Histopathological Diagnosis

PREPARATION OF HISTOLOGICAL SPECIMENS

WASHING

- Following fixation, the tissues should be washed from 3 to 6 hours.

- The fixed tissues are washed in running tap water to remove the fixative from them.

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

- Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin.
- First, the water from the tissues must be removed by dehydration.This is usually done with a series of alcohols; say **70% to 95% to 100%.** The organic solvent must replace the water gradually to prevent turbulence at the interface between water and pure ethanol.
- Turbulence could cause damage or distortion to cellular components.
- Sometimes the first step is a mixture of formalin and alcohol.

Clearing

- The next step is called "clearing" and consists of removal of the dehydrant with a substance that will be miscible with the embedding medium (paraffin).
- The commonest clearing agent is **xylene**.
- Toluene works well, and is more tolerant of small amounts of water left in the tissues, but is 3 times more expensive than xylene.

Clearing

- Chloroform used to be used, but is a health hazard, and is slow.
- Methyl salicylate is rarely used because it is expensive, but it smells nice (it is oil of wintergreen).
- Excessive exposure to clearing reagents may cause excessive hardness or shrinkage.

- 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 .

Embedding

- The tissue is infiltrated with the embedding agent, almost always **paraffin.**
- Nearly 100 years ago, the method of embedding tissues in paraffin was developed.
- Paraffin is a derivative of crude petroleum.
- Most paraffins suitable as embedding media melt between 52° and 58°C.
- Since most paraffin have a melting point between 52-58°C, it must infiltrate the cells while it is hot.

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.

Procedure of Paraffin-embedded Section and Pathologic Examination

Specimen of tissues or organs by excision (biopsy or operation)

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Cutting

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Tissue Treatment Procedure (Dehydration → Paraffin embedding → Section → Staining → Sealing)

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Microscopic examination → Reports signed out

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Application of modern technologies (IHC, PCR, FISH, EM, etc.) for specific requirement → Reports signed out

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Placing on files (Blocks; Slides; Documents, etc.)

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.
- The most common applications of microtomes are :

1- Traditional histological technique:

2- Cryosection:

3- Electron microscopy:

4- Botanical microtomy: