Histopathological techniques

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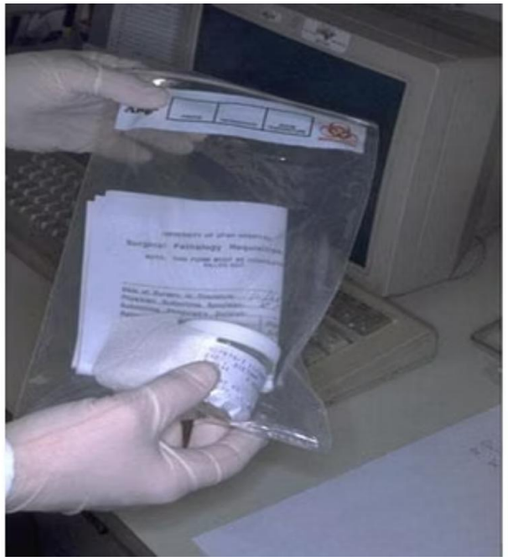
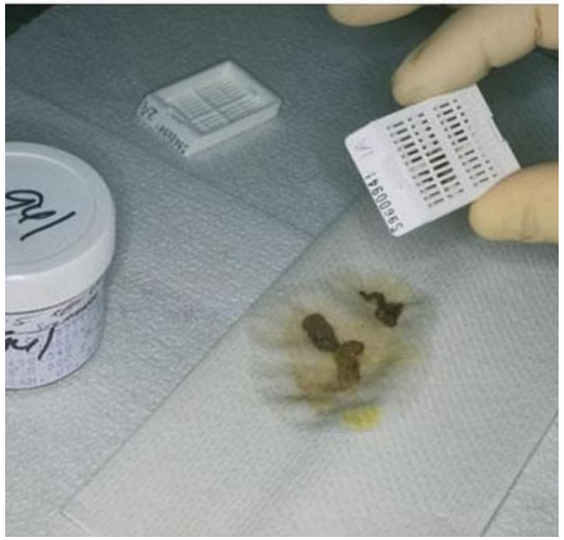
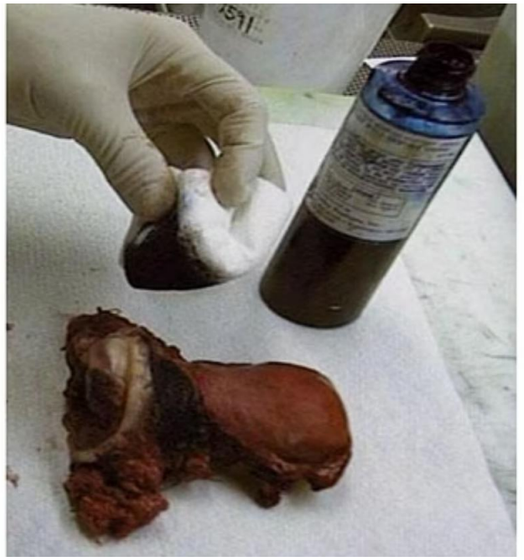
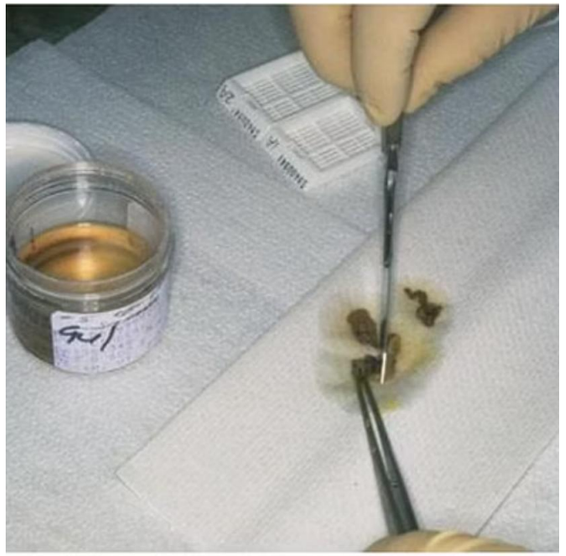
Tissue Preparation for Light Microscope

Tissue specimens received in the surgical pathology laboratory have a request form that lists the patient information and history along with a description of the site of origin.









1- Fixation

Purpose

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

Timing

Fixation should be carried out as soon as possible after removal of the tissues to prevent autolysis.

Best Practice

There is no perfect fixative, though formaldehyde comes the closest.

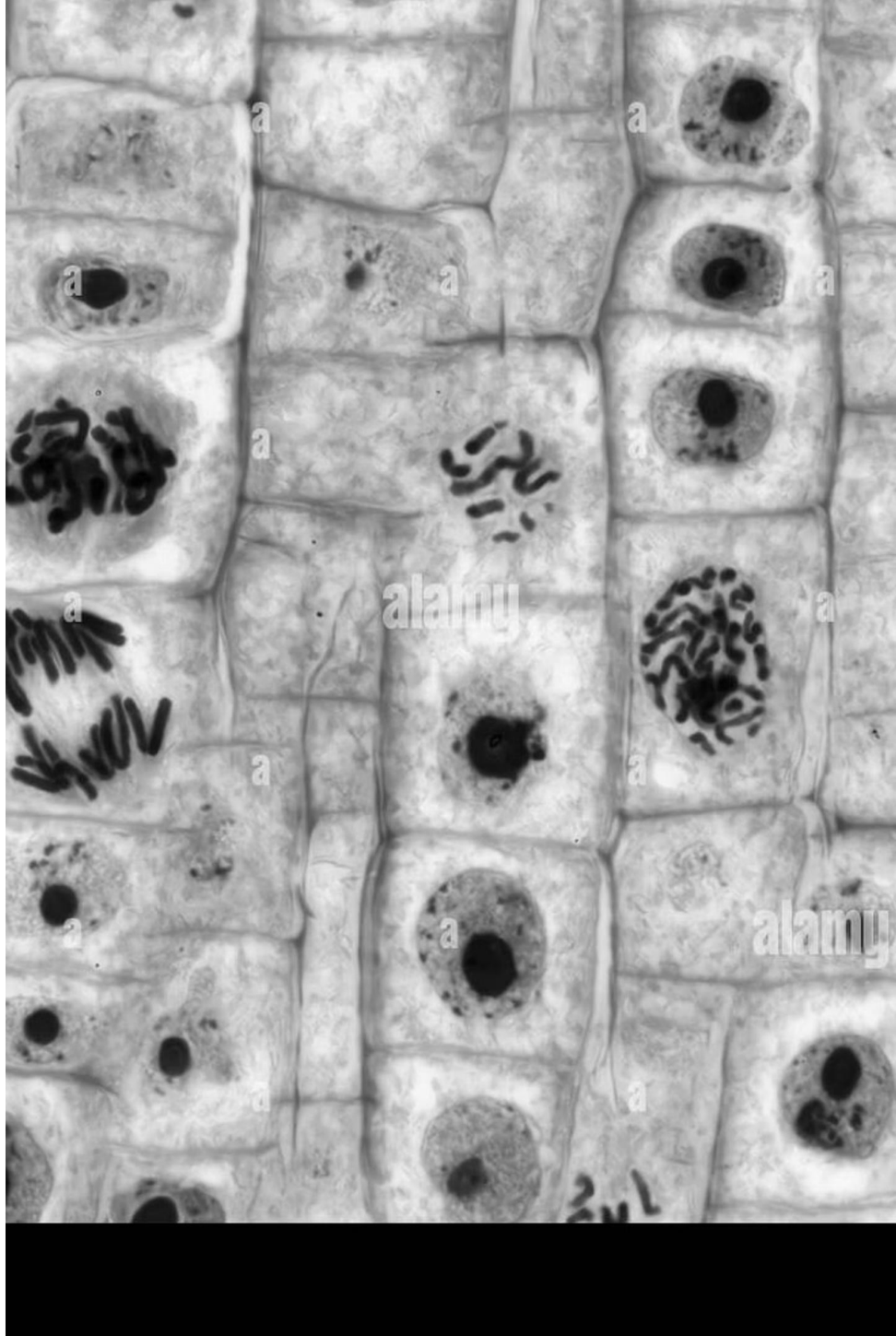
Variety

Therefore, a variety of fixatives are available for use, depending on the type of tissue present and features to be demonstrated.

Types of fixatives

There are five major groups of fixatives, classified according to mechanism of action:

- Aldehydes
- Mercurials
- Alcohols
- Oxidizing agents
- Picrates



Aldehydes:

Include formaldehyde (formalin) and glutaraldehyde.

- It is good for immuno-histochemistry techniques.
- Formalin penetrates tissue well, but is relatively slow.
- The standard solution is 10% neutral buffered formalin.
- Mercurials fix tissue by an unknown mechanism.
- They contain mercuric chloride and include such well-known fixatives as Zenker's.
- These fixatives penetrate relatively poorly and cause some tissue hardness, but are fast and give excellent nuclear detail.
- Alcohols: including methyl alcohol (methanol) and ethyl alcohol (ethanol).
- However, they are very good for cytologic smears because they act quickly and give good nuclear detail.

Oxidizing Agents:

Include permanganate fixatives (potassium permanganate), dichromate fixatives (potassium dichromate), and osmium tetroxide.

Picrates:

include fixatives with picric acid.

