James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

# STANDARD OPERATING PROCEDURE

| | | | |
|---|---|---|---|
| **Title:** | **Sectioning of Paraffin Embedded Tissue** | | |
| **Procedure:** | BB_HIST.002.01 | **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 standard operating procedure (SOP) establishes the standard for sectioning of paraffin embedded tissues performed byBiobank personnel that have been trained in the use of the manual and semi-automated rotary microtomes.

## Responsibilities

This procedure is applicable to the following:

- Biobank personnel who are responsible for sectioning paraffin embedded tissues.

## Safety

For safe handling of human biological material refer to BB.001.01, “Handling Biohazardous Materials”. Wear PPE (lab coat, gloves, goggles, etc.) when working with biohazardous materials and chemical substances. Refer to Material Safety Data Sheets (MSDS) for safe handling, disposal and storage of chemical substances.

For microtome use, Biobank personnel must complete safety and practical training on the site-specific model(s) of microtome. Follow equipment manual or site procedures for maintenance, use and safety.

## Definitions

| | |
|---|---|
| Microtome | An instrument used to cut a specimen, as of organic tissue, into thin sections for microscopic examination. |
| MSDS | Materials Safety Data Sheets |
| PPE | Personal Protective Equipment |
| SOP | Standard Operating Procedure. Document used to control the method and requirements by which personnel will perform their activities. |

## Materials and Equipments

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 procedure.

| | |
|---|---|
| Microtome (i.e.Leica RM2255, 2245) | Forceps |
| Feather Microtome blades | Probes |
| Knife disposal boxes (blue) | Tissues or Kim wipes |
| Water bath | Brushes (Fine or thick: to removed paraffin waste) |
| Waste tray | 60ºC oven |
| PARAGARD (to remove wax residue) | Glass slides ( choose appropriate slides for test/procedure) |

## Procedures

- **Note:** Only qualified Biobank personnel that have been trained in the use of the site model(s) of microtome by Histology manager or other designee may use the microtome.

1. Set up water bath with water. Let water bath warm up to optimal temperature of around 40 to 45ºC (takes approximately 15 minutes).
2. Check that microtome is on stable surface, free of vibrations with no loose levers/ knobs.

3. Place blocks to be sectioned on cold plate or ice tray and let cool for at least 20 minutes.
4. Label corresponding slide(s) for blocks to be cut. Choose appropriate slides for the specific procedure/test.
5. For automated microtomes: turn on microtome with ON/OFF switch. The switch may be located at the rear, depending on the make and model of the microtome.
6. Adjust/check that the section thickness is appropriate for the procedure/test (H&E sections are usually cut at 4 um thick to produce a wax ribbon and suitable for histological evaluation).
7. Insert a microtome blade into the knife holder and secure in place. If the blade is not in use, have a knife guard in place.
8. Clamp the block into the block holder; 1) then using the advance or retract controls/dials to bring the block closer to the cutting edge of the blade or 2) move the knife base closer to the block.
9. Use the block holder position adjustment controls/dials to adjust so the block's surface is parallel to the knife edge.
10. Unlock the rotary handle on the right-hand side of the microtome.
11. At this point, the block may 1) need to be trimmed to the appropriate surface or depth to get a full section of the tissue or 2) be at the right depth and so can proceed with sectioning.
12. Return the block to the cold plate or ice tray for cooling after some time of trimming and/or sectioning.
13. Clamp block into the block holder.
14. The knife may need to be moved to an unused section for production of satisfactory morphology of tissue without knife marks and/or tears.
15. To begin sectioning: turn the rotary wheel away from you.
16. As the rotary wheel turns away from you, the block will come in contact with the microtome blade. A ribbon of wax will form at the site of the blade holder. As the ribbon becomes longer, use an appropriate tool (forceps, probe, blunt knife, etc.) or your fingers to hold the ribbon away from the microtome.
17. Place the wax ribbon onto water bath. A wrinkle free ribbon should be obtained.
18. Use forceps or other suitable tool to separate the sections from the floating wax ribbon.
19. Place a labeled slide at an angle at one edge of the wax section. Gently pull at an angle so the wax section will adhere to the slide in a wrinkle-free fashion.
20. Place the slides in a slide holder or slide rack. Dry the slides as required for the staining procedure/ test (air dried at room temperature, 37 or 60ºC oven).