SOP on Histology Slide Preparation

Department of Anatomy

Faculty of Medicine

UWUSL

Standard Operating Procedure (SOP) on Histology Slide Preparation

Issued By,
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Purpose

To ensure uniform preparation of high-quality histology slides for teaching and laboratory purposes.

(1) Scope

Applicable to histology laboratories and pathology laboratories.

(2) Materials Required

(A) Chemicals

- 10% formalin (neutral buffered)

- Ethanol solutions

- Xylene

- DPX mounting medium

- Paraffin wax

- Hematoxylin and eosin stains (H & E)

(B) Equipment

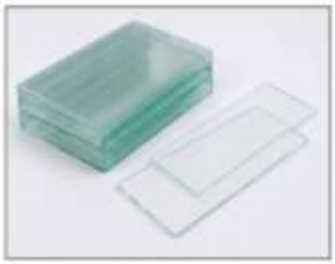
- Tissue processor

- Rotary microtome

- Tissue embedding machine

- Water bath

- Glass slides



- Cover slips

- Plastic cassettes

- Scalpels

- Plastic pipettes

- Labels and markers

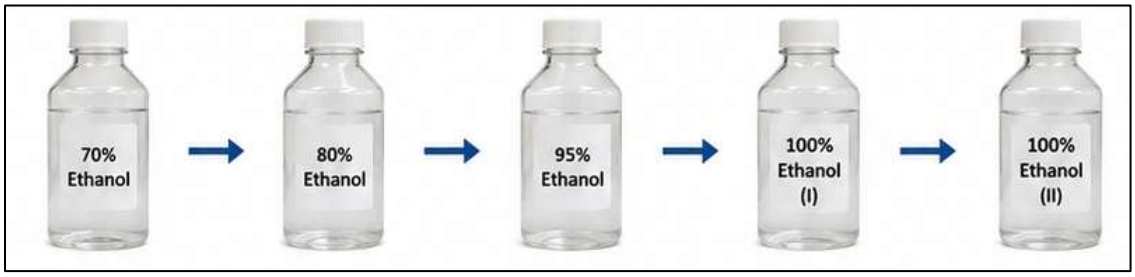
(3) Procedure

Step 1: Fixation – Preserve tissue in 10% formalin.

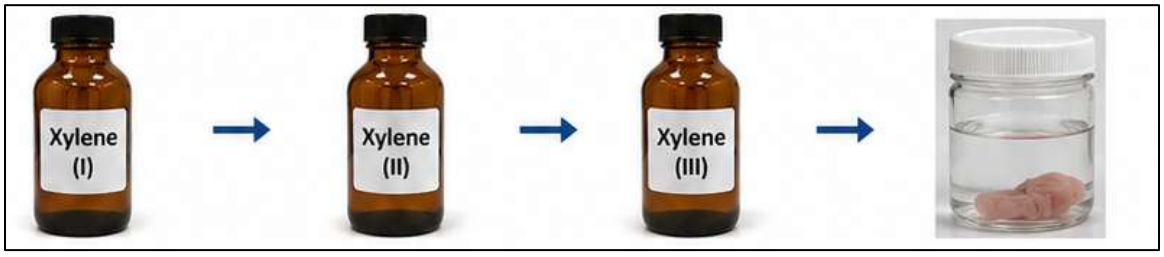
- The tissue specimen is immediately placed in 10% formalin to preserve its structure and prevent decomposition.
- Fixation stabilizes cellular proteins and prevents autolysis and bacterial growth.
- Proper fixation is essential for maintaining tissue morphology during further processing.

Step 2: Dehydration & Clearing

- The fixed tissue is passed through ascending concentrations of alcohol using a tissue processing machine, usually from 70% to 100% ethanol.
- This process removes water from the tissue.
- Gradual dehydration minimizes tissue shrinkage and distortion.

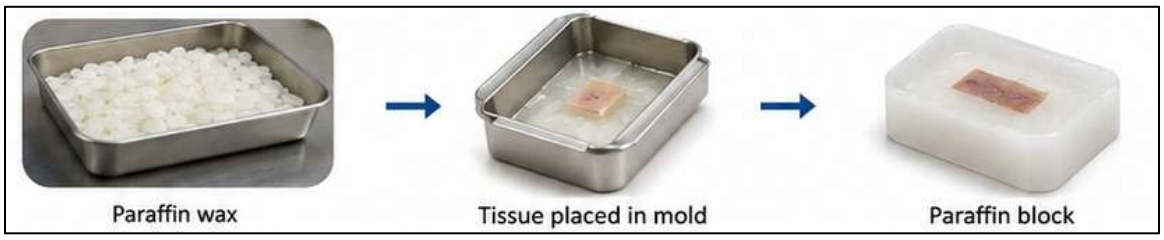


- After dehydration, alcohol within the tissue is replaced with xylene or another clearing agent using the same tissue processing machine.
- Xylene makes the tissue transparent and prepares it for wax infiltration.
- This step is necessary because paraffin wax is soluble in xylene but not in alcohol.



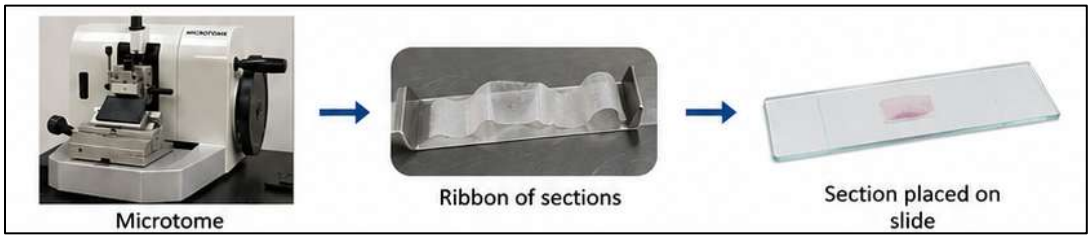
Step 4: Embedding – Embed tissue in paraffin wax using the tissue embedding machine.

- The cleared tissue is infiltrated with molten paraffin wax and then embedded in a wax block.
- Embedding provides support and firmness to the tissue for thin-section cutting.
- Proper orientation of the tissue during embedding is important for accurate microscopic examination.



Step 5: Sectioning – Cut thin sections using a microtome.

- The paraffin block is trimmed, and thin tissue sections are cut using a microtome, usually at 4–5 µm thickness.
- Thin, even sections are essential for clear microscopic visualization.



Step 6: Immerse in the water bath

- After sectioning with a microtome, the paraffin ribbon is gently placed on a warm water bath maintained at about 40–45°C.
- The warmth helps the tissue sections flatten and removes wrinkles or folds.
- Once the section spreads properly, it is carefully picked up onto a clean glass slide.
- The slide is then dried on a hot plate or in an incubator at 37–60°C for about 15–30 minutes to ensure proper adhesion and to remove excess water.

Step 7: Staining – Perform hematoxylin and eosin staining.

(A) Deparaffinization

Sections mounted on glass slides are placed in xylene to completely remove paraffin wax. This step makes the tissue accessible to aqueous stains.

Time:

- Xylene I – 5 minutes
- Xylene II – 5 minutes

(B) Hydration

The slides are passed through descending grades of ethanol to rehydrate the tissue gradually. Finally, the slides are rinsed in water.

Time:

- 100% ethanol – 2 minutes
- 95% ethanol – 2 minutes
- 70% ethanol – 2 minutes
- Running tap water – 2 minutes

(C) Hematoxylin Staining

Slides are immersed in hematoxylin.

Hematoxylin is a basic stain that binds to acidic structures such as the nucleus and nucleic acids, staining them blue-purple. This helps identify nuclear details clearly.

Time:

- Hematoxylin stain – 5–10 minutes

(D) Washing and Bluing

Excess hematoxylin is washed off with water. Bluing agents or alkaline tap water convert the reddish-purple hematoxylin to a crisp blue color.

Time:

- Tap water wash – 2 minutes
- Bluing step – 1 minute

(E) Eosin Staining

Slides are stained with eosin, which colors the cytoplasm, connective tissue, and extracellular components pink.

Time:

- Eosin stain – 1–2 minutes

(F) Dehydration

The stained slides are dehydrated again using ascending alcohol concentrations to remove water from the tissue.

Time:

- 70% ethanol – 1 minute
- 95% ethanol – 1 minute
- 100% ethanol – 2 changes, 1 minute each

(G) Clearing

Alcohol is replaced with xylene, making the tissue transparent and ready for mounting.

Time:

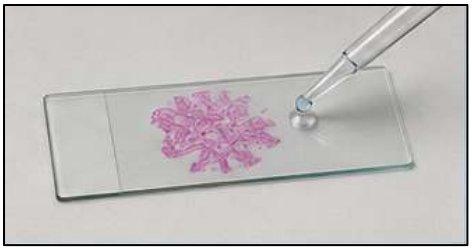
- Xylene I – 2 minutes
- Xylene II – 2 minutes

(H) Mounting

A drop of DPX mounting medium is placed on the section and covered with a cover slip. The slide is then allowed to dry.

Time:

- Mounting procedure – 2–3 minutes
- Drying time – several hours to overnight



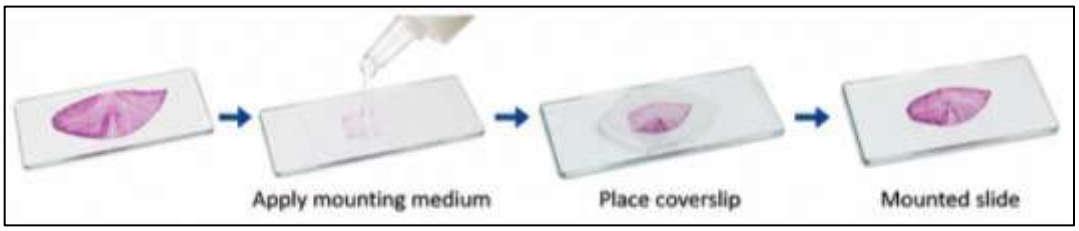
Approximate Total Time

The complete H&E staining procedure usually takes about 30–45 minutes, excluding drying time.

- Staining enhances contrast and allows different tissue structures to be identified under the microscope.

Step 8: Mounting – Apply coverslip with mounting medium.

- After staining, a coverslip is placed over the tissue section using DPX mounting medium.
- Mounting protects the stained section from damage and preserves it for long-term storage.
- Proper mounting also improves optical clarity during microscopic examination.



(4) Quality Control

- Ensure tissue sections are wrinkle-free.
- Confirm adequate staining quality.
- Label slides correctly.

(5) Safety Precautions

- Wear gloves and a lab coat.
- Handle xylene and formalin in a ventilated area.
- Dispose of sharps safely.

References

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