Foremost among these is Bouin's solution.

2-Factors affecting fixation

There are a number of factors that will affect the fixation process:

01

Buffering

Fixation is best carried out close to neutral pH, in the range of 6-8.

02

Penetration

6-8 tissues depends upon the diffusability of each individual fixative, which is a constant.

03

Volume

The volume of fixative is important. There should be a 10:1 ratio of fixative to tissue.

04

Temperature

Increasing the temperature, as with all chemical reactions, will increase the speed of fixation.

05

Concentration

Concentration of fixative should be adjusted down to the lowest level possible.

06

Time interval

Also very important is time interval from removal of the tissues to the fixation.

Tissue Processing

The technique of getting fixed tissue into paraffin is called tissue processing. The main steps in this process are dehydration and clearing.

1. Dehydration

Gradual removal of water from the tissue using ascending grads of ethyl alcohol to prevent tissue shrinking.

2. Clearing

Replacement of alcohol in tissue by clearing fluid like xylene, benzene, or acetone.

3. Embedding:

Tissues are impregnated in paraffin

4. Cutting:

Paraffin block are cut by microtome using metal knife, into thin sections ~ 6µ

6. Mounting:

Sections spread on the hot plate and mounted on glass slides.

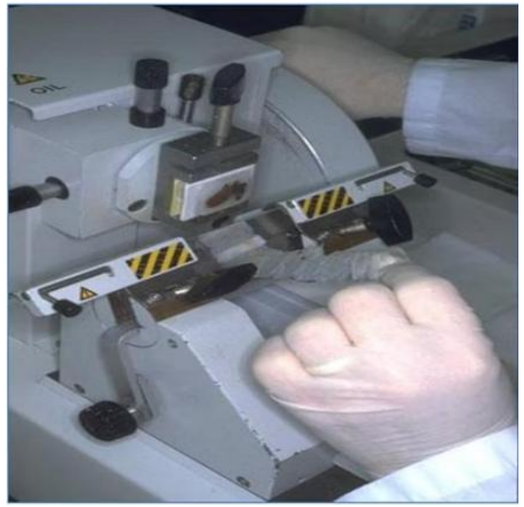
7. Staining:

Variable stains are used for specific tissues.

