Document Title: Microtomy – Paraffin Blocks and Section Cutting

Document Number: TB SOP111

Staff involved in development: *Job titles only*	Senior R&D Manager, Tissue Bank Team Leader, Tissue Bank Coordinators, Clinical Project Managers.
Document author/owner:	Tissue Bank Team/Senior R&D Manager
Directorate:	Research and Development
Department:	Research and Development
For use by:	Tissue Bank Staff
Review due:	February 2027

Summary of Amendments

Version Number	Modification:
V1	**Reviewed and updated SOP PRO/TE/TBR/011**

Key Points of this Document

1 Purpose and Contents

a. This document defines the Trust's procedure for microtomy of formalin fixed paraffin embedded tissue blocks, section cutting and section mounting.

b. The document details safe operating instructions for section cutting and mounting of sections onto glass microscope slides. It includes molecular requirements and creation of tissue curls and cleaning procedures.

2 Roles & Responsibilities

a. Staff involved in microtomy and section mounting must comply with the requirements set out in Section 4.

3 Policy

a. This SOP is mandatory and, as per the Trust's Information Governance and Records Management framework, non-compliance with it may result in disciplinary procedures.

4 Procedure

4.1 Introduction

a. Paraffin section cutting involves the use of extremely sharp knives or blades to cut very thin sections of tissue ready for mounting onto glass slides, prior to staining. Once the slides are stained, they will be examined microscopically.

4.2 Training

a. Training in this procedure will be by a competent member of the RPH Tissue Bank team.

b. Following a period of supervision (depending on the individual needs of the trainee) there will be an informal assessment.

4.3 Microtomes

a. The Royal Papworth Research laboratory contains two Thermo Finesse E+ rotary microtomes (Both are for standard Tissue Bank/Project section cutting and require deep cleaning prior to molecular work).
b. The "in use" box of blades is located on top of the microtomes.
c. Please note if cutting for trials, please refer to study specific protocols.

4.4 Microtome safety

a. The blades are extremely sharp and must always be left covered by the knife guard when the cutting has finished.
b. Before adding or removing blocks from the block holder, make sure the hand wheel is locked by the lever on top and the flip up knife guard is covering the blade.
c. When leaving the work area, place the signage "Microtome in use, Blade in position" on the tray to remind you and alert other staff.

4.5 Thermo Shandon Finesse E+ - for routine sectioning or molecular sectioning

a. The Thermo Shandon Finessse E+ Microtome has an electronic motor to advance the block to and from the blade. The handwheel must be manually turned for trimming and sectioning.
b. Changing the blade: Make sure the handwheel is locked before loosening the black lever on the right of the blade holder to remove the used blade and dispose of it in the bottom of the blade pack. Make sure the handwheel is locked. Slide a new blade into the holder and retighten the black lever.
c. The blade must be removed when giving the machine a thorough clean. Always ensure that the brake is on before changing the block or blade and when the cutting has finished.
d. To move the blade along during cutting, release the black lever on the left of the blade holder and move the entire blade holder block. The blade should never extend past the plate of the blade holder.
e. To advance a block to the blade, use the keypad on the left of the machine. Use the arrow keys to move the block to and from the blade. Single arrow keys result in a slow movement speed, the double arrow keys are a faster speed and if depressed will return the block holder to its full extent of that speed.
f. 'Trim' and 'Section' thickness can be selected using the keypad on the front right of the machine. Use the + and – keys to increase the thickness for each cutting type.
g. The light bulb button on the front keypad switches on a light strip under the blade holder block, which illuminates the controller unit**.**
h. Before putting the specimen in the block holder, make sure that the knife guard is covering the blade and the hand wheel is locked. Ensure that there is no excess wax on the sides, ends and back of cassette (particularly important when cutting blocks from outside sources). Make sure there is no wax build up in block holder.
i.

N.B. The red button on the top of the machine is the emergency brake for the advance/retract functions. Depress the button and the block holders travel will stop. To release the emergency brake, twist the button.

4.6 Specimen orientation

a. If required, unlock the hand wheel and move the block holder slightly until the specimen is directly above and behind the blade holder, then lock the handwheel.
b. Line the block up to the blade holder and check that it is aligned to the blade by looking down from above the block.
c. To orient the specimen, push down the Tilt Clamp Lever on the right side of the specimen holder (behind the block clamp).
d. On top of the block holder is the vertical tilt control, which moves the holder up and down.
e. On the left side of the block holder is the horizontal tilt control which moves the block side to side.
f. Move both tilt controls until the block is roughly in line with the blade; gently turn the hand wheel to confirm the orientation is correct. Lock the Tilt Clamp lever. Uncover the blade. Slowly turn the hand-wheel and see where the blade cuts the wax block. If required fine tune the orientation further repeating 6.9.

4.7 Trim block

a. Trim block if block has not been cut before. How deep to trim the block will depend on the block requirements. (Tissue Bank blocks do not need to be full face however the section needs to be deep enough to identify tissue type and microscopic detail for selection purposes).
b. When trimming in a block use a slightly increased thickness (6-10µm) than for section cutting.
c. Blowing on the tissue block can help identify how deep the tissue has been cut. NB. DO NOT blow on sections if cutting for molecular work.
d. It should be possible to determine when full face has been reached by looking at the face of trimmed the block. Exposed tissue will appear matte or ‘dull’ in appearance. If still uncertain if the tissue has reached full face, then take a section on a slide, stain with Haematoxylin and Eosin (H&E) and then compare the slide and tissue by block checking.
e. Some tissue types will require very gentle trimming at low microns (e.g., heart). If while trimming, white ‘scuffed’ areas appear on the tissue surface, this indicates the tissue is being damaged by trimming. If this occurs, slow down the speed and reduce the trimming thickness.

4.8 Section Cutting and Mounting

a. Make blocks cold by placing them on ice or placing them in the freezer prior to cutting.

b. Fill water bath with distilled water and check that the thermostat dial is set at 45°C. If sectioning is required quickly then add 1/3 boiling distilled water on top of the cold. (Distilled water is the middle tap of the laboratory sink)
c. The yellow light at the front of the water bath indicates the waterbath is coming up to temperature. It will go off once the set temperature is reached. The red light will come on if the water bath has over heated.
d. Put the lid of the water bath on until it has reached the required temperature.
e. Check the surface of the water is clean and clear of any tissue debris. This can be done by floating a clean tissue over the surface and bringing it towards the edge of the bath and then discard it in the laboratory yellow waste bin. If there are sections on the bath then dispose of tissue in Human waste clear autoclave bags in red boxes.
f. Make sure there are no bubbles at the bottom of the water bath, these should be removed before sectioning by tapping the waterbath or using a clean brush.
g. Set the thickness of section cutting following 5.6
h. If cutting a small piece of tissue, cut the wax around the tissue in the block into a trapeze shape, using a blade. This will make ribboning the block easier and will enable you to fit several sections on one slide. When the block is in the holder, and blade is exposed, use the handle to start cutting the block. Use forceps to float the ribbon from the microtome onto the surface of the water.
i. Floating the sections onto the water bath will allow them to flatten and any creases to unfold. Some tissue e.g., pleura should be left longer on the water bath.
j. If sections are fatty and dispersing on the waterbath too quickly then sections can be floated on cold distilled water or 70% IMS/IDA and picked up on a slide. The slide can then be transferred to the waterbath, held flat so that it is just touching the water surface, to remove any creases.
k. If tissue is tough or calcified, soak the block in 70% IMS/IDA, and make sure that the block is cold before cutting.
l. If there is scoring of the ribbon tissue move the blade along the microtome. When the entire blade is used, put the blade in the back of the blade container. **Do not keep any un-sheathed blades on top of the microtome.**
m. If there is a lot of blood in tissue or it is friable, then float the block onto the waterbath for a few minutes and return the block to the ice. Alternatively, water can be added to the ice tray. Try not to trim in too much before taking a section.
n. If block requires (surface decal) try using IDA. If required discuss with requesting researcher as this may affect DNA and immunohistochemistry (IHC) staining. Use a timer when soaking the block in IDA. Try to minimise the amount of time the block is in IDA and be prepared to take the first sections when returning to the microtome.
o. When sections are floating on the water bath surface, divide the ribbon as appropriate.
p. Use clean chilled forceps to gently tease the sections apart.
q. The glass slide used to pick up the section will be project dependent. Generally normal slides are for H&E staining and DNA testing, while positively charged slides tend to be used for IHC or special stains.
r. Dip the clear glass end of the slide into the water whilst holding the written end, carefully move it alongside the section.
s. Tilt the slide to an angle towards the section (labelled side facing the water).
t. Draw the slide from the water and the surface tension should bring the tissue section with it.

u. If there is excess water under the tissue, stand the slide upright against the oven on an absorbent material. This will take a matter of minutes to drain.
v. Dry slides according to project protocol. Sections for H&E staining require a minimum of 15 min at 60°C. Sections for IHC and special stains should be in the oven for a minimum of 1 hr at 60°C. DNA slides need to be air dried at room temperature or at 37°C, normally overnight.

4.9 Cleaning microtomes and cutting equipment

a. As the microtome is required for molecular work it needs to be cleaned thoroughly after every use. When the microtome is not in use the blade guard must be covering the blade.
b. Use the big white brush to remove as much wax as possible from the machine, especially in the tray which collects the wax trimmings. Brush the floor to make sure there is no wax trimming on the floor. Put all trimmings into a human waste autoclave bag within red boxes. Change bags regularly as wet paper towels smell.
c. Use forceps to take any wax from specimen holder.
d. The tray that collects the wax trimmings can be removed and cleaned using hot running water to remove any excess wax.
e. Use gloves to spray a small amount of the paraclear over the microtome and use tissue to wipe off the wax. Move the blade and put in back of blade container if cleaning near blade holder. **Do not put blades on top of the microtome.** Particular areas to clean are the front plate, behind the blade block, hand-wheel handle, blade holder levers and thickness control dial/ control pad. Also wipe down the bench to make sure there is no residual wax.
f. Remove water from the water bath at the end of each day and wipe off any excess wax.
g. Clean the waterbath, by wiping with tissues and 70% IDA making sure there is no wax left on the rim.
h. Put the cover over the microtome when finished.
i. Only if excess build-up of wax is present clean the forceps and brushes by soaking in xylene for 5min and then allow to air dry overnight in the fume hood of the lab.
j. Ensure any wax on the floor is removed after each cutting episode.

4.10 Molecular Cutting

a. Wear gloves throughout and try to do all molecular cutting at the beginning of the day when the water bath is clean. Removed existing water and clean out the water bath removing all the wax and wiping with 70% IDA before starting.

b. Spray the bench and the microtome with 70% IMS/IDA and wipe down with tissue.
c. Get a fresh clean ice tray from the freezer to put wax block on.
d. Always wear gloves when preparing slides for cutting molecular work.
e. Use slides that have been provided by a researcher or use slides that have 'For molecular use'. Always check that the slides are used within their expiry date.
f. If handwriting on the slides is required wearing gloves, place a new piece of tissue on the bench.
g. Orientate the block following section 5.9.
h. Wear gloves while cutting. Make sure you use a fresh part of the blade for cutting each block. If necessary, change the blade. Always make sure the forceps you use are clean.
i. Cut sections to the thickness that the research project requires. DO NOT BLOW ON SECTIONS
j. When changing to a new block change the water bath, and carefully wipe the front plate and surrounding areas with 70% IMS/IDA-soaked tissue before continuing to the next block.
k. Put sections in a clean rack; if necessary, wipe rack with 70% IMS/IDA.
l. Dry sections according to the researcher's instructions, some require over night at room temperature others require heating in the oven at a specific temperature and time.
m. Put slides in the slide mailer that is clean, rinse with 70% IMS/IDA before use.
n. If the blade holder is dirty, it can be removed and cleaned using paraclear, if unsuccessful due to excess build-up of wax take the blade holder to the chemical fume hood and with a brush wipe with xylene. Make sure that the blade is removed. Keep the blade holder in the fume hood overnight until all the xylene has been removed.
o. Use wax remover to clean block holder and hand wheel with paraclear if required.
p. Always clean the microtome, water bath and surrounding area in between cases. To remove wax deposits use a small amount of the industrial wax remover (i.e. Paraclear or QPath Clean Lab) and finish with wiping 70% IMS/IDA.

4.11 Cutting Curls/Scrolls

a. In addition to sections picked up on slides, curls or scrolls may be requested by researchers. As with sections, these may be cut at various microns following the steps in section 5. Curls/scrolls are then placed into vials for transportation. Ensure steps in section 9 are followed and sterile vials are used if material will be subject to molecular work.

5 Risk Management / Liability / Monitoring & Audit

a. The R&D SOP Committee will ensure that this SOP and any future changes to this document are adequately disseminated.

b. The R&D Department will monitor adherence to this SOP via the routine audit and monitoring of individual clinical trials and the Trust's auditors will monitor this SOP as part of their audit of Research Governance. From time to time, the SOP may also be inspected by external regulatory agencies (e.g. Care Quality Commission, Medicines and Healthcare Regulatory Agency).

c. In exceptional circumstances it might be necessary to deviate from this SOP for which written approval of the Senior R&D Manager should be gained before any action is taken. SOP deviations should be recorded including details of alternative procedures followed and filed in the Investigator and Sponsor Master File.

d. The Research and Development Directorate is responsible for the ratification of this procedure.

Further Document Information

Approved by: *Management/Clinical Directorate Group*	Research and Development Directorate
Approval date: *(this version)*	Current approved version date
Ratified by Board of Directors/ Committee of the Board of Directors:	STET
Date:	N/A
This document supports: *Standards and legislation*	Medicines for Human Use (Clinical Trials) Regulations 2004 and all associated amendments. UK Policy Framework for Health and Social Care Research (2023) Human Tissue Act 2004
Key related documents:	Trust Research Policy Trust Policy DN1 Document Control Procedures Activity Location Guide **Risk Assessments** RAC/RD/TBR/022 – Microtomy and Section Mounting RAC/RD/TBR/008 – Loss of Traceability RAC/RD/TBR/005 – Labelling (Datix 2400) **COSHH** COSHH/RD/TBR/011 - Alcohol (IDA) COSHH/RD/TBR/038 - Wax (Paraffin) COSHH/RD/TBR/032 - Paraclear (Q path clean lab) COSHH/RD/TBR/039 – Xylene

Equality Impact Assessment: Does this document impact on any of the following groups? If YES, state positive or negative, complete Equality Impact Assessment Form available in Disability Equality Scheme document DN192 and attach.

Groups	Disability	Race	Gender	Age	Sexual orientation	Religious & belief	Other

Yes/No	NO	NO	NO	NO	NO	NO	NO
Positive/Negative							
Review date:			February 2027				