Royal Papworth Hospital
NHS Foundation Trust

Document Title: Tissue Fixation, Processing and Embedding

Document Number: TB SOP135

Staff involved in development: *Job titles only*	Senior R&D Manager, R&D Operational Manager, Clinical Project Managers, Tissue Bank Team leader
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Summary of Amendments

Version Number	Modification:
V1	New SOP. Reviewed and updated SOP's PRO/TE/PTH/002, PRO/TE/PTH/006 and PRO/TE/PTH/007

Key related documents:	Trust Policy DN001 Document Control Procedures Activity Location Guide FRM122 FFPE Sample Processing Log **SOP'S** SOP095 Laboratory Biological Safety Cabinet SOP107 TB Fresh Tissue SOP111 TB Microtomy and Section Mounting SOP114 TB Extraction Equipment & Biological Safety Cabinet SOP130 TB Waste Management and Tissue Disposal SOP134 Research Tissue Banks Transport of Human Tissue

	HLRI Medicine Safety Manual **Risk Assessments** RAC/RD/TBR/007 - Transport of relevant material RAC/RD/TBR/004 - Fresh samples RAC/RD/TBR/018 – Spillage RAC/RD/TBR/025 – Biological Safety Cabinet RAC/RD/TBR/032 – Waste Management and Tissue Disposal RAC/RD/TBR/037- Handling, processing and Freezing Fresh Tissue in the HLRI. **COSHH** COSHH/RD/TBR/011 – Alcohol IDA COSHH/RD/TBR/039 – Xylene COSHH/RD/TBR/038 – Wax COSHH/RD/TBR/021 – 10% Buffered Formalin

Key Points of this Document

1 Purpose and Contents

a. This document defines the Trust's procedure for fixing, processing and embedding human tissue samples within the HLRI histology laboratory.

a. The document details the requirements for safe and correct handling and processing of human tissue in the laboratory.

2 Roles & Responsibilities

a. Staff involved in tissue handling and processing must comply with the requirements set out in Section 4.

b. Training in this procedure will be by a competent member of the RPH research team.

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

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 Fixing Fresh Tissue

a. Fresh tissue is a biohazard. Always wear a laboratory coat, gloves and eye protection when handling any unfixed sample. The Pathology labs in the HLRI are designated Containment Level 2. Any biological waste generated is treated accordingly and sent to be autoclaved followed by incineration.

b. Tissue cassettes are transferred in Hartman's solution from CUH cut up laboratory or RPH Hospital to HLRI histology laboratory in a specimen bucket and green pathology transfer bag.

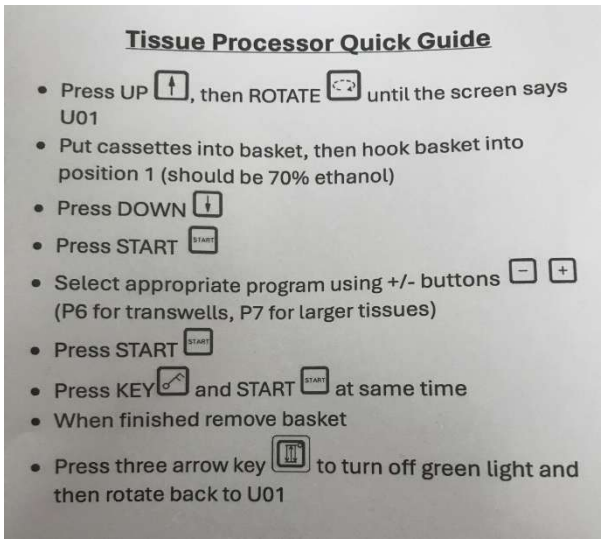
c. Cassettes containing fresh tissue are handled in a class II biological safety cabinet.

d. Tissue cassettes are transferred into the 10% neutral buffered formalin trough(s) in the CAS BioMat 2 class II cabinet, ensuring enough 10% neutral buffered formalin to cover the cassettes and allow adequate fixation (~10x volume of tissue). Once transferred under the Biological Safety Cabinet (BSC), the trough containing cassettes and formalin should be removed from the cabinet and placed on the AFOS table. Tissue samples should be left to fix in formalin overnight before processing the following day. Record date and time into formalin on FFPE sample processing log (FRM122).

4.1.1 Processing fixed tissue

a. The HLRI processor does not contain a formalin retort. As the first retort contains 70% Industrial Denatured Alcohol (IDA), it is necessary to remove excess formalin by transferring formalin-fixed tissue cassettes from the formalin trough into the 70% IDA tough on the AFOS table, for a minimum of 1 hour, prior to loading cassettes onto the processor. This will prevent excessive formalin contamination of the 70% IDA in retort 1. Record transfer date and time into 70% IDA on the FFPE Sample Processing log (FRM122)

b. Load tissue cassettes into the metal processor basket following the steps on the Tissue Processor Quick Guide below. Record the date and time tissue is loaded onto the processor on FFPE Sample Processing log (FRM122). NB. There is also a laminated copy of the guide next to the processor.



c. Selecting the appropriate programme: There are pre-programmed protocols set up on the processor. Most RPH tissue samples will be processed using Program 7 for routine tissues. A longer programme is available for very fatty samples. Routinely, Tissue Bank will load the processor in the evening and run the desired programme overnight. The processing run will begin immediately and finish in the early hours of the morning. Cassettes will be held in the molten wax chamber until a member of staff unloads the processor in the morning. See tables below for protocol steps.

Table 1.

Program 7 Routine tissues (~9.5 hrs)			
Station	**Reagent**	**Vac**	**Duration**
1	70% ethanol	No	30 mins
2	80% ethanol	Vac	1 hour
3	95% ethanol	Vac	1 hour
4	100% ethanol	Vac	45 mins
5	100% ethanol	Vac	45 mins
6	100% ethanol	Vac	45 mins
7	xylene	Vac	45 mins
8	xylene	Vac	45 mins
9	xylene	Vac	45 mins
10	paraffin	Vac	45 mins
11	paraffin	Vac	45 mins
12	paraffin	No	45 mins

Table 2.

Program 1 - fatty tissues (~15 hrs)			
Station	**Reagent**	**Vac**	**Duration**
1	70% ethanol	Vac	1 hour
2	80% ethanol	Vac	1 hour
3	95% ethanol	Vac	1 hour 15 mins
4	100% ethanol	Vac	1 hour
5	100% ethanol	Vac	1 hour 30 min
6	100% ethanol	Vac	1 hour 30 min
7	xylene	Vac	1 hour
8	xylene	Vac	1 hour 30 min
9	xylene	Vac	1 hour 30 min
10	paraffin	Vac	1 hour
11	paraffin	Vac	1 hour 30 min
12	paraffin	Vac	1 hour 30 min

4.2 Embedding processed tissue

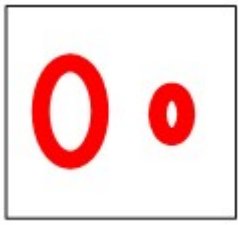
a. Tissue removed from the processor requires embedding in paraffin wax to enable section cutting. The laboratory has an embedding centre for this purpose.

b. Following routine overnight processing, remove the basket of cassettes from the molten wax retort the following morning (following the instructions on the Tissue Processor Quick Guide) and transfer into the molten wax chamber in the embedding centre.

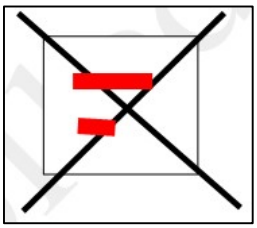
c. Prior to embedding, consider if the sample requires specific orientation as below:

On end, i.e. small vessels, so that the lumen and all layers of the vessel wall are visible in cut sections. Vessels may be bisected to reduce their height to aid 'on end' embedding.

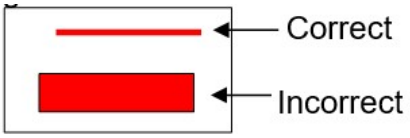
Correct Orientation

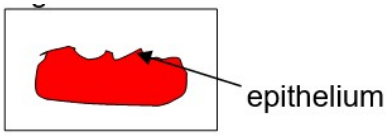


Incorrect Orientation



On edge, i.e. valves or samples with epithelial layer, so that a cross section of all layers of the wall can be seen in cut sections.





Flat, most large tissues, i.e. lung background, tumour, myocardium.

Embed flat so that surface of entire tissue is on one plane. Tissue on an uneven plane will result in difficulty achieving 'full-face' when cutting, with some areas of the tissue cutting out before other areas are exposed.

d. Once orientation has been considered, place the tissue cassette on the hot plate and carefully remove the lid. Select a suitable sized mould (tissue should not touch the edges of the mould) from the heated mould chamber and fill the mould recess with molten wax using the dispenser.

Place the mould on the cold spot and using the heated forceps, set the piece(s) of tissue in the correct orientation on the base of the mould, ensure samples are pressed down firmly, ensuring all tissue is on the same plane. NB. This is essential with very small samples as some pieces may be cut away before others have been exposed at all. Place the now empty cassette case on the mould, above the tissue and gently press down on the cassette case. Top up with wax, checking for air bubbles and ensuring the mould does not touch the hot plate again as this may cause the pieces to float away from the base of the mould. Place the mould on the cold plate to solidify. Clean the forceps between every sample, to prevent cross-contamination between samples.

e. When blocks are completely cold, they can be removed from the moulds. This could take up to 30 minutes. If they do not lift out easily then they must be left longer to set further. The excess wax around the edges of the cassettes can be removed by running along the hot plate. Replace moulds in heated mould chamber.

f. Blocks must then be placed into a container deep enough to safely contain the blocks for transport into the blocks/slides storage in the laboratory, ready for sectioning as per SOP 111 Microtomy and Section Mounting. Blocks must not be carried loose by hand or in pockets.

g. If re-embedding is required (for example to re-orientate, further tissue dissection required) the wax block can be placed back into a metal mould in the heated chamber of the embedding centre. Place the block into an appropriately sized metal mould, to capture the tissue as the wax melts. Leave until wax is completely melted before re-embedding as required, following steps above.

4.3 Waste Disposal

a. Follow HLRI waste disposal routes for all waste reagents and tissue, as per the HLRI Medicine Safety Manual.

5 Risk Management / Liability / Monitoring & Audit

a. The Tissue Bank Organisational Group (TBOG) 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) (Amendment) Regulations 2025 (and any subsequent amendments) UK Policy Framework for Health and Social Care Research version 3.3 (07/11/17) and authorised amendments thereafter Human Tissue Act 2004

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Groups	Disability	Race	Gender	Age	Sexual orientation	Religious & belief	Other
Yes/No	NO	NO	NO	NO	NO	NO	NO
Positive/Negative							

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