Document Number: SuRF-HIS-004.02 **Title: Cryostat-Microtome Use, Cleaning and Decontamination** **Version: 2.0** **Author: Melanie McMillan**

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Reviewed annually	

SOP History		
Number:	**Date:**	**Reason for Change:**
01	01/06/2013	Original
02	19/02/2014	Update procedure

1.0 Purpose:

The purpose of this Standard Operating Procedure (SOP) is to describe the current procedure for cryostat-microtome use, cleaning and decontamination.

2.0 Scope:

This SOP applies to all Histology staff, students, visitors and any other supervised/trained individuals involved in this procedure within the Shared University Research Facilities (SuRF), based in the Queen's Medical Research Institute (QMRI), Edinburgh.

3.0 Responsibilities:

This document is a guide only – on site training is essential before use

3.1 All staff involved in this procedure are responsible for ensuring that methods are followed in accordance with this SOP after suitable training.

3.2 All staff must have read and signed any relevant risk assessment document(s) relating to this SOP before performing this procedure.

3.3 All staff are strongly advised to have an up-to-date Hepatitis B vaccination.

4.0 Procedure:

4.1 Safety considerations

- Gloves must be worn at all times:- Tissues are not fixed and there may be a potential danger of infection.
- When freezing tissue with solid carbon dioxide ("dry ice") protective gloves must be worn.
- When freezing tissue in liquid nitrogen wear protective cryogenic gloves and a face-mask or goggles.
- Remember that the knife is very sharp and is therefore a potential hazard.

High risk samples are not permitted in the Histology laboratory

4.2 Equipment: Cryostat

A cryostat-microtome (herein simply referred to as a cryostat) is used to cut histological slides. It is usually used in a process called frozen section histology. The cryostat is essentially a microtome, placed in a freezer.

A microtome is a sectioning instrument that allows for the cutting of extremely thin slices of material, known as sections. Microtomes are an important device in microscopy preparation, allowing for the preparation of samples for observational under transmitted light or electron radiation. Steel blades are used to prepare sections of human or animal or plant tissues for light microscopy.

The cryostat is usually a stationary upright freezer, with an external wheel for rotating the microtome (see photograph below). The temperature can be varied, depending on the tissue being cut – usually from -20^{o}C to -30^{o}C. The freezer is powered by electricity. To minimize unnecessary warming all necessary mechanical movements of the cryostat can be achieved by hand via a wheel mounted outside the chamber. The precision of the cutting is in micrometres (µm). Tissue are sectioned as thin as 1µm. Usual histology slides are mounted with a thickness of about 7µm.

Specimens that are soft at room temperature are mounted on a cutting medium (commercially available product such as OCT) on a metal “chuck”, and frozen to cutting temperature (for example at -20^{o}C). Once frozen, the specimen on the chuck is mounted on the microtome. The crank is rotated and the specimen advances toward the cutting blade. Once the specimen is cut to a satisfactory quality, it is mounted on a warm (room temperature) clear glass slide, where it will instantaneously melt and adhere. The glass slide and specimen is air dried or stored until required at -20^{o}C and stained.

The centre currently uses a Leica 1850 or a Leica 1900 cryostat-microtome. These are floor standing models, located in room E1.24 These cryostat-microtomes are adapted for use with disposable blades.

After use the excess frozen tissue trimmings and sections should be brushed from the cryostat-microtome and put in the waste bin. Always brush upwards near the blade and do not brush the cutting edge of the blade. The blade should be removed before cleaning for safety and ensure fingers do not come into contact with the cutting surface of the blade. The cryostat-microtome should then be properly cleaned with 100% ethanol.

4.3 Equipment: Disposable blades

Used blades should be placed into the bottom of the blade container that forms part of the blade dispenser. **Always ensure fingers are kept away from the sharp cutting surface of the blade.**

When starting a new blade, start sectioning from one end of the blade and as each part becomes blunt work along the length of the blade.

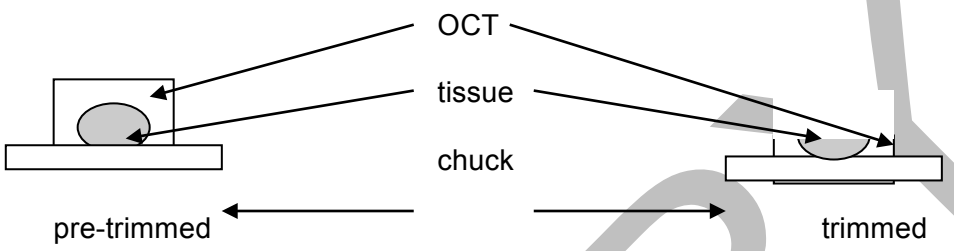
4.4 Method: Freezing the tissue

- Place a drop of OCT compound onto a chuck.
- Place tissue on top of the OCT.
- Place the chuck upright into a box containing dry ice.
  (Wear protective gloves).
- Once frozen, place the chuck into the cryostat-microtome taking care to avoid the knife (the cryostat-microtome has a holding bay located to the inside left of the chamber).
- Allow the block to come up to operating temperature (usually -20oC).

