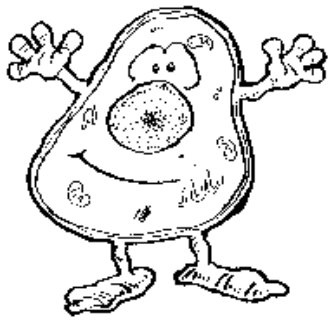


PREPARATION OF HISTOLOGICAL SPECIMENS

- Histotechniques
- Tissue Processing
- Tissues taken for diagnosis of disease processes must be processed in the histology laboratory to produce microscopic slides that are viewed under the microscope by pathologists.
- Specimen Accessioning
- Tissue specimens received in the surgical pathology laboratory have a request form the surgeon.
- The specimens are accessioned by giving them a number .

- Gross Examination
- Tissues removed from the body for diagnosis arrive in the Pathology Department and are examined by a pathologist, pathology assistant, or pathology resident. Gross examination consists of describing the specimen and placing all or parts of it into a small plastic cassette which holds the tissue while it is being processed to a paraffin block. Initially, the cassettes are placed into a fixative.

TISSUE FIXATION

- **Fixation** is a complex series of chemical events that differ for 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.
- Fixation is coming by reaction between the fixative and protein which form a gel, so keeping every thing as their in vivo relation to each other.

Factors affect fixation:

- PH.
- Temperature.
- Penetration of fixative.
- Volume of tissue.

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

Types of fixative:

- There are five major groups of fixatives, classified according to mechanism of action:
- Aldehydes
- Mercurials
- Alcohols
- Oxidizing agents
- Picrates

TISSUE PROCESSING

the aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut , and yet soft enough not to damage the knife or tissue.

Stages of processing:

1- Dehydration.

2- Clearing.

3- Embedding.

Dehydration

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

-Alcohols. 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.
- In the paraffin wax method, dehydration from aqueous fixatives is usually initiated in 60%-70% ethanol, progressing through 90%-95% ethanol, then two or three changes of absolute ethanol before proceeding to the clearing stage.

Types of dehydrating agents:

Ethanol, Methanol, Acetone.

- Duration of dehydration should be kept to the minimum consistent with the tissues being processed. Tissue blocks 1 mm thick should receive up to 30 minutes in each alcohol, blocks 5 mm thick require up to 90 minutes or longer in each change. Tissues may be held and stored indefinitely in 70% ethanol without harm

Clearing

- replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium.
- **Choice of a clearing agent depends upon the following:**

  - The type of tissues to be processed, and the type of processing to be undertaken.
  - The processor system to be used.
  - Intended processing conditions such as temperature, vacuum and pressure.
  - Safety factors.
  - Cost and convenience.
  - Speedy removal of dehydrating agent .
  - Ease of removal by molten paraffin wax .
  - Minimal tissue damage .

- **Some clearing agents:**

- Zylene.
- Toluene.
- Chloroform.
- Benzene.
- Petrol.

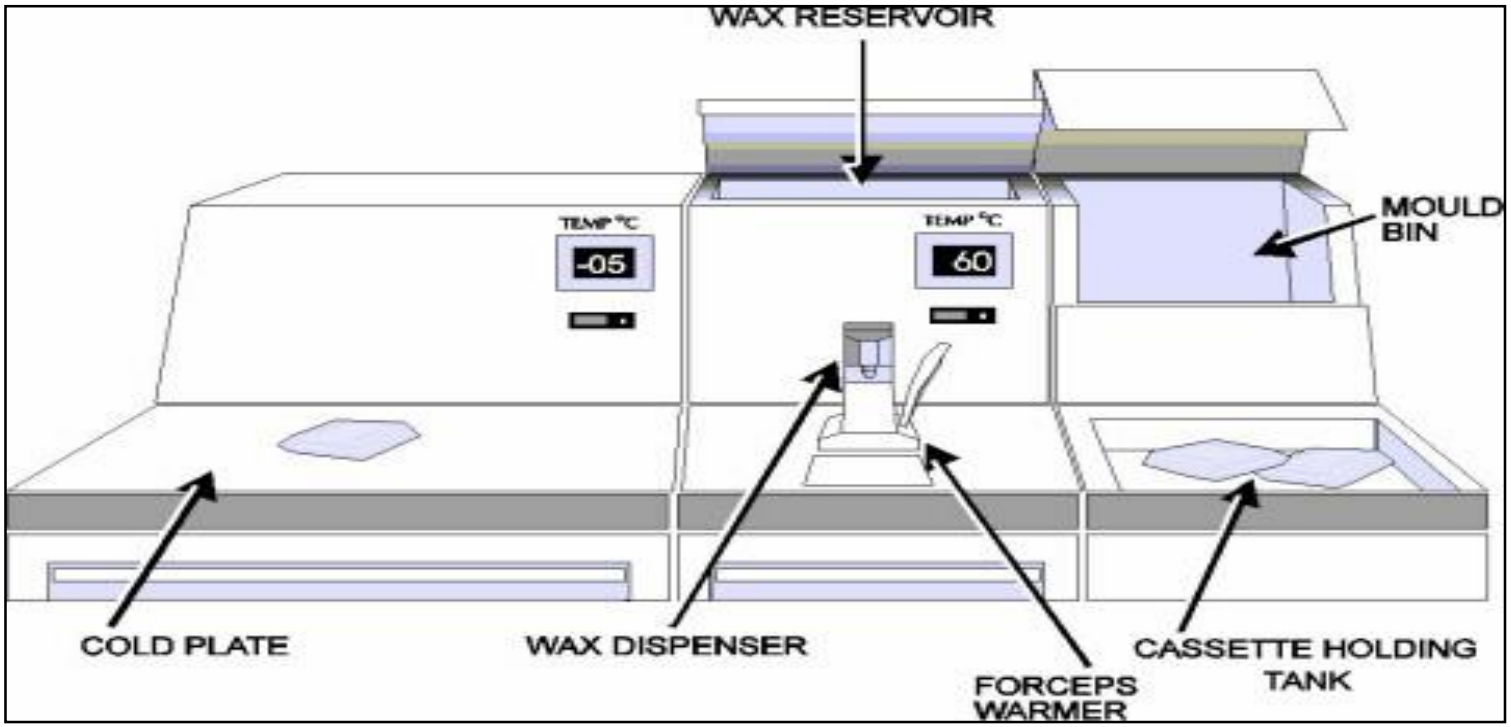
Embedding

- 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.
- **Paraffin wax**

  properties :
- Paraffin wax is a polycrystalline mixture of solid hydrocarbons produced during the refining of coal and mineral oils. It is about two thirds the density and slightly more elastic than dried protein**.** Paraffin wax is traditionally marketed by its melting points which range from 39°C to 68°C.
- The properties of paraffin wax are improved for histological purposes by the inclusion of substances added alone or in combination to the wax:
  - improve ribboning.
  - increase hardness.
  - decrease melting point
  - improve adhesion between specimen and wax

Precaution while embedding in wax

- The wax is clear of clearing agent.
- No dust particles must be present.
- Immediately after tissue embedding, the wax must be rapidly cooled to reduce the wax crystal size.



- **There are four main mould systems and associated embedding protocols presently in use :**

  1- traditional methods using paper boats

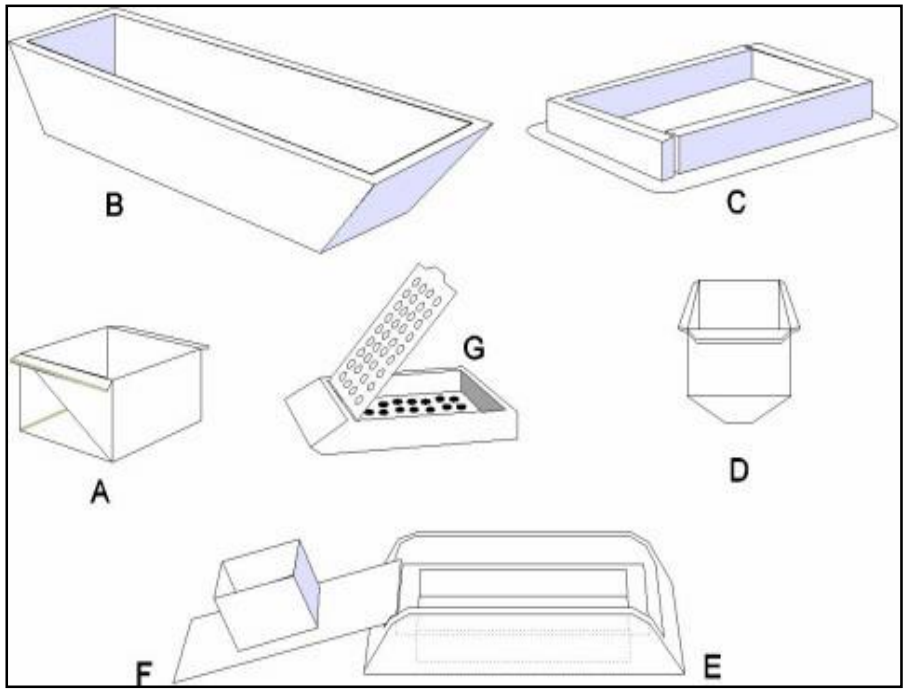
  2- Leuckart or Dimmock embedding irons or metal containers

  3- the Peel-a-way system using disposable plastic moulds and

  4- systems using embedding rings or cassette-bases which become an integral part of the block and serve as the block holder in the microtome.

Tissue processing
Embedding moulds:

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



- **ORIENTATION OF TISSUE IN THE BLOCK**
  Correct orientation of tissue in a mould is the most important step in embedding. Incorrect placement of tissues may result in diagnostically important tissue elements being missed or damaged during microtomy.
- elongate tissues are placed diagonally across the block
- tubular and walled specimens such as vas deferens, cysts and gastrointestinal tissues are embedded so as to provide transverse sections showing all tissue layers
- tissues with an epithelial surface such as skin, are embedded to provide sections in a plane at right angles to the surface (hairy or keratinised epithelia are oriented to face the knife diagonally)
- multiple tissue pieces are aligned across the long axis of the mould, and not placed at random

Processing methods and routine schedules

- **Machine processing**
- **manual processing**

CUTTING

- **using the microtome**

- A **microtome** is 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.

- **Cryosection**:
- **water-rich tissues are hardened by freezing and cut frozen; sections are stained and examined with a light microscope. This technique is much faster than traditional histology (5 minutes vs. 16 hours) and are used in operations to achieve a quick diagnosis. Cryosections can also be used in immunohistochemistry as freezing tissue does not alter or mask its chemical composition as much as preserving it with a fixative.**

- **Electron microscopy**:

- after embedding tissues in epoxy resin, a microtome equipped with a glass or diamond knife is used to cut very thin sections (typically 60 to 100 nanometers). Sections are stained and examined with a transmission electron microscope. This instrument is often called an **ultramicrotome.**

- **Botanical microtomy**:

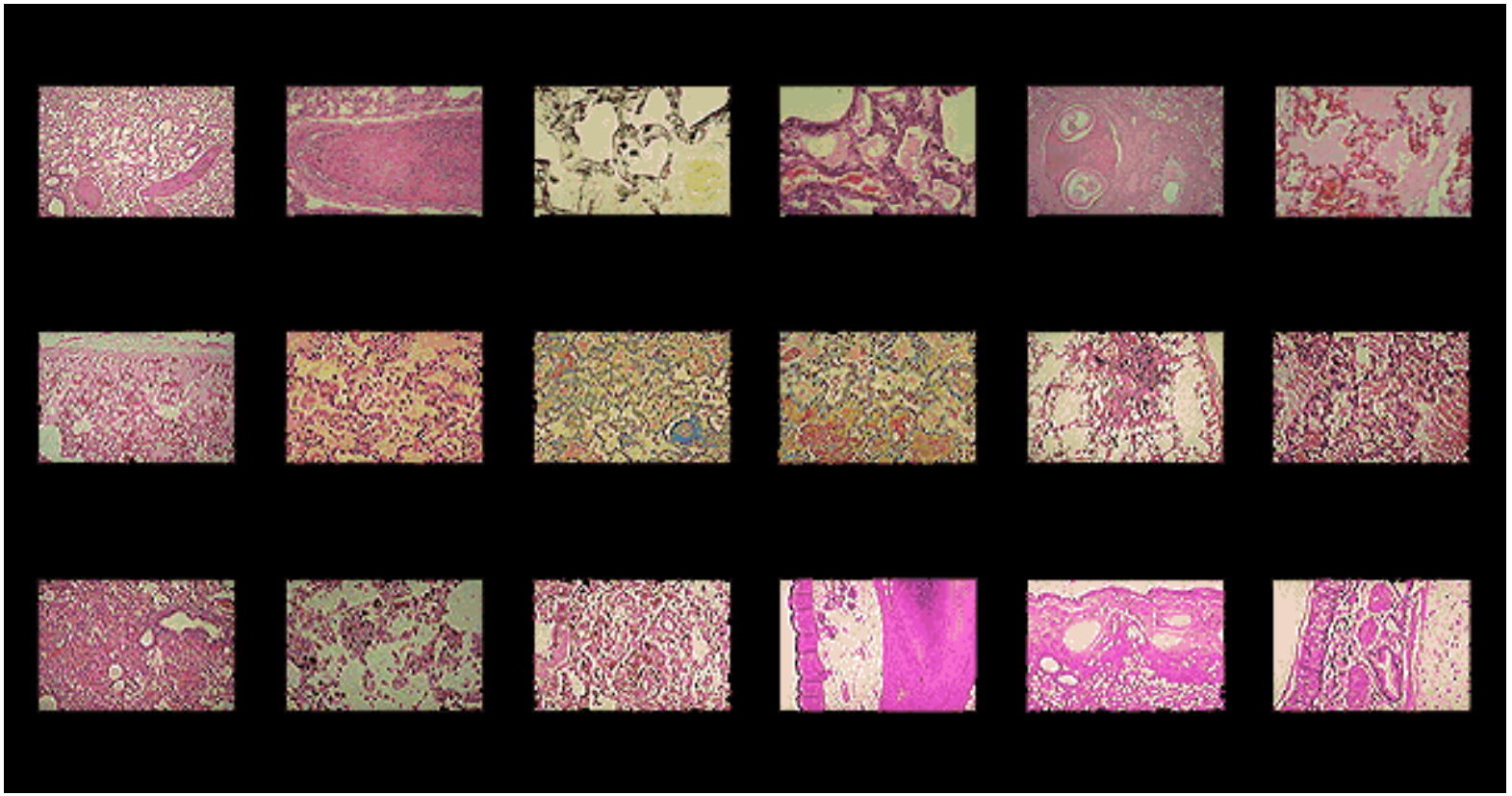
- **hard materials like wood, bone and leather require a sledge microtome. These microtomes have heavier blades and cannot cut as thin a regular microtomy.**

- **Microtome blades are extremely sharp, and should be handled with great care. Safety precautions should be taken in order to avoid any contact with the cutting edge of the blade.**

Microtome knives

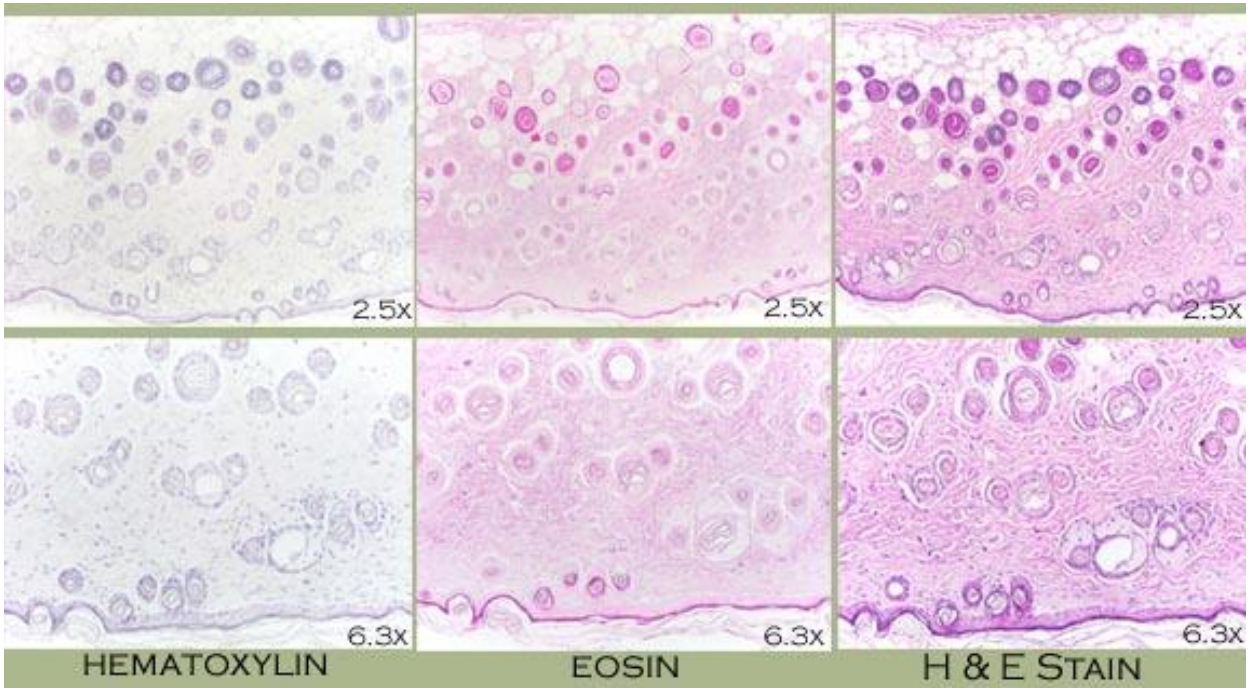
- **STEEL KNIVES**
- **NON-CORROSIVE KNIVES FOR CRYOSTATS**
- DISPOSABLE BLADES
- GLASS KNIVES
- DIAMOND KNIVES

STAINING



Hematoxylin and Eosin (H & E)

H & E is a charge-based, general purpose stain. Hematoxylin stains acidic molecules shades of blue. Eosin stains basic materials shades of red, pink and orange. H & E stains are universally used for routine histological examination of tissue sections.



Fixation

Any well fixed tissue.

Staining Procedure

1- Deparaffinize (30 min .in oven at 70 c) and xylen two change with 5 min,for every step.
2- graded Alcohols,(100%,90%,80% and 70% ,2min.for each step.
3- Mayer's or harris hematoxylin for 5 minutes
4- Wash in running tap water for 2 minutes
5- Counterstain with eosin from 1 seconds to 2 minutes .
6- Dehydrate in graded alcohols (70%,80%,90% and 100%) 2 min.for each step.
7- Clear in xylene, two changes of 2 minutes each
8- Mount in Permount or DPX.
9-coverslip and examination under microscope.

Results

Nuclei - blue - with some metachromasia
Cytoplasm - various shades of pink-identifying different tissue components

Staining machine