

Document Numbe	er: SASoM/EQUIP/070.v2
Title:	Use and maintenance of the Leica CM1860 Cryostat
Version:	v2
Author:	Peter Mullen

Effective from:	19/11/2018	
Valid to:	18/11/2023	

SOP History		
Number	Date	Reason for Change
v1	19/11/2013	Original
V2	19/11/2018	Update and change of author

1.0 Purpose –

The purpose of this SOP is to outline the principles of the routine use of the Leica CM1860 Cryostat in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope -

This SOP applies to routine use and maintenance of the Leica CM1860 Cryostat within the SASoM.

3.0 Responsibilities -

It is the responsibility of all users of the Leica CM1860 Cryostat within the SASoM to comply with this SOP.

4.0 Procedure -

*****All users are required to be trained and signed off by supervisory personnel before use.*****

The cryostat is a piece of equipment for sectioning frozen tissues, with automatic and adjustable movement of the specimen, according to desired thickness, and a fixed knife. <u>It</u> is not to be used for Human Samples due to the potential risk of Hep B contamination within biologiocal / biopsy samples. If you require equipment for sectioning human tissue, please discuss with the lab manager.



This cryostat uses disposable blades only. Ensure there are no blades in the cryostat before use, and remove any blades after use.

1. Turn on light by pressing the light button at the red arrow.



4. Preparation of the sample. Using an appropriate tissue mould (can also be formed aluminium foil if sufficient), embed tissue in OCT embedding media in necessary direction to allow desired cutting plane. Leave the tissue in OCT for approximately 30 minutes before freezing. Tissue (in OCT) can then be frozen on dry ice or inside the cryostat chamber.

5. Preparation of the specimen disc. Place specimen disc inside cryochamber in an empty slot on the quick freeze shelf, it will start to cool immediately. Add a sufficient amount of OCT to the specimen disc and orient and mount your specimen (removed from mould or foil). Once the specimen is securely frozen to the disc, it is ready for sectioning. (There is a Peltier element for very quick freezing. This can be enabled by pressing the button indicated by the red arrow below. It takes about



60 seconds to activate. If using, place the blue slots in the quick freeze shelf and apply the heat extractor to the specimen/specimen disc. After approximately 30 seconds, the specimen should be completely frozen to the disc. Remove heat extractor and tissue is ready for sectioning.)



6. Insert specimen disc into specimen head. Lock handle of handwheel (on right side of cryostat) in the upper position. Loosen the clamping screw on the specimen head, insert the shaft of the specimen disc into the hole of the specimen head and tighten the screw. Ensure that the tissue is oriented in the necessary direction/plane for desired cutting.

7. Inserting the blade into blade holder - Fold the anti-roll guide system to the left – while doing so, hold the lever (**not** the adjusting screw of the anti-roll guide) so that the height of the anti-roll guide remains unchanged. Open the clamping lever by turning it counterclockwise. Carefully insert the blade from above or from the side between the pressure plate and the blade rest. Make sure that the blade is inserted so that it is centered and lies evenly along the ledge. Clamp the clamping lever by turning it clockwise. Fold the anti-roll guide system back to the right (toward the blade) using the lever.

8. Angle of sectioning – the angle of the knife holder is set to a standard cutting angle sufficient for most applications. If this needs to be adjusted, an Allen screw will need to be inserted on the left side of the knife holder base to unlock it – adjustment is then done manually with relocking of the holder base with an Allen key. <u>Note: If this angle is adjusted for use, please return it to original position when done sectioning for the next user.</u>

Position of sectioning – the knife holder can also be moved from left to right, mainly to fully utilise a blade over the course of cutting a large quantity of tissue (generally not necessary for a single block). In this case, left-right blade holder adjustment is possible if the lever on the right side of the blade holder base is unlocked by pushing in and twisting the lever. The base can then be moved and relocked into position. The base can also be moved forward toward the tissue specimen – this is done by unlocking the lever on the left side of the base holder.

9. Begin sectioning by unlocking the handwheel to advance the tissue to the blade at the desired thickness. Use a brush to brush off unwanted tissue sections or OCT from the guide plate. The anti-roll plate should be used and adjusted with great care. It will need to be aligned so that it is near flush with the blade. It can be adjusted with the black screw at the position closest to the user.



10. If cutting more than one tissue block, you can use the same disposable blade, HOWEVER, place the safety plate over the blade when changing your specimen disc.

11. When finished sectioning, unclamp blade and carefully remove using magnet at end of brush, or by removing it manually. Dispose in disposal part of blade box or into sharps bin. Remove specimen disc from holder and retract specimen holder back to starting point. Clean specimen holder of any remaining tissue or embedding media, using 70% ethanol. Clean entire working surface inside the cryostat by wiping with paper towel and 70% ethanol. (Report to Mary Wilson or Melissa Andrews if cryostat has not cleaned by previous user.)

5.0 Personal protection -

Howie coat and lab gloves must be worn at all times.

6.0 Training –

All users have to be trained before using the Cryostat by a designated person before using on their own.

7.0 Related documents –

- 7.1 Equipment manual (Leica_CM1860_CryostatManual_1v3)
- 7.2 Risk assessments (RA-GEN-041-Cryostat)



8.0 Approval and sign off -

Author:				
Name:	Peter Mullen			
Position:	Research Assistant			
Signature:	Date:			
Management Approval:				
Name:	Mary Wilson			
Position:	Laboratory Manager			
Signature:	Date:			
QA release by:				
Name:	Alex MacLellan			
Position:	QA Manager			
Signature:	Date:			