Automated tissue processor

Sectioning

- Once the tissues have been embedded, they must be cut into very thin sections (4 to 6 microns) that can be placed on a slide.
- This is done with a microtome. The important thing for proper sectioning is a very sharp knife.



Frozen Sections

- Frozen sections are performed with an instrument called a cryostat.
- The cryostat is just a refrigerated box containing a microtome.
- The temperature inside the cryostat is about -20 to -30 C.
- The tissue sections are cut and picked up on a glass slide.
- The sections are dried and then stained.

Staining

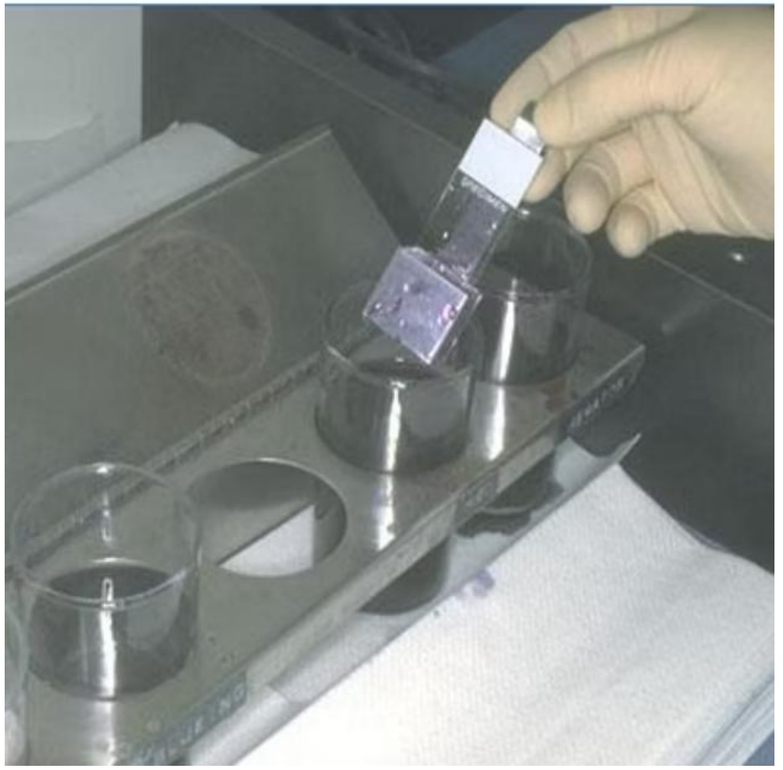
The embedding process must be reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections.

Therefore, before any staining can be done, the slides are "deparaffinized" by running them through xylene then, to alcohols and lastly to water.

There are no stains that can be done on tissues containing paraffin.

