue: Tissue Fixation, Processing, and Paraffin Embedding**		
Procedure:	BB_HIST.001.01	**Supercedes:**	none
Originator and Date:	Crystal Leung 29OCT2008	**Effective Date:**	29OCT2008
Review Frequency:	annually	**Approved By:**	The iCAPTURE Centre Privacy Team
Total Number of Pages: 5			

Revision History		
Date	**Reviewer**	**Summary of revision**
20Apr2009	Crystal Leung	Reformatted to iCAPTURE format

Purpose

The purpose of this document is to outline standardized procedures for Biobank personnel to follow when preserving tissue in formaldehyde fixation and paraffin embedding (FFPE). Formaldehyde is the most widely used universal fixative as it preserves a wide range of tissues and tissue components. Formaldehyde fixed and paraffin embedded (FFPE) tissues can easily be stored under normal laboratory conditions for a long period of time. The method is effective for preserving histological morphology of the tissue specimen.

Responsibilities

This procedure is applicable to the following:

- Biobank personnel

Safety

Universal precautions are a method of infection control in which all human tissue, blood and body fluids are treated as if they are infectious. The SOP does not cover detailed

safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals. Refer to BB.001.01 (Biohazardous Material Handling).

Definitions

Biohazardous materials	Human tissue, cells, body fluids, or culture materials that may contain infectious or other hazardous materials.
Clearing	Removal of the dehydrant with a substance that will be miscible with the embedding medium (paraffin). Most common clearing agent is xylene.
Dehydration	Removal of water from tissue. Usually done by a series of increasing concentrations of alcohols.
Fixation	To preserve tissues in an as life-like a state as possible.
Preservation	Use of chemical agents to prevent or retard biological or physical deterioration of a specimen.
SOP	Standard Operating Procedure. Document used to control the method and requirements by which personnel will perform their activities.
Tissue processing	Fixed tissue is processed into a paraffin block. The main steps of this process are dehydration and clearing.
Wax penetration	Tissue is infiltrated by an embedding agent (molten paraffin wax) and embedded in paraffin wax. This hardens the tissue to allow for sections, usually 4 to 5 µm, to be cut.

Materials and Equipments

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products.

Clean absorbent (blue) pads for covering bench surface	Biospecimen Collection/Processing Worksheets (see Appendix I for sample form)
Clean Forceps	Neutral pH Phosphate buffered 10 % Formalin
Clean Scalpels for trimming tissue	Alcohol (ethanol)
Containers for fixing tissue	Xylene
Labels for tissue cassettes, containers	Paraffin
Tissue cassettes	Tissue Processors
Needle/sharps disposal unit	Embedding centers
Markers, ink and pens	

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.

1. **Fixation in Formalin**
    a. Treat all tissue as potentially infectious.
    b. Fixation is performed by the trained laboratory personnel designated by the BC Biobank.
    c. Have materials and equipment ready. Have as many labeled containers, cassettes or vials as needed and ready.
    d. Fixation of tissue should be undertaken as soon as possible. Optimally, tissue should be fixed within 4 hours from resection.
    e. Cut the tissue before fixation to ensure adequate penetration of the fixative.
    f. It is recommended that tissue 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.
    g. Place the sections of tissue in labeled cassettes and secure on cassettes lids. Place the cassettes in 10% neutral pH phosphate buffered Formalin. It is important that the fixative is buffered to avoid the formation of formaldehyde pigment on blood rich tissues.
    h. Perform fixation at room temperature (<25º C).
    i. 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).
    j. Record time from resection to fixation.
    k. Optimally, duration of fixation should be overnight to 24 hours but no more than 36 hours. Under-fixation is a greater risk but also avoid over fixation as it can create problems for immunohistochemical methods.
    l. Record time tissue spent in fixative.
    m. After fixation for 24 to 36 hours the tissue cassettes are ready for processing in an automated tissue processor.

2. **Tissue Processing (Dehydration, Clearing and Wax Impregnation)**
    Note: Processing location site may have an automated paraffin tissue processor which has standardized processing times. However, use the following Table 1 stations and solutions as a guide.

a. After formalin fixation, the following stations and times for dehydration, clearing, and wax impregnation can be used as a guide for tissue processing. Start at Station 3 for tissue that has been fixed overnight or longer.
b. Dehydration processes tissues through a series of increasing concentrations of alcohols to remove water.
c. A clearing agent, the most common is xylene, then treats the tissue to remove the alcohol.
d. The final step is wax impregnation of the tissue. Preferably, a low melting point paraffin is used as it will improve quality of nucleic acids.
e. Tissues are usually processed with an average 14 hour overnight cycle.

STATION	TIME	TEMPERATURE (ºC)	SOLUTION
1	60 min	37ºC	10% Formalin
2	60 min	37ºC	10% Formalin
3	60 min	37ºC	70% ALCOHOL
4	60 min	37ºC	95% ALCOHOL
5	60 min	37ºC	95% ALCOHOL
6	60 min	37ºC	100% ALCOHOL
7	60 min	37ºC	100% ALCOHOL
8	60 min	37ºC	100% ALCOHOL
9	60 min	37ºC	XYLENE
10	60 min	37ºC	XYLENE
11	30 min	58-60ºC	PARAFFIN
12	30 min	58-60ºC	PARAFFIN
13	30 min	58-60ºC	PARAFFIN
14	30 min	58-60ºC	PARAFFIN

Table 1 – Tissue processing – stations and solutions

3. Paraffin Embedding

a. After completion of tissue processing, the labeled cassettes are removed from the tissue processor and placed on the embedding center's hot plate. The cassettes are opened one at a time at the embedding center.
b. Remove the tissue from the cassette and place in an appropriate sized heated mould.

c. Hold the tissue specimen down with heated forceps while partially filling the mould with molten paraffin. Secure the tissue by quickly cooling the base of the mould. Place the cassette on top of the mould.
d. Place the labels as appropriate on top of cassette and fill to just below the top edge of the cassette with paraffin.
e. Cool the blocks on a cooling area to set the paraffin for 30 minutes
f. Remove blocks from the mould.
g. Scrape excess wax from the blocks.
h. The blocks are now ready to be sectioned and/or stored.
i. 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
j. Record storage location.

References

i. *Canadian Tumor Repository Network*; SOP #8.3.00, Preservation of Tissue: Paraffin Embedding, 2007.
ii. *The University of British Columbia, Department of Health, Safety and Environment*; Laboratory Biosafety Reference Manual, 4th Edition 2001