Preservation of Tissue in Paraffin-Embedded Blocks

Category:	**Materials Handling and Documentation**		
SOP number:	04.009	Version	1.0
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1.0 PURPOSE

The purpose of this SOP is to establish standardized procedures for the preservation of tissue samples in paraffin-embedded blocks at Nourah's Tissue Biobank. This technique is critical for the long-term storage of tissue samples, enabling future histological analysis and research.

2.0 SCOPE

This SOP applies to all personnel involved in the processing, embedding, and preservation of tissue samples in paraffin blocks within Nourah's Tissue Biobank. It covers the steps required to properly fix, transport, process using the Thermo Excelsior ES Tissue Processor, embed using the Thermo HistoStar embedding machine, and document tissue samples using the LabVantage LIMS system.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel of Nourah's Tissue Biobank members

Biobank Personnel	Responsibility
Pathologist/Pathologist Assistant	Responsible for selecting, fixing, and preparing tissue samples for paraffin embedding, ensuring they meet the necessary criteria.
Laboratory Technicians	Responsible for receiving fixed tissue samples from the pathology lab, performing automated tissue processing using the Thermo Excelsior ES Tissue Processor, embedding in paraffin using the Thermo HistoStar embedding machine, labeling, and documentation according to this SOP.
Biobank Manager	Responsible for overseeing the paraffin embedding process, ensuring compliance with this SOP, and addressing any issues that arise.
Quality Assurance (QA) Officer	Responsible for auditing the paraffin embedding process, ensuring adherence to protocols and regulatory requirements.

4.0 MATERIALS, EQUIPMENT, AND FORMS

Listing of the materials, equipment, and forms being used to achieve the goals of the SOP, this list will mainly contain necessary materials and, or recommendations that may be substituted by alternative or equivalent materials more suitable at the time of testing.

Material to be used	Site
10% neutral buffered formalin (for fixation)	
Tissue cassettes for processing	
Thermo Excelsior ES Tissue Processor	
Thermo HistoStar Embedding Machine	
Dehydration reagents (e.g., ethanol, xylene)	
Paraffin wax (embedding medium)	
Tissue embedding molds	
Microtome for sectioning (if sectioning is needed for QC)	
Labels and markers	
Forceps and scalpel	
PPE (gloves, lab coat, face mask)	
Biohazard waste containers	

5.0 POTENTIAL HAZARDS

In this part of the SOP, we explain the potential hazards from chemicals and methodologies used in this procedure. It will also contain information on how to handle these chemicals and the level of biosafety

Material	Safety and handling

6.0 PROCEDURES

This section outlines the steps involved in the preservation of tissue samples in paraffin-embedded blocks at Nourah's Tissue Biobank. These procedures ensure the long-term preservation of tissue morphology and molecular integrity, facilitating future research and histological analysis. Tissue samples are fixed in 10% formalin, transported from the pathology lab to the biobank lab, processed using the Thermo Excelsior ES Tissue Processor, and embedded using the Thermo HistoStar embedding machine.

6.1 PREPARATION

1. The pathologist or pathologist assistant selects tissue samples suitable for paraffin embedding, based on research or diagnostic needs.
2. The samples are placed in a pre-labeled cassettes and are immediately immersed in 10% neutral buffered formalin for a minimum of 24 hours to ensure proper fixation. This fixation step is crucial for preserving tissue morphology.
3. After fixation, the tissue samples in their labeled cassettes are securely packaged and transported from the pathology lab to the biobank lab.
4. Ensure that the samples are transported under appropriate conditions to prevent any damage or degradation.

6.2 AUTOMATED TISSUE PROCESSING WITH THERMO EXCELSIOR ES

1. Place the labeled cassettes containing fixed tissue samples in appropriate baskets and load them into the Thermo Excelsior ES Tissue Processor.
2. Ensure that all cassettes are securely placed in the processor to prevent any movement during the processing cycle.
3. Ensure that all chemicals needed to run the instrument are filled, then start the routine program to run overnight and is scheduled for 8:30 AM. the next day during weekdays or 8:30 A.M. on Sunday for weekend runs.
    a. Routine Program:
        i. Fixation with 10% Formalin (30 Mins)
        ii. Second Change of 10% Formalin (30 mins)
        iii. Dehydration with 75% Ethanol (1 hour)
        iv. Dehydration with 90% Ethanol (1 hour)
        v. Dehydration with 95% Ethanol (1 hour)
        vi. Dehydration with first change of 100% Ethanol (1 hour)
        vii. Dehydration with second change of 100% Ethanol (1 hour)
        viii. Dehydration with third and final change of 100% Ethanol (1 hour)
        ix. Clearing with first change of Xylene (1 hour)
        x. Clearing with second change of Xylene (1 hour)
        xi. Clearing with third and final change of Xylene (1 hour)
        xii. Infiltration with first change of Paraffin Wax (1 hour)
        xiii. Infiltration with second change of Paraffin Wax (1 hour)
        xiv. Infiltration with third and final change of Paraffin Wax (1 hour)
4. Ensure that the tissue samples are fully infiltrated with paraffin to avoid any air pockets or incomplete preservation..

6.3 EMBEDDING TISSUE IN PARAFFIN BLOCKS USING THERMO HISTOSTAR

1. Once the automated processing is complete, transfer the paraffin-infiltrated tissue samples from the cassettes to tissue embedding molds using the Thermo HistoStar Embedding Machine.
2. Orient the tissue samples correctly within the molds to facilitate optimal sectioning later.
3. The Thermo HistoStar machine will dispense molten paraffin into the molds, covering the tissue completely.
4. Allow the paraffin to cool and solidify at room temperature or by placing the molds on a cold plate, ensuring the tissue is fully embedded in a solid block.
5. Once the paraffin has solidified, trim excess paraffin from the blocks to prepare them for storage or sectioning.
6. Store the paraffin blocks in a designated storage area at room temperature, ensuring they are properly labeled and recorded in the LIMS.

6.4 LABELING AND DOCUMENTATION

1. Label each paraffin block with the necessary information, including patient identifier, tissue type, date, and any specific processing details.
2. Verify that the labeling is legible and securely attached to the blocks.
3. Enter the details of the paraffin-embedded tissue samples into the LabVantage LIMS system immediately after embedding.

4. Document the fixation, processing using the Thermo Excelsior ES, embedding using the Thermo HistoStar, and any observations regarding the condition of the tissue into the LabVantage LIMS system.

6.5 STORAGE AND TRANSPORT

1. Store the paraffin-embedded tissue blocks at room temperature in a dry, well-ventilated area.
2. Update the storage location and conditions in the LabVantage LIMS system for accurate tracking.
3. If the paraffin blocks need to be transported, place them in appropriate containers to prevent damage during transit.
4. Record the transport details in the LIMS, ensuring traceability during transportation.

6.6 INCIDENT MANAGEMENT

1. In case of any deviations from the SOP (e.g., temperature excursions, delays), document the incident and notify the Biobank Manager immediately.
2. Implement corrective actions as necessary to mitigate any impact on sample integrity.
3. Complete an incident report detailing the deviation, corrective actions taken, and any follow-up measures required.
4. Submit the report to the QA Officer for review and documentation.

7.0 REFERENCES

1. CTRnet SOPs “08.03.006 Sectioning of tissue - Paraffin and OCT Embedded Tissue”
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4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).
   http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories
5. National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
   http://bioethics.georgetown.edu/nbac/hbm.pdf
6. US National Biospecimen Network Blueprint
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8.0 REVISION HISTORY

SOP No.	Date Revised	Author	Summary

9.0 APPENDICES