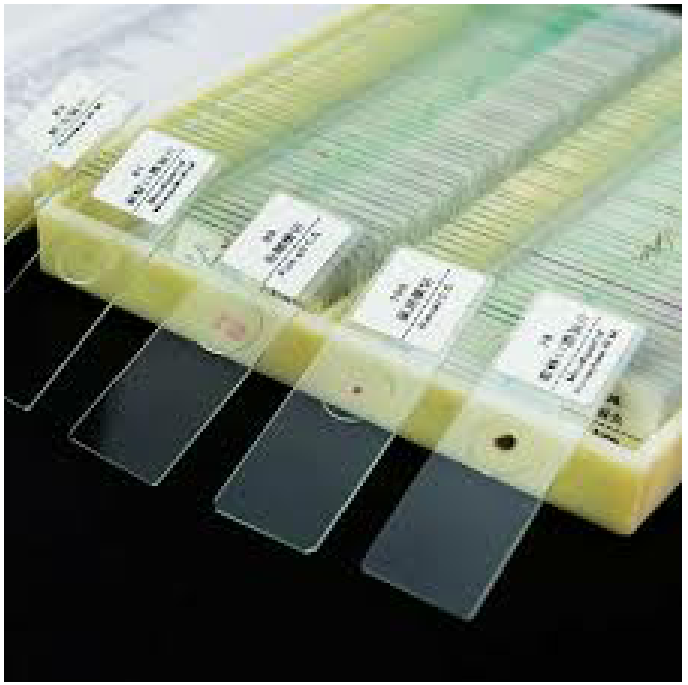


Preparation of tissue glass slide

- **Biopsy**:- its histological sample taken from living body (i.e through operation) in order to diagnose the disease. It should contain both the affected parts and intact parts of tissues, the latter should be have:-

1- appropriate size for fixation(1 cm)

2-fixed immediately without washing

3- make the term for it with sharp scalp and avoid any destruction to piece of tissue

Autopsy (necropsy):- histological sample from determined (dead body)

The steps for preparation

1- fixation: aim of fixation

A- prevent petrifaction of tissue by saprophytic bacteria

B- prevent autolysis by lysozymes

C- keep tissue components as possible as it in the living body

Types of fixation:

A- 10% neutral buffered formalin composed of

8.5gm sod. Chloride, 9.0 gm sod. phosphate monobasic(anhydrous), 6.5 gm sod. Phosphate dibasic, 37-40% formaldehyde solution, Distal water

B- ethyl alcohol

C- Carnoys solution

D- Bouins solution

2- processing of tissue sample:

These processes can be done automatically by histokinnete or manually , which include following steps

A- dehydration by gradual concentration of ethyl alcohol

70% ethanol for 2 hr , 80% ethanol for 2 hr , 90% ethanol for 2 hr,

95% ethanol for 2hr, 100% ethanol for 2hr

B- replacement of alcohol by xylol or (chloroform)

Xylene (or chloroform) for 2 hr

Xylene (or chloroform) for 2 hr

The aim of this step is predisposing tissue to next step

C- liquid paraffin (56 ° C) 2 hr

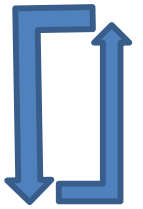
liquid paraffin (56 ° C) 2 hr

The aim of this step in order to penetrate components with paraffin

- **3- Blocking : done with**

- Liquid paraffin (56° C)

-2 of L shape metal pieces



- Stay in room temp. for 15 min
- Kept in refrigerator over night

- 4- Microtomy:
- The microtome 5-6 micrometer (thickness of tissue slices) micrometer= 1×10^{-6} meter

- water bath 37 ° C
- Brush the slide with a drop of egg albumin or glycerin
- Keeping slides in over 37 ° C over night or 60 ° C for 2 hr

5- Staining:

A- removing paraffin from tissue by

- Xylene 1-30 min
- Xylene 1-30 min

B- Rehydration by:

100% ethyl alcohol → 2 min

90% ethyl alcohol → 2 min

80% ethyl alcohol → 2 min

70% ethyl alcohol → 2 min

D.W for washing 2 min.

C- Staining with routine stain hematoxylen and eosin(H& E)

- **hematoxylen** for 15 min give acid structure(nuclei) blue color

Washing with tap water 10 min clear

Acid alcohol just quick dipping 1 or 2 dip (1 part of acetic acid in 99 part of 100% ethyl alcohol)

- **Eosin** for only 5 min give enoplasmic reticulum red color

Clearing by

Xylene 15-30 min

Xylene 15-30 min

Covering with cover slips: put small drop of canada balsam on clear cover slip