

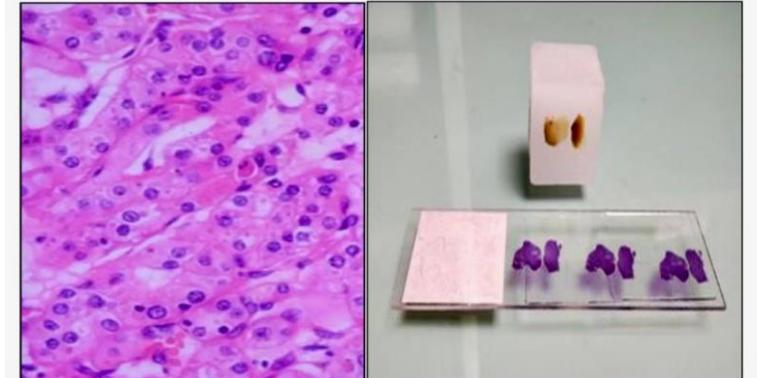


LEARNING OBJECTIVES

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:

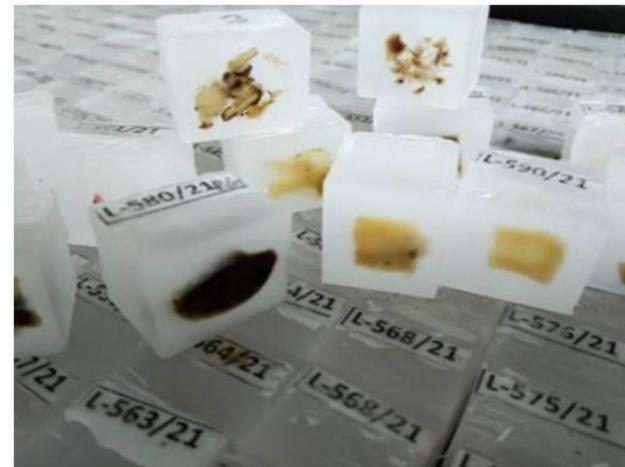
- Makes tissue transparent
- Allows paraffin wax to penetrate the tissue

C. Embedding

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

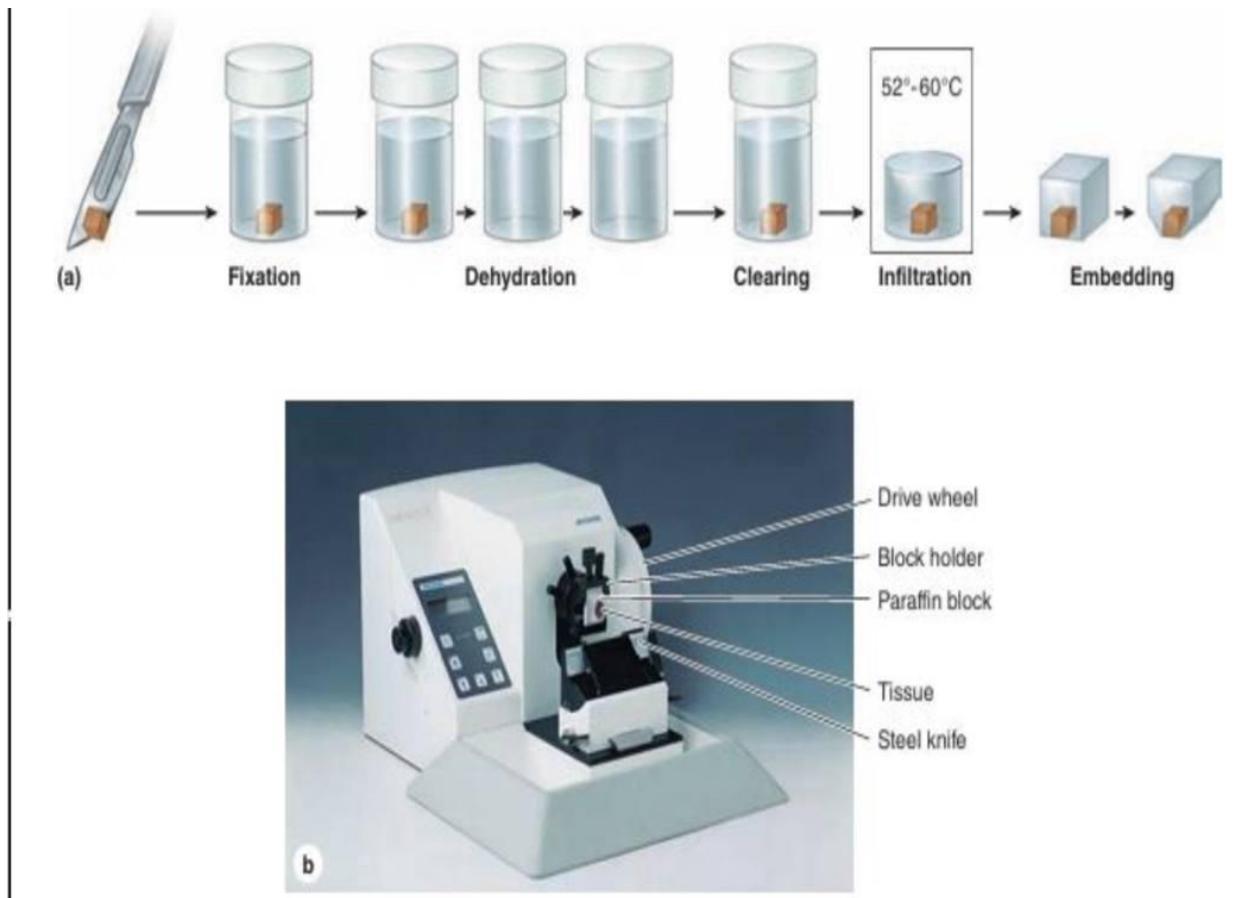
Purpose:

- Provides firm support
- Allows cutting of very thin sections



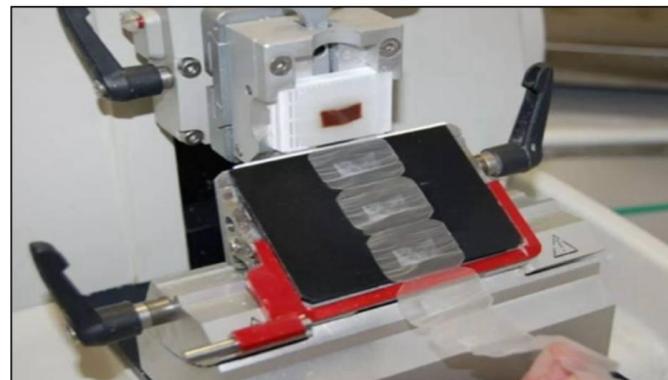
4. Sectioning

- 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:

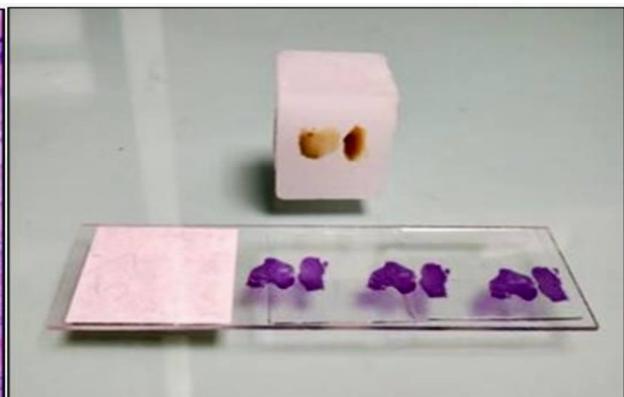
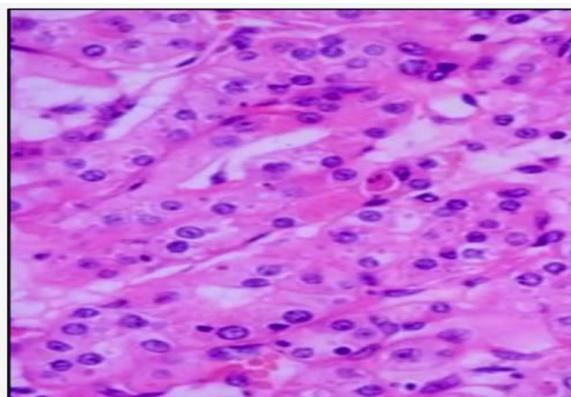
- Hematoxylin and Eosin (H&E)

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