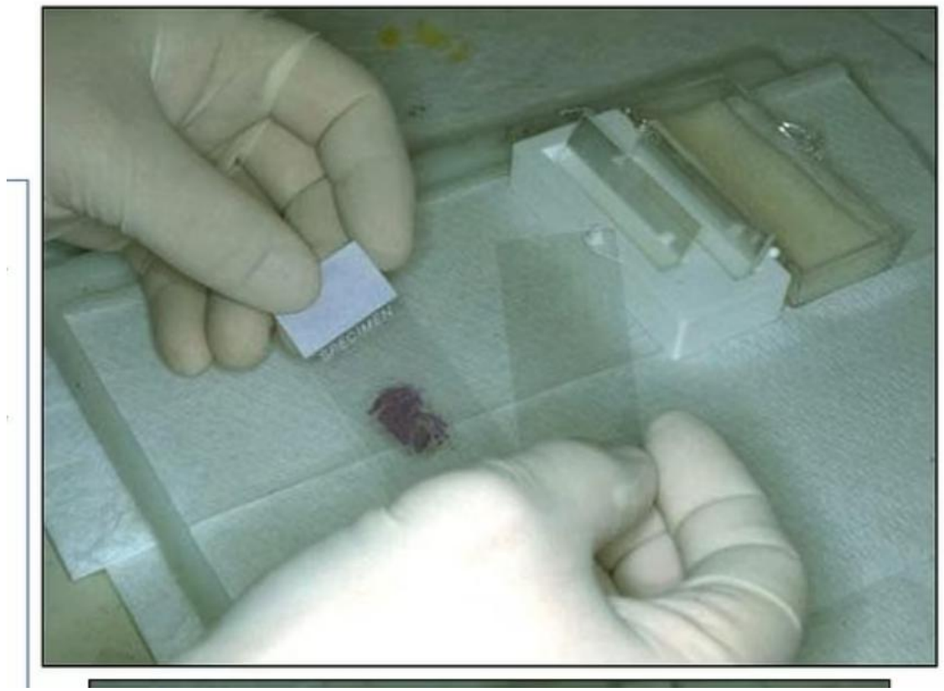
Automated stainer

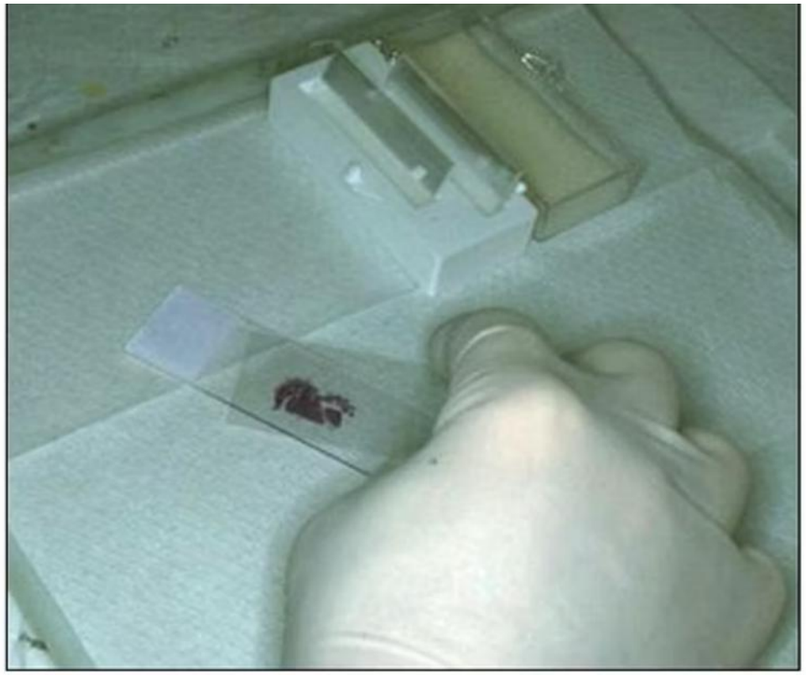
Frozen sections are stained by hand, because this is faster for one or a few individual sections.



Coverslipping

The stained section on the slide must be covered with a thin piece glass to protect the tissue from being scratched, and to preserve the tissue section for years to come.





procedure

01

Deparaffinize

Deparaffinize the section : flame the slide on burner and place in the xylene. Repeat the treatment.

02

Hydration

Hydrate the tissue section by passing through decreasing concentration of alcohol baths and water. (100%, 90%, 80%, 70%)

03

Hematoxylin

Stain in hematoxylin for 3-5 minutes

04

Wash

Wash in running tap water until sections "blue" for 5 minutes or less.

05

Differentiate

Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 5 minutes.

06

Blue again

Wash in running tap water until the sections are again blue by dipping in an alkaline solution (eg. ammonia water) followed by tap water wash.

07

Eosin

Stain in 1% Eosin Y for 10 minutes

08

Rinse

Wash in tap water for 1-5 minutes

09

Dehydrate

Dehydrate in increasing concentration of alcohols and clear in xylene

10

Mount

Mount in mounting media

11

Observe

Observe under microscope