Pathology Lab

Tissue processing

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By :

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Definitions:

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.

Clinical pathology: It is the bridge between science and medicine. It underpins every aspect of patient care, from diagnostic testing and treatment advice to using cutting-edge genetic technologies and preventing disease.

Histo-techniquse(tissue processing) : 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 .

Etiology: in the fields of biology and medicine, refers to the **cause or causes** of a disease. Etiologies of disease may be intrinsic, extrinsic or idiopathic.

Pathogenesis: is the process by which a disease or disorder develops. It can include factors which contribute to the onset of the disease or disorder and to its progression and maintenance. The word comes from Ancient Greek (pathos) 'suffering, disease' and (genesis) 'creation'.

Biopsy It is a medical procedure involving the removal of cells or tissues from the body for the histopathological examination.
This piece of tissue or organ taken from a living human being is generally examined under microscope by a pathologist.

A variety of biopsy techniques can be applied:

- When only a sample of tissue is removed, the procedure is called an **incisional** biopsy or core biopsy (a wedge of tissue is taken).
- When an entire mass or suspicious area is removed, the procedure is called an **excisional** biopsy.
- When a sample of tissue or fluid is removed with needle, the procedure is called a **needle biopsy(core needle biopsy)** also a sample can be collected by devices that bite a sample.

A clear margins or **negative margins** means that no disease was found at the edge of biopsy specimen.
A **positive margins** means that disease was found, and wider excision may be needed, depending on the diagnosis.

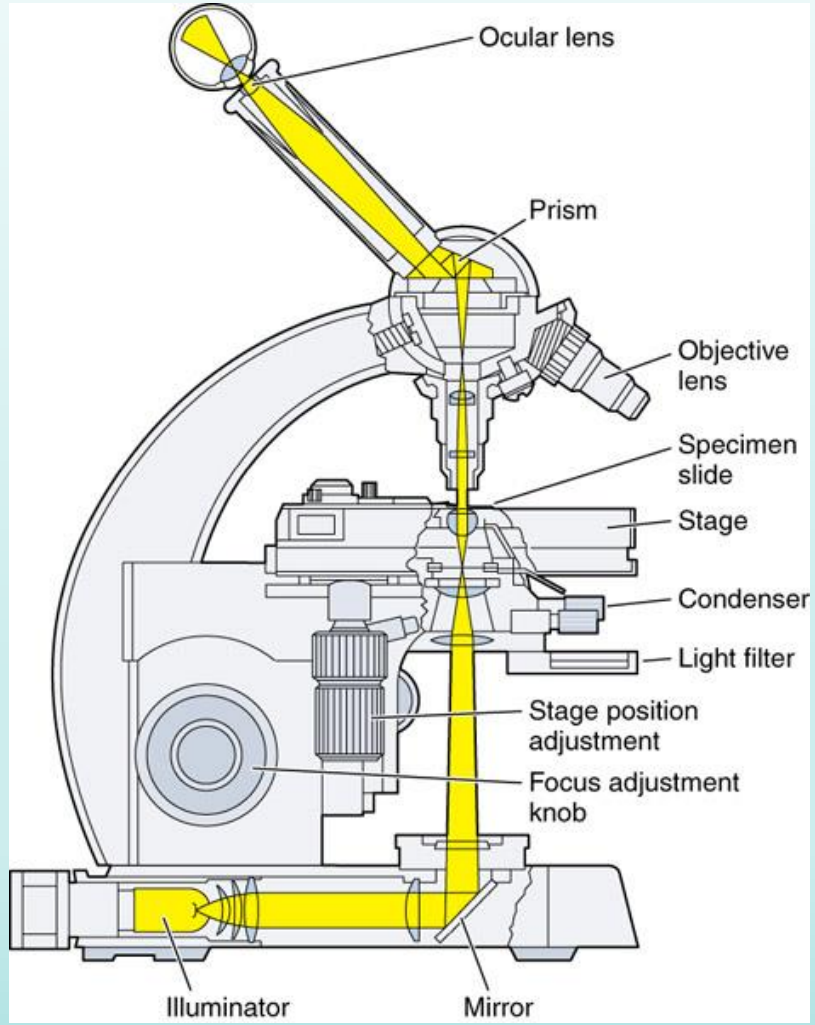
Autