

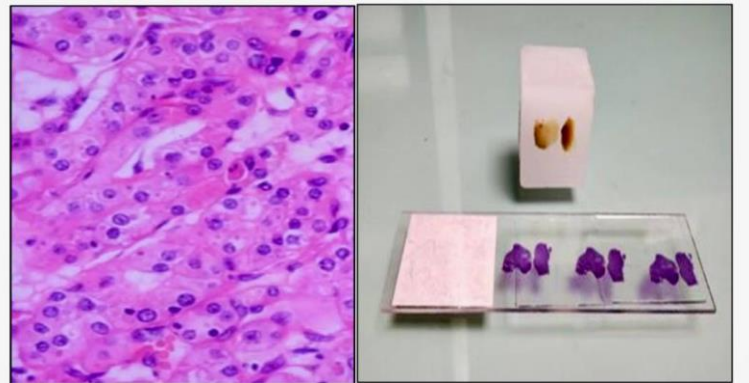


LEARNING OBJECTIVES

By the end of this section, you will be able to understand:

Tissue Preparation for Histological Study

1. Fixation
2. Cassette placement
3. Processing
4. Sectioning
5. Staining.



Tissue Preparation for Histological Study

Histology is the study of body tissues and how they are arranged to form organs.

Tissues consist of:

- Cells
- Extracellular Matrix (ECM)

Functions of ECM:

- Supports cells
- Transports nutrients
- Removes waste products
- Helps in tissue organization

To study tissues under a light microscope, tissues must be prepared in a special way because most tissues are too thick for light to pass through them.

Why Tissue Preparation Is Needed

- Most tissues are thick and opaque
- Light microscopy requires thin, transparent sections
- Proper preparation preserves normal tissue structure

Basic Steps of Tissue Preparation

Tissue preparation involves five main steps:

1. Tissue Fixation

Definition:

Fixation is the process of preserving tissue structure by preventing:

- Autolysis (self-digestion)
- Putrefaction (bacterial decomposition)

Key Points:

- Tissue must be placed in fixative **immediately after collection**

- **Most common fixative:**

10% Neutral Buffered Formalin

- Formalin to tissue ratio: **10 : 1**

- Fixation time: 24–48 hours

Purpose of fixation:

- Preserves cellular details
- Maintains tissue morphology



2. Specimen Transfer to Cassettes

After fixation:

- Tissue is trimmed using a scalpel
- Size should fit easily into a labelled tissue cassette
- Tissue should not completely fill the cassette
- Cassettes are stored in formalin until processing



Purpose:

- Easy handling
- Proper identification
- Uniform processing

3. Tissue Processing

This step prepares the tissue for sectioning by forming a paraffin block.

A. Dehydration

- Removes water and formalin from tissue
- Done using increasing concentrations of alcohol

Purpose:

Water must be removed because paraffin wax is not water-soluble.

B. Clearing

- Alcohol is removed using an organic solvent
- Commonly used solvent: Xylene

Purpose:

- Makes tissue transparent
- Allows paraffin wax to penetrate the tissue

C. Embedding

- Tissue is infiltrated with molten paraffin wax
- Wax surrounds the tissue and then solidifies
- Forms a solid paraffin block

Purpose:

- Provides firm support
- Allows cutting of very thin sections



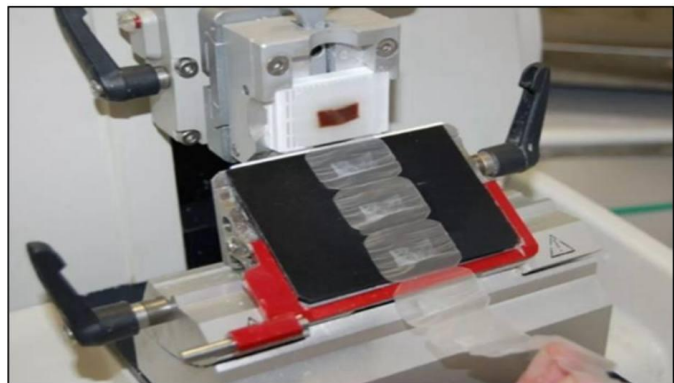
4. Sectioning

- Paraffin block surface is trimmed to expose tissue
- Block is chilled before cutting
- Tissue is cut using a microtome



Important Details:

- Section thickness: about **5 μm**
- Sections appear as a ribbon



Slide Preparation

- Sections floated on a warm water bath
- Picked up onto glass slides
- Slides are labelled
- Dried at 37°C to melt excess wax—

5. Staining

Most tissues are colorless, so staining is required.

Common stain:

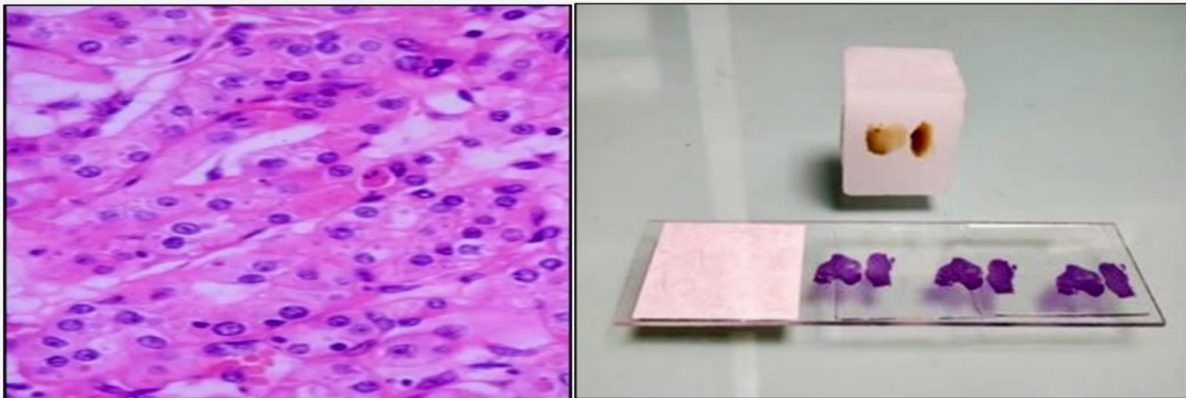
- Hematoxylin and Eosin (H&E)

Purpose of staining:

- Provides contrast
- Makes cells and tissue structures visible

After staining:

- A coverslip is placed over the section to protect the specimen



Picking up floating sections on slide and drying

