Tissue Processing Steps

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❖**Histology:** It is the branch of science which deals with the gross & microscopic study of normal tissue

❖**Histopathology:** It is the branch of science which deals with the gross & microscopic study of tissue affected by disease.

❖**Histo-techniquse**: The techniques for processing the tissue, whether biopsies, larger specimen removed at surgery, or tissue from autopsy so as at to enable the pathologist to study them under the microscope .

Protocols followed in Histo-techniques

1) Receipt & Identification.

2) labeling of the specimen with numbering.

3) fixation.

4)Washing and dehydration.

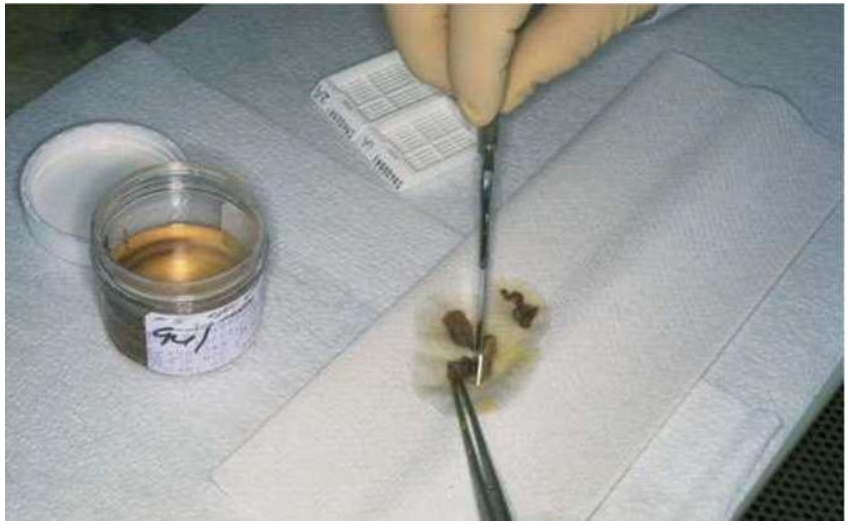
5) clearing.

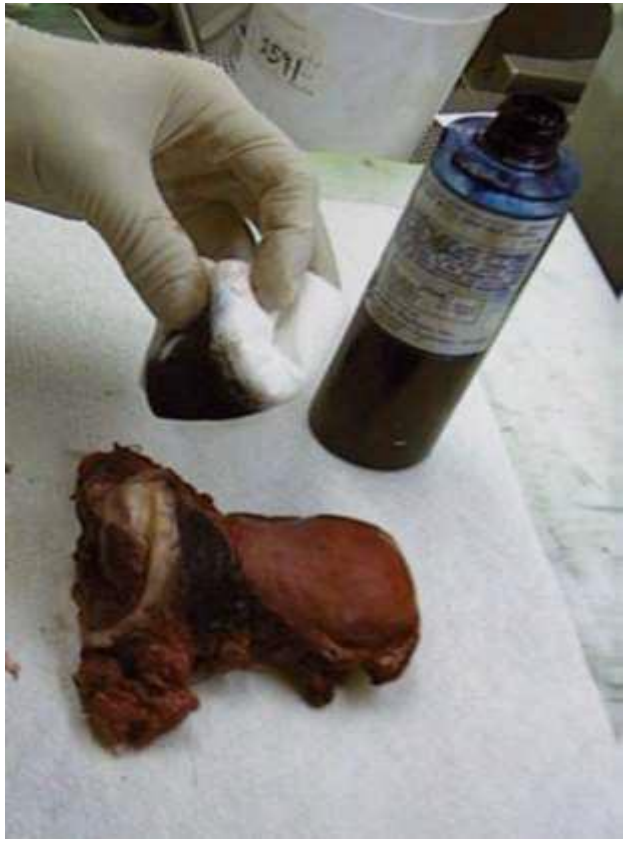
6) Infiltration and Embedding.

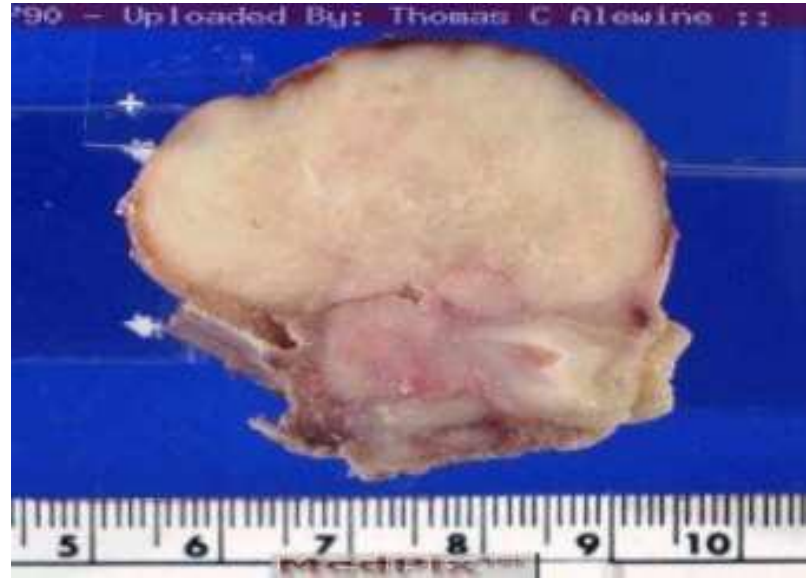
7) section cutting.

8)staining

1- Receipt & Identification: Tissue specimen received in the surgical pathology laboratory have a request form that list the patient information and history along with a description of the site of origin.







2-labeling of the specimen with numbering. The specimen are accessioned by giving them a number that will identify each specimen for each patient.

3-fixation: It is a process in which a specimen is treated by exposing it to a fixative for a particular period of time (24-48h) in order to facilitate the succeeding step.

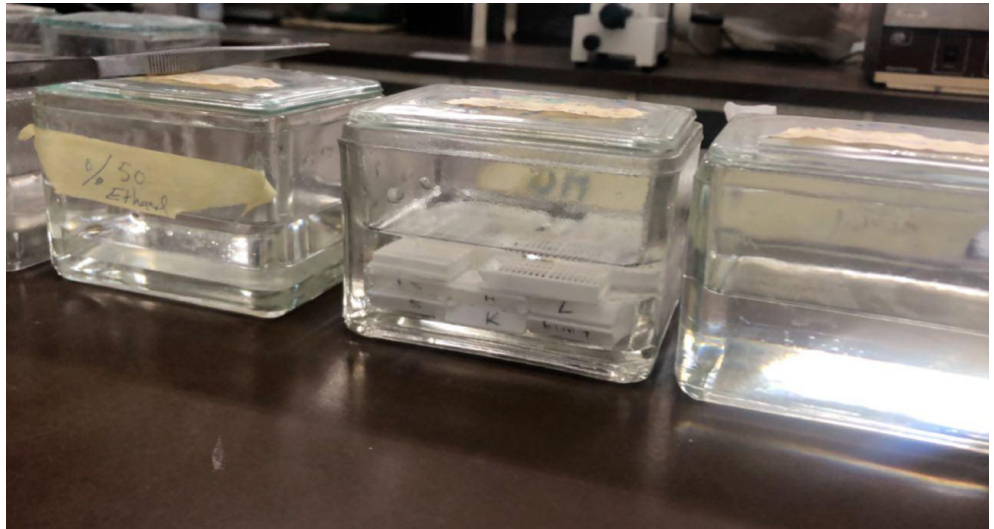
- Tissue samples should be transferred into fixative immediately after collection solution or by freezing. Although there are many types of fixative, most specimens are fixed in 10% formalin

- The purpose of fixation is to preserve tissue permanently in as life-like a state as possible.

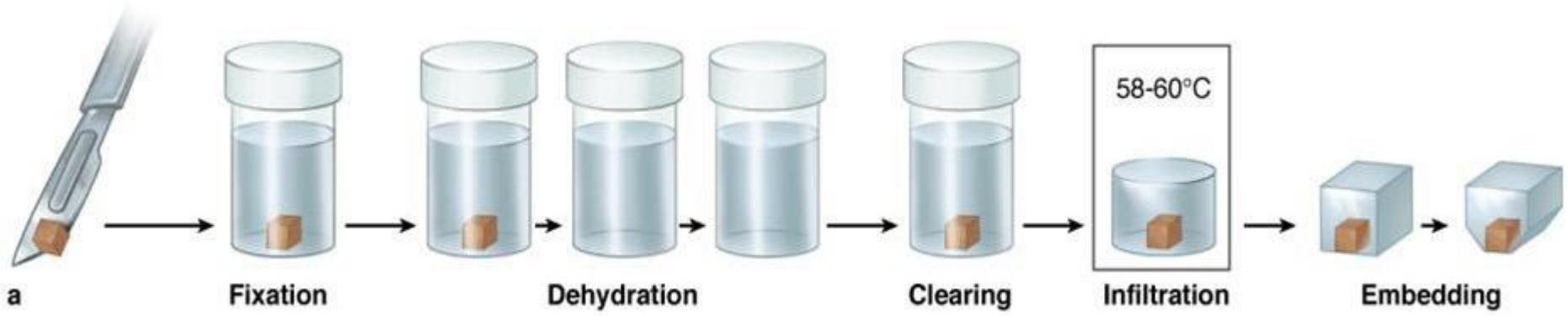
- The fixative should be 15-20 times more in volume then the specimen. The bite should of size of approximately 2x2 cm & 4-6 micrometer in thickness for optimum fixation to take place.

4-Dehydration

Most of the water in a specimen must be removed before it can be infiltrated with wax. This process is commonly carried out by immersing specimens in a series of alcohol (ethanol) solutions of increasing concentration until pure, water-free alcohol is reached. A series of increasing concentrations is used to avoid excessive distortion of the tissue. A typical dehydration sequence for specimens not more than 4mm thick would be 60% ethanol (30 min- 1h) 70% ethanol (30 min-1h), 90% ethanol (1h), 100% ethanol (1h) 100% ethanol over night



5-Clearing This solvent will displace the ethanol in the tissue, then this, in turn, will be displaced by molten paraffin wax. This stage in the process is called “clearing” and the reagent used is called a “clearing agent”. popular clearing agent is xylene, and multiple changes are required to completely displace ethanol we can use two change for 20 min and another one for 45min



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6-Infiltration and Embedding paraffin wax (60-65 C): This is the next step after clearing. The clearing agent within the tissue is removed by the process of diffusion. The tissue space is now infiltrated with the embedding media. Usually molten wax is used as the embedding medium. After specimens are infiltrated with the embedding agent usually paraffin wax. The tissue becomes surrounded by a large block of molten paraffin wax, creating “block”. Once the block solidifies


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B


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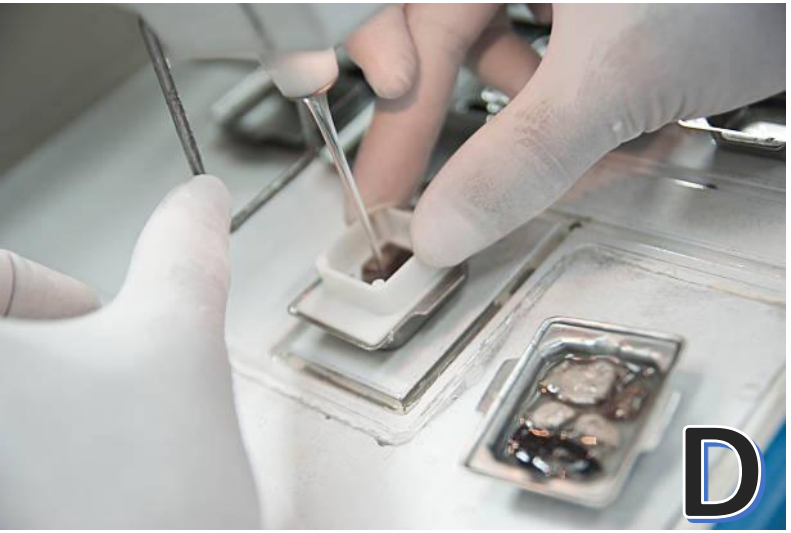
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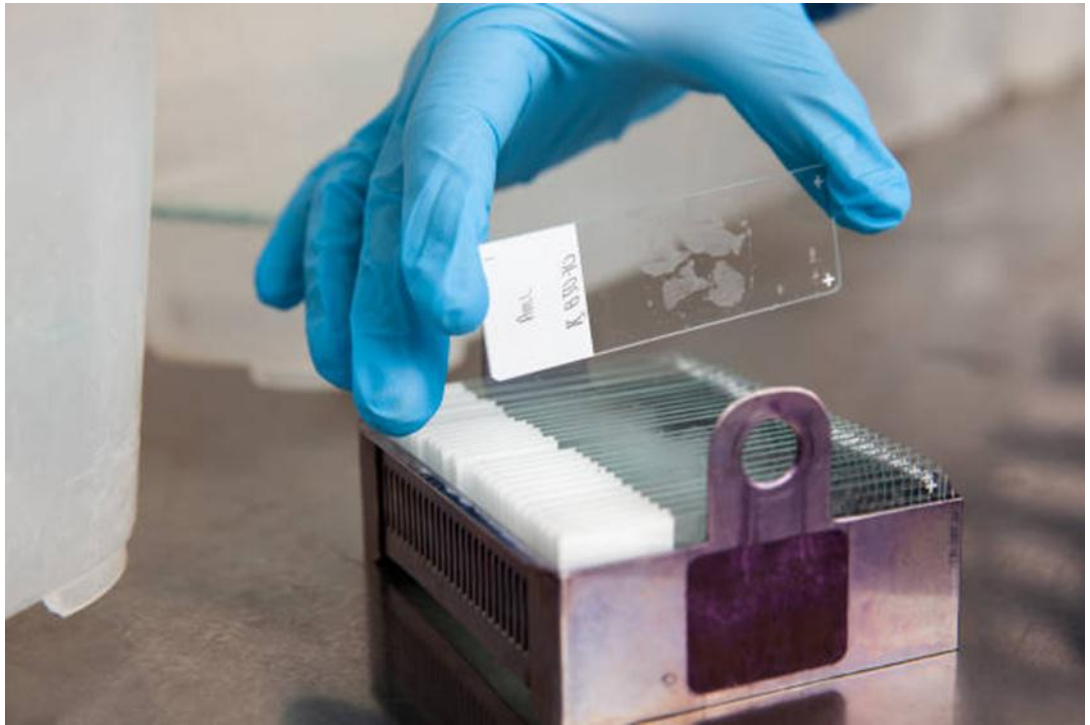
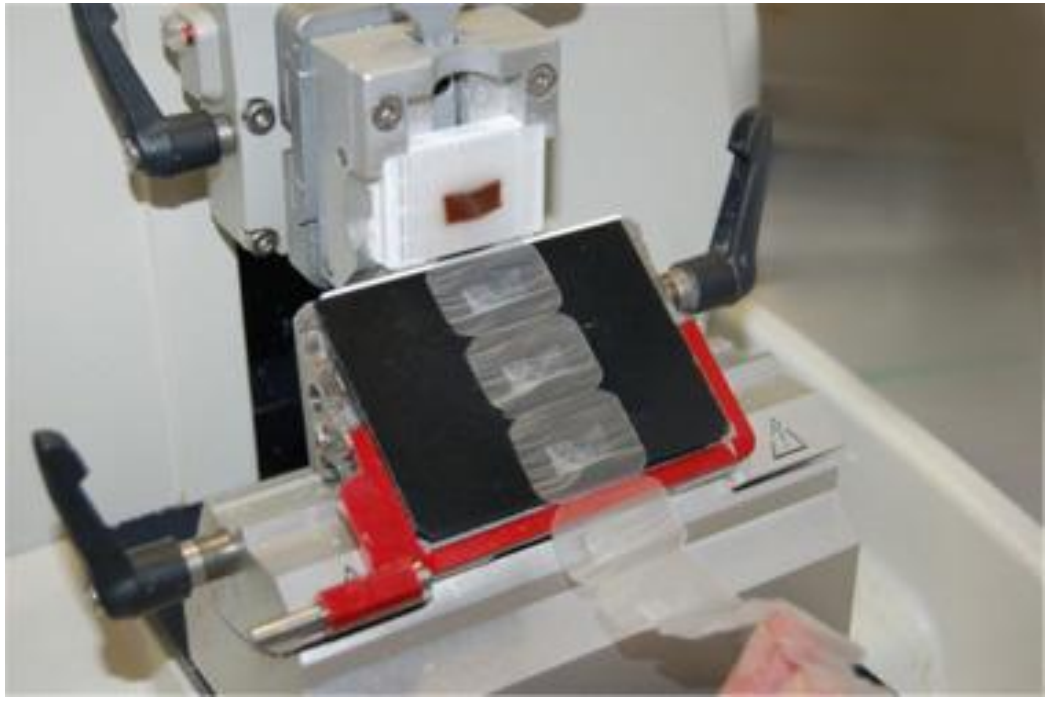
E


F

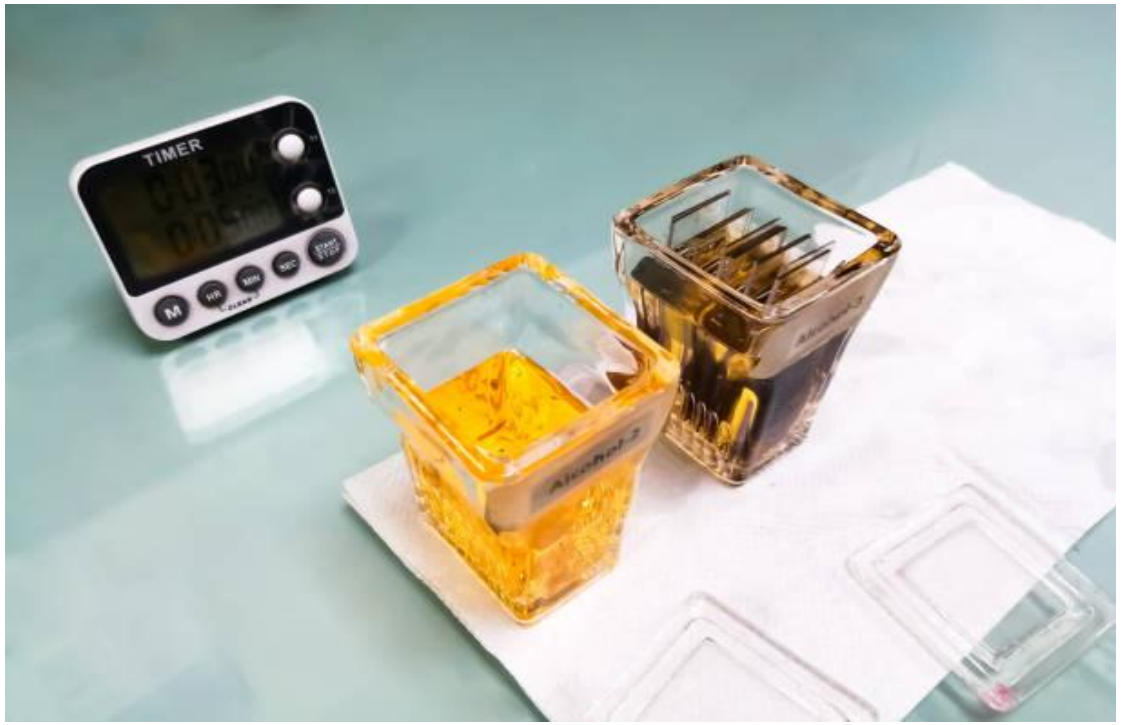
7- Sectioning Blocks:

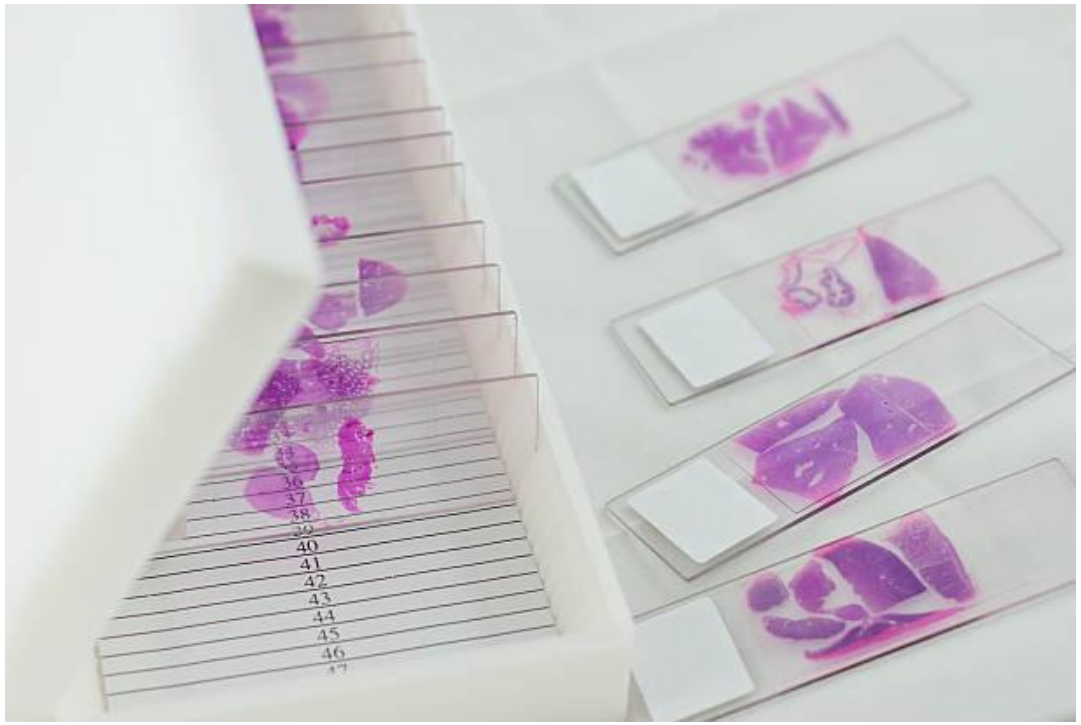
After Embedding sample are chilled on a refrigerated plate or ice tray for 10 minutes before sectioning. A microtome is an instrument used to slice extremely thin tissue sections off the block in the form of a ribbon. The microtome can be cut at different thicknesses, but most tissues are cut at around 5 µm. →Later, the tissue ribbons are carefully transferred to a warm water bath. Here they are allowed to float on the surface and can then be scooped up onto a slide placed under the water level. Slides should be clearly labelled, and then allowed to dry upright at 37oC for a few hours to gently melt the excess paraffin wax, leaving the tissue section intact.





8-Staining: Most cells are transparent, and appear almost colourless when unstained. Histochemical stains (typically haematoxylin and eosin) are therefore used to provide contrast to tissue sections, making tissue structures more visible and easier to evaluate. Following staining, a cover slip is mounted over the tissue specimen on the slide, using optical synthetic grade glue (DPX), to help protect the specimen





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