Histopathological Diagnosis

PREPARATION OF HISTOLOGICAL SPECIMENS

Different histological methods

There are 3 main techniques which are used in preparing microscopical sections from tissues:

- The paraffin technique (It is the most common method)
- The celloidin technique (It is the most perfect method)
- The freezing technique (It is the most rapid method)

- Tissues from the body 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.
- The persons who do the tissue processing and make the glass microscopic slides are histotechnologists.

Histopathological Diagnosis

- **Biopsy**: parafin embedded tissue section

Incisional biopsy

Excisional biopsy

- **Surgical excision:** parafin embedded tissue section

Organ or tissue with the tumours

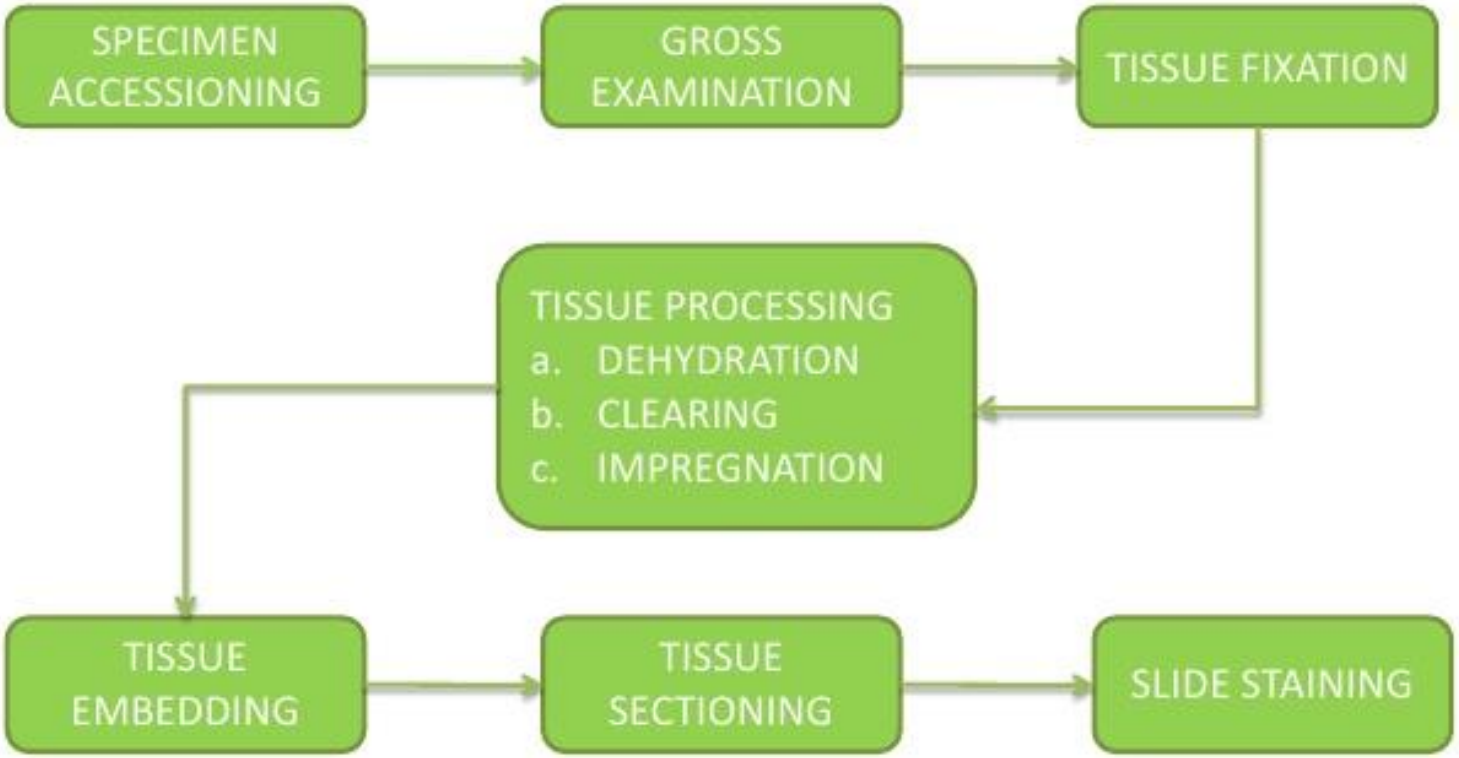
Regional lymph nodes

- **Intraoperative consultation**

Frosen section

- **Tissues from necropsy**

HISTOLOGY PROCEDURE



Gross Examination

- Tissues removed from the body for diagnosis arrive in the Pathology Department and are examined by a pathologist.
- 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.
- Biological tissues are generally rather soft, making it quite difficult to cut acceptably thin sections directly from the fresh or fixed tissues.

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.

TISSUE FIXATION

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.
- Types of fixative: Acetic acid, **Formaldehyde**, Ethanol, Glutaraldehyde, Bouin, Methanol and Picric acid.

- As soon as tissues are removed from the body, the vascular supply is lost, haemoglobin leaches from the red blood cells and the tissues begin to die.
- Fixation inhibits autolysis and putrefaction, hardens tissue and allows easy manipulation of soft tissues such as friable tumours and brain.

- The most widely used fixative in cellular pathology is *formalin.*
- When formalin comes into contact with fresh tissue, the haemoglobin becomes converted to the brownish tan pigment haematin.
- This conversion is observed when fresh tissue (which is normally a red-pink colour) is placed into 10% formalin at room temperature. Over a relatively short period of time, a change in surface colour to grayish brown occurs and this change can be seen in the images below.

TISSUE FIXATION

Factors affect fixation:

- PH. The pH values of the different fixatives vary. In general, the hydrogen ion concentration is usually adjusted by a suitable buffer to physiological pH, usually between pH 6-8. Many buffer systems are available for use in fixation; the most common ones are phosphate, chloride and carbonate (or bicarbonate) salts.

TISSUE FIXATION

Factors affect fixation:

- Temperature. Fixation of surgical specimens is carried out at room temperature, although 0-4 degc will slow down autolysis. However, chemical reactions, including those of fixation, are more rapid at higher temperatures.
- Formalin heated to 60 degrees Centigrade is sometimes used for the rapid fixation of urgent biopsy samples, but the risk of tissue distortion is increased.

TISSUE FIXATION

Factors affect fixation:

- Penetration of fixative. The penetration of fixatives into tissue is clearly an important phenomenon.
- As this process is relatively slow, the tissue samples should either be very small or very thin in order to obtain optimum fixation.
- The greatest factor influencing the rate of fixation is the thickness of the tissue.
- Whole organs should therefore be sliced to expose the cut surfaces to the fixative as soon as possible after removal.

TISSUE FIXATION

Factors affect fixation:

- Penetration of fixative.
- Volume of tissue.

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

TISSUE FIXATION

Factors affect fixation:

- Volume of tissue. Tissues commonly alter in volume due to changes in membrane and ion transport. Some intercellular substances such as collagen swell when they are fixed.
- Tissues fixed in 10% formalin and embedded in paraffin wax can shrink up to 33%.
- Concentration. This appears to be determined by factors of cost, effectiveness and solubility. The staining of tissues has been shown to alter with the concentration of the fixative employed. Formalin is generally used as a 10% solution.

TISSUE FIXATION

Factors affect fixation:

- Duration: The fixation time is dependent on sample size; for small biopsies 2-6 hours may suffice, but larger tissue samples usually require overnight fixation.
- Whole organs are usually sliced to ensure adequate fixation. If tissues are fixed in 10% formalin for 24 hours, then most of the fixative can be washed out in water. Although formaldehyde reacts rapidly with tissue components, the reaction is largely reversible.
- Prolonged fixation in formaldehyde is known to cause shrinkage and hardening of tissues..

FIXATION AND DECALCIFICATION OF BONE

- Bone specimens should be sawn into thin slices using fine tooth saws prior to decalcification.
- The method is usually carried out between the stages of fixation and processing and is essential for good section preparation and is used for bone and other tissues that may contain calcified areas.
- Several agents have been used to decalcify tissues, each having advantages & disadvantages.

FACTORS INFLUENCING DECALCIFICATION

- Concentration - this will affect the rate at which calcium is removed. The concentration will be depleted as it combines with calcium so large volumes and regular renewal is recommended.
- Temperature - increased temperature will speed up decalcification but also increase the rate of tissue damage.
- Agitation - gentle agitation may help to increase the rate.
- Fluid access - the decalcifier should have access to all tissue surfaces to enhance penetration and removal of calcium.

A GOOD DECALCIFYING AGENT MUST:

- * Complete remove the calcium
- * Have a reasonable speed of action
- * Cause minimal tissue damage
- * Produce non-impairment of subsequent staining