HISTOPATHOLOGICAL TECHNIQUE

Specimen collection (Sampling):

Sample or specimen means an organ or tissue which has a lesion.

General consideration during collection of specimen:

1- Sample must be collected as quickly as possible just after death to avoid PM changes.
2- You must collect organ or tissues without crushing or squeezing.
3- User a sharp scalpel or knife to avoid tearing of tissues.
4- Size of the sample is about 2X1X0.5 cm to allow rapid penetration of the fixative.
5- The sample should be including a part from the lesion with adjacent normal tissues to identify the organ.
6- Wash of the sample to facilitate the penetration of the fixative.
7- Amount of the fixative solution must be 15 – 20 times volume of the sample usually 10 times.
8- Clean wide mouth bottle enough for sample or in a plastic bottle.
9- The sample should be frozen only in case of neoplasm or histochemical study.

Fixation

Principle objects of the fixative:

1- Penetrate the tissue rapidly to prevent PM changes.
2- Harden the tissue by coagulates the contents of the cells into insoluble substance.

3- Protect tissue against shrinkage during dehydration and embedding and sectioning.

4- Preserve tissue elements by stopping tissue enzymatic pr system.

Most Common Fixative And Fixing Solutions

Alcohol:

- Recommended for glycogen in the animal tissues.
- **Formula** :
- 95 % or absolute alcohol (absolute alcohol 9 volume with formalin 1 volume).

Carnoy's fluid:

- Recommend for glycogen in the animal tissues especially if used at 3–5 hr
- **Formula** :
- Alcohol (absolute) 60 ml
- Chloroform 30 ml
- Glacial acetic acid 10 ml

Neutral formalin 10 %

- Formalin (formal) is a trade name for the liquid resulting from combination of formaldyde gas and water.
- Full strength of formalin is 37 to 40 %.
- **Formula**:
- Formaldyde 10 ml
- Distilled water 90 ml
- Formalin recommended for animal tissues and material.Also for frozen sectioning and for celloidin embedding.

Advantages of formalin :

1- Cheap and penetrate the tissues rapidly.
2- It does not cause over- hardening of the tissues even with long period of immersion.
3- It can be used for a variety of staining methods and can penetrated and preserve the fatty tissues.

Disadvantages of formalin:

- It has unpleasant odor , irritating effect especially to the eye , nasal mucosa and produce an allergic reaction to the skin of the hand.

1- Formation of formalin pigment (black or dark brown precipitates) derived from leaked hemoglobin.
2- Formalin is slightly acid and to maintain neutral reaction, calcium carbonate or lead oxide should be added in excess.

Buffered neutral formalin (pH 7):

- Recommended for pathological samples especially for hemoglobin and hemosiderin pigment with prevention of formalin pigments.
- **Formula**:
- Formalin 100 ml
- Sodium phosphate dibasic (unhydrous) 6.5 gm
- Sodium phosphate monobasic 4.0 gm
- Distilled water 900 ml
- Produce fixed tissues in the above for 1-2 days or longer.

Formalin ammonium bromide:

- Recommended for animal tissues and CNS.
- **Formula** :
- Formalin 15 ml
- Distilled water 85 ml

- Ammonium bromide 2 gm

Zenker's fluid:

- A popular fixative .
- **Formula** :
- Potassium dichromate 2.5 gm
- Mercuric chloride 5 gm
- Sodium sulfate 1 gm
- Distilled water 100 ml
- Glacial acetic acid 5 ml
- Add 5 ml of glacial acetic acid to 95 ml of the above solution just before use.
- Fixation time is about 24 hours.

Bouin's solution:

- **Formula**:
- Saturated aqueous solution of picric acid 750 ml
- Formalin 250 ml
- Glacial acetic acid 50 ml
- Fixation time about 4-12 hours according to the size of the specimens.

Washing

- Fixative must be removed to ensure proper staining.
- When use Zenker's fluid , you must wash in running water for 15-24 hours.
- After fixation in 10 % formalin, tissue must be washed carefully in water then placed in 70 % alcohol.
- When tissues fixed in Bouin's solution , excess fixative washed by 70 % alcohol.
- Tissues fixed in Carnoy's fluid are transferred directly into absolute alcohol.

Trimming

- The specimens are cut in the final form to demonstrate the lesions when sectioning.

Dehydration:

- It is the removal of the extracellular of free water from fixed tissues used gradual strengths of alcohol starting at 70 % and end at 100 % alcohol.
- Tissues should be gradual transferred from water to alcohol to avoid distortion of the tissues.
- N.B. Long treatment in the higher concentration of alcohol above 80 % makes the tissues brittle and difficult to cut while too long treatment in the lower dilution of alcohol under 70 % macerates the tissues.

Clearing

- It means production of transparency of the specimen by removal of the fluid from it
- **Clearing agents :**
- Xylene , toluene , cedar wood oil , chloroform , benzene , amyle acetone and methyl benzoate.

Embedding

- After clearing of the tissues, it is necessary to infiltrate the specimen into a supporting medium.
- Paraffin is widely used. It is usually used two or three serial transport at 45 – 50 oC for 1 hour to ensure infiltration.

Casting , blocking or embedding

- It is enclosing of the tissues in a solid mass of the embedding medium in a paper box (boat shape strip metal or plastic embedding molds) after filling of the boats with melted paraffin .
- The paper boat should be about twice in the thickness of the specimen and then transport to cold water bath.

Cutting and mounting of the paraffin sections

- By using of microtomes either rotatory , Cambridge , sliding or sledge microtome.

Method of decalcification

There are different methods of decalcification;

1- **Acid alcohol:**

Aqueous solution of 5 % Nitric acid :

Change the solution every day for 1-4 days.

2- **Formic acid:**

- **Solution 1:**

Formic acid	500 ml
Distilled water	500 ml

- **Solution 2:**

Sodium citrate	200 ml
Distilled water	1000 ml

- Using a combination of equal parts.