

PREPARATION OF HISTOLOGICAL SPECIMENS

- **What is Histology?**

Histology: is the scientific study of the fine detail of biological cells and tissues (composition, and function of tissues) using microscopes to look at specimens of tissues that have been carefully prepared using special processes called "histological techniques"

- **What is Tissue:**

A group or layer of cells that perform specific functions. Human body tissue consists of groups of cells with a similar structure working together for a specific function. There are four main types of tissue in a body

- **Why we should perform tissue preparations?**

Most fresh tissue specimens are colorless and squishy. They provide little useful information. For scientific or diagnostic purposes, tissue specimens must undergo substantial alteration in preparation for viewing under a microscope.

- **There are four steps in tissue preparation.**

- Fixation stabilizes and preserves the tissue.
- Embedding converts the tissue into a solid form which can be sliced ("sectioned").
- Sectioning (slicing) provides the very thin specimens needed for microscopy.
- Staining provides visual contrast and may help identify specific tissue components.

The most common mode of routine tissue preparation involves fixation with buffered formaldehyde, embedding in paraffin, sectioning into slices about 5 micrometers in thickness, and staining with hematoxylin and eosin.

1-Tissue fixation

Fresh tissue samples must be preserved for future examination and the resulting specimen is described as **fixed tissue**.

Fixation is a complex series of chemical events that differ according to the different groups of substance found in tissues.

- **The aim of fixation:**

- 1- To prevent autolysis and bacterial attack.
- 2- To fix the tissues so they will not change their volume and shape during processing.
- 3- To prepare tissue and leave it in a condition which allow clear staining of sections.
- 4- To leave tissue as close as their living state as possible, and no small molecules should be lost.

- **Factors affect fixation:**

- 1- PH.
- 2- Temperature.
- 3- Penetration of fixative.
- 4- Volume of tissue.

According to previous factors we can determine the concentration of fixative and fixation time.

- **Types of fixative:**

Acetic acid, Formaldehyde 10%, Ethanol, Glutaraldehyde for EM, Methanol and Picric acid

- **Tissue processing**

Series of soluble replacements

- Washing :Removing the formaline by put the container which contain the tissue Under tap water for 48 hours
- Dehydration: replace with ethanol, acetone (alcohol)
- Clearing: replace with xylene

Stages of processing:

- 1- Dehydration.
- 2- Clearing.

❖ **Dehydration**

To remove fixative and water from the tissue and replace them with dehydrating fluid .

- There are a variety of compounds many of which are alcohols. Several are hydrophilic so attract water from tissue.
- To minimize tissue distortion from diffusion currents, delicate specimens are dehydrated in a graded ethanol series from water through 10%-20%-50%-95%-100% ethanol.
- Types of dehydration agent ethanol , methanol ,acetone

❖ **Clearing**

Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium.

- Some clearing agents :Xylene ,Toluene for gland ,xylene ,petrol ,benzene and chloroform .

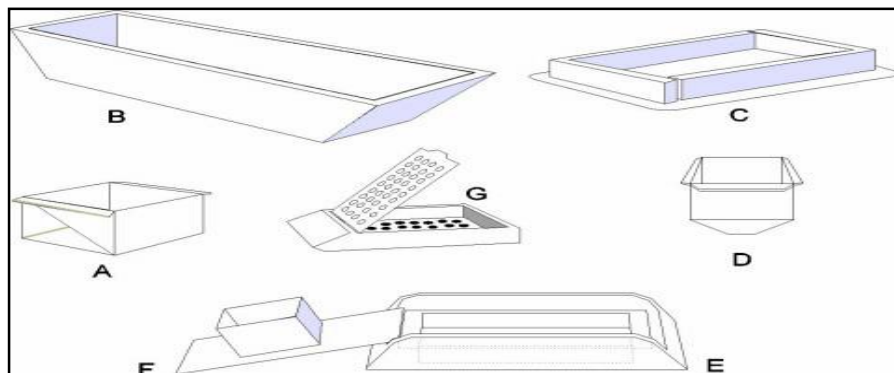
2-Embedding

Is the process by which tissues are surrounded by a medium such as agar, gelatin, or wax which when solidified will provide sufficient external support during sectioning.

There are four main systems and associated embedding protocols presently in use but the most popular is the traditional methods using paper boats

Embedding moulds:

- (A) paper boat;
- (B) metal boat mould;
- (C) Dimmock embedding mould;
- (D) Peel-a-way disposable mould;
- (E) Base mould used with embedding ring (F) or cassette bases (G).

**3-Sectioning (Cutting)**

Sectioning is the production of very thin slices from a tissue sample. The tool used for sectioning is called a microtome (tom = to cut, as in appendectomy).

- 3 dimensions --> 2 dimensions
- Planes of Section
- whole mount (unsectioned)
- cross section
- longitudinal section
- random

Microtome Usage

- **The microtome**

A mechanical instrument used to cut biological specimens into very thin segments for microscopic examination. Most microtomes use a steel blade and are used to prepare sections of animal or plant tissues for histology.

Microtome knives

- Steel knives
- Non-corrosive knives for cryostats
- Disposable blades
- Glass knives
- Diamond knives

Sections for routine light microscopy are typically 5-10µm (micrometers, microns= 10^{-6} meter) in thickness. Exceptionally thin sections may less

than 2 μ m thick. For electron microscopy, sections are typically 50-100 nanometers (10⁻⁹ meter) in thickness.

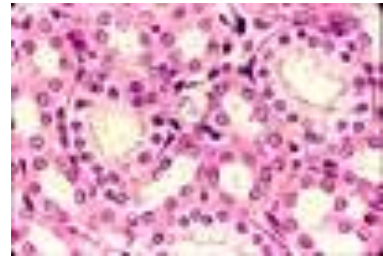
4- Staining

Most cells are essentially transparent, with little or no intrinsic pigment. Even red blood cells, packed with hemoglobin, appear nearly colorless when unstained, unless packed into thick masses. Stains are used to confer contrast, to make tissue components visibly conspicuous. Certain special stains, which bind selectively to particular components, may also be used to identify those structures. But the essential function for staining is simply to make structures easier to see.

Note: You should generally use specific aspects of actual structure (location, size, shape, texture) to identify cells and tissues, rather than color. Color can offer additional information if used wisely, but is unreliable by itself.

- **H&E stain(hematoxylin and eosin):**

Routine histology uses the stain combination of hematoxylin and eosin, commonly referred to as H&E. Hematoxylin is a basic stain with deep purple or blue color. Structures that are stained by basic stains are described as basophilic ("base-loving"). Chromatin (i.e., cell nuclei) and ribosomes are basophilic. With H&E staining, basophilic structures are stained purple.



Eosin is an acidic stain with a red color. Structures stained by acid stains are described as acidophilic (or eosinophilic) and include collagen fibers, red blood cells, muscle filaments, mitochondria. With H&E staining, acidophilic structures are stained red or pink.

Note: Some cell structures do not stain well with aqueous dyes and so routinely appear clear. This is especially so for those which are hydrophobic, containing fat. Included in this category are adipocytes, myelin around axons, and cell membranes of the Golgi apparatus.

HISTOLOGY PROCEDURE