4.5 Method: Block Trimming

- Before sections can be taken from a block, excess OCT has to be trimmed from the surface of the cutting face. This process is termed “trimming” or “facing-in” and is performed inside the cryostat-microtome.
- Make sure the safety catch is on.

- Place the block into the chuck holder and tighten the screw to ensure that the chuck does not move.
- Position the face of the block close behind the blade edge. Do this by advancing the block holder to the blade (the controls for this are located outside the cryostat-microtome on the left hand side).
- Align the block face with the blade edge. **Keep fingers away from blade edge.**
- Set cryostat-microtome section thickness to 20µm, or use the cryostat-microtome advance mechanism (located outside left of the cryostat-microtome)
- Continue rotating the cryostat-microtome handle until the surface OCT has been removed and the full face of the tissue can be seen.



4.6 Method: Sectioning

- Once the block has been trimmed apply the cryostat-microtome safety catch.
- Ensure that a new piece of sharp disposable blade is used to cut sections.
- Set the cryostat-microtome cutting thickness to 5 or 10µm.
- Release the safety catch.
- Rotate the cryostat-microtome handle until a full face is achieved.
- Dispose of unwanted sections using a brush.
- Place the guide plate (clear glass plate located to the left of the knife holder) over the knife holder or use the edge of a brush to keep the frozen sections from curling up.
- Rotate handle in a steady continuous motion to cut a section.
- Lift up the guide plate to reveal the flat section and place a room temperature microscope slide gently on top of the section **(take care not to touch the knife edge).**
- Inspect the section and compare with the block to make sure there is a full face. If a full face is not achieved initially then the block should be trimmed further.
- Sections containing large folds or tears should be discarded.

4.7 Post treatment

- Once a satisfactory section has been obtained, several options are available:-
- Sections may be placed into a slide holder and stored at -80°C until used.
- Sections may be “fixed” in a number of reagents including 70% ethanol, ice-cold methanol or ice-cold acetone for 5 minutes and stored at -80°C or -20°C (this is dependent upon the final use of the section, to be determined by the end user).

- If light microscopy staining to view tissue architecture is required, we routinely fix the sections in 70% ethanol followed by staining with Haematoxylin and Eosin (see SuRF-SOP-052)
- Remove the chuck from the cryostat-microtome (reverse order of above, **taking care not to touch the sharp blade**).
- Remove the tissue from the chuck and place it into a container or wrap up in foil. Store this at -80^{o}C for future use.
- If the tissue is no longer required fix it in 4% formaldehyde for 24 hours and discard it into the clinical waste bag.

4.8 Cryostat cleaning

- Remove the knife from the holder and clear away all the waste shavings inside the cryostat-microtome.
- Clean the inside of the chamber with 100% IMS.

If it is subsequently discovered that the sample was infectious follow the decontamination procedure

4.9 Decontamination procedures:

- Routine decontamination is not necessary after each section cutting session unless it is found that high-risk samples have inadvertently been handled. Decontamination is, however, required prior to servicing of the cryostat.
- For decontamination, the cryostat-microtome is switched off from the mains electrical supply and cotton wool soaked in 4% (v/v) Trigene in tap water or 1% (w/v) Vircon in tap water placed over the cryostat-microtome. This is left overnight before servicing.

5.0 Related documents / references:

- RA1: SuRF-RA1-004: Cryostat-microtome Use, Cleaning and Decontamination.
- COSHH: Surf-COSHH-004: Cryostat-microtome Use, Cleaning and Decontamination.
- Manufacturer's manual / instructions
- The University of Edinburgh Health & Safety Policy / Codes of Practice (available on University's Health and Safety Department website)
- College of Medicine and Veterinary Medicine Health and Safety Manual (available on University's Health and Safety Department website)
- Bancroft J.D., and Gamble M. Theory and Practice of Histological Techniques 6th Edition (2008) Churchill Livingstone, Elsevier Limited

6.0 Approval and sign off:

Author:		
Name:	Melanie McMillan	
Position:	Responsible Scientist	
Signature:		Date:
Management Approval by:		
Name:	Mike Millar	
Position:	Facility Manager	
Signature:		Date:
QA Release by:		
Name:	Robin Sellar	
Position:	QA Manager	
Signature:		Date:

7.0 Verification by users:

Sign below to indicate you have read and understood the activity outlined above and the risk control measures that you must implement, use and / or wear. Ensure you have received sufficient information, instruction and training to enable you to conduct this activity with the minimum of risk to yourself and others.

Signature:	**Date:**

Signature:	Date:

Signature:	Date: