

University of Anbar
College of Dentistry
Department of Basic Sciences
Practical Histology (2024-2025)



Lab 1

Tissue Processing



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Microscopic examination of tissues requires the preparation of very thin sections. This is necessary because most tissues and organs are too thick for light to pass through. Therefore, they must be processed and sectioned into thin, translucent slices suitable for observation under the light microscope.

Fresh tissues are extremely delicate and prone to distortion or damage during sectioning. To avoid this, tissues must first be chemically preserved (a process known as **fixation**) and mechanically supported to withstand the cutting process. Sections are then cut using a precision instrument called a **microtome**.

The **ideal microscopic preparation** should retain the tissue's **original structure and molecular composition**, mimicking its state in the living body as closely as possible.

Steps of processing

- 1- **Fixation**
- 2- **Dehydration**
- 3- **Clearing**
- 4- **Wax infiltration**
- 5- **Embedding**
- 6- **Sectioning**
- 7- **Staining**

1-Fixation: small pieces of tissue should be promptly and adequately treated immediately after removal from the body and placed in solutions of chemicals (fixatives like formalin) that preserve by cross-linkage proteins and inactivating degradative enzymes and preventing cell autolysis.

Ideally specimens should remain in fixative for long enough to allow the fixative to penetrate into every part of the tissue .

2- Dehydration: The water is extracted from the fragments to be embedded by bathing them successively in a graded series of mixtures of ethanol and water (usually from 70% to 100% ethanol) .

Ethanol is miscible with water in all proportions, so that the water in the specimen is progressively replaced by the alcohol.

3- Clearing: Involves the replacement of ethanol with a solvent miscible with the embedding medium (wax), since wax and ethanol are largely immiscible.

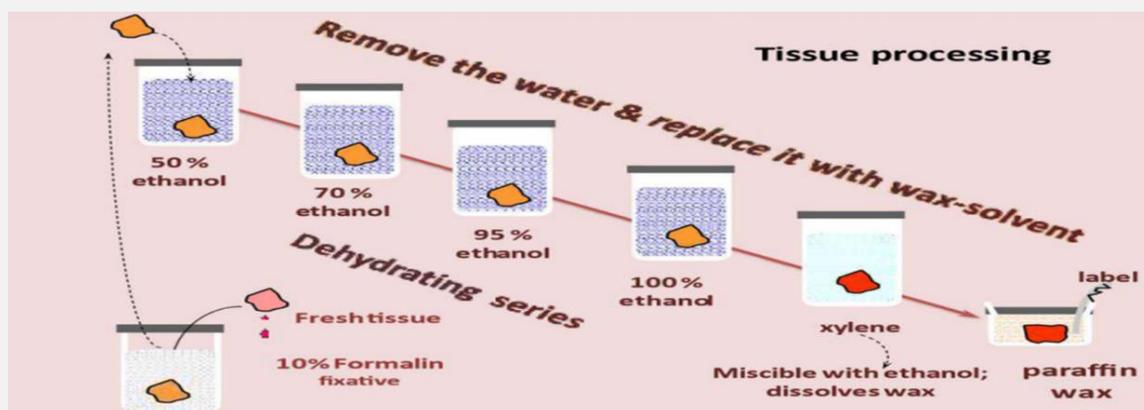
In paraffin embedding, the solvent used is usually xylene; an intermediate solvent that is fully miscible with both ethanol and paraffin wax .

This solvent will displace the ethanol in the tissue, and this in turn, will be displaced by molten paraffin wax. As the tissues are infiltrated with the solvent, they generally become transparent.

4- Wax infiltration: Includes the exchange of xylene with an embedding agent .

Once the tissue is impregnated with the solvent, it is placed in melted paraffin in the oven, typically at 58–60°C. The heat causes the solvent to evaporate, and the spaces within the tissues become filled with paraffin. A typical wax is liquid at 58-60°C, and can be infiltrated into tissue at this temperature then allowed to cool to 20°C where it solidifies to a consistency that allows sections to be consistently cut.

Although many different reagents have been used for this purpose over many years, the paraffin wax-based histological waxes are the most popular.

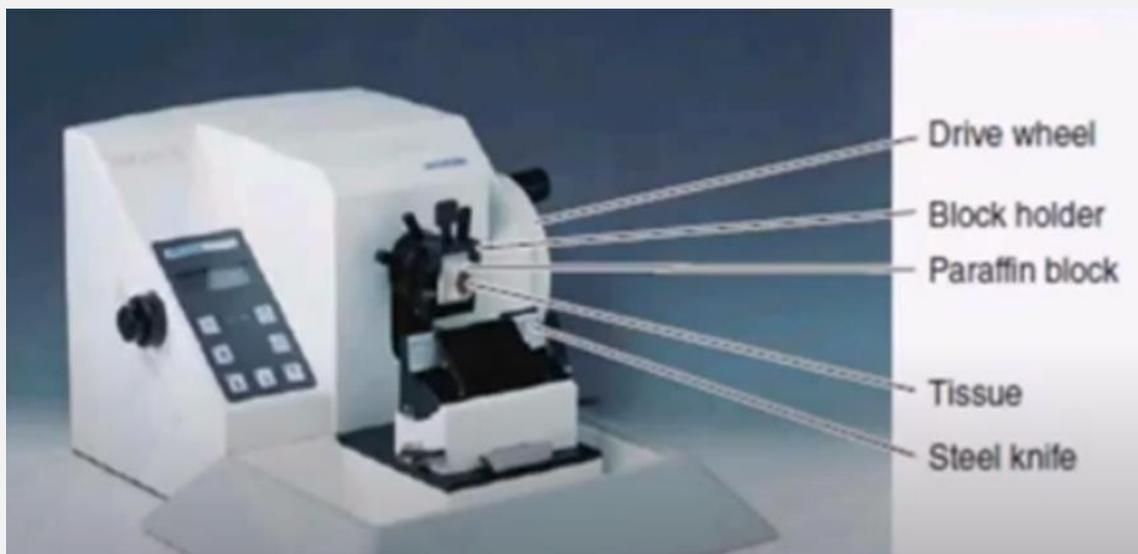


5-Embedding: After tissue has been dehydrated, cleared and infiltrated with paraffin, it must be formed into a “block” which can be clamped into a microtome for section cutting.



For indefinite storage of a block, an identification number is written on a small piece of paper and attached to one side of the block.

6-Sectioning: The paraffin block is then cut by using a microtome.



8- Staining: In order to reveal structural details some form of staining is required. The routine stain used universally as a starting point in providing essential structural information, is the hematoxylin & eosin (H&E) stain . When it is properly performed, it has the ability to demonstrate a wide range of normal and abnormal cell and tissue components, and yet, it is a relatively simple stain to carry out on paraffin sections. With this method, cell nuclei are stained blue, and cytoplasm and many extracellular components in shades of pink.

Light Microscopy Stains

Stain name	The stained structure
Hematoxylin	acts as a basic molecule and stains cellular regions rich in acidic macromolecules (DNA or RNA) a purplish blue color .
Eosin	acts as an acidic stain that binds to basic macromolecules such as collagen and most cytoplasmic proteins, especially those of mitochondria. Eosin stains regions rich in such structures a pinkish red color .
Hematoxylin and Eosin (H&E)	Sharp blue nucleus and red cytoplasm staining
Pararosaniline–Toluidine Blue (PT)	This dye combination stains chromatin shades of purple and cytoplasm and collagen a lighter violet . Toluidine blue is also commonly used for differential staining of cellular components, particularly cytoplasmic granules.
Mallory Trichrome	Mallory's trichrome stain also called Mallory's Triple Stain is a stain (aniline blue, acid fuchsin, and orange G.). nuclei staining purple ; cytoplasm, keratin, and erythrocytes staining bright red or orange .collagen staining bright or light blue . Mallory trichrome is particularly useful in demonstrating cells and small blood vessels of connective tissue..
Picrosirius-Hematoxylin (PSH)	The dye sirius red in a solution of picric acid stains collagen red and cytoplasm a lighter violet or pink , with nuclei purple .
Wright-Giemsa Stain	These are two similar combinations of stains that are widely used on fixed cells of blood or bone marrow smears. Nuclei stain purple and erythrocytes stain uniformly pink or pinkish orange .
Silver or Gold Stains	Used to stain filamentous structures in neurons and fibers of reticulin (type III collagen). By these techniques these filaments stain dark brown or black .

<p>Weigert's resorcin fuchsin, aldehyde fuchsin, and orcein.</p>	<p>To distinguish between elastic structures from collagen, most of which stain the elastin-rich structures brown or shades of purple</p>
<p>Oil red O and Sudan black</p>	<p>When special preparation techniques are used to retain lipids of cells, such as in frozen sections, lipophilic dyes are used to demonstrate lipid droplets and myelin.</p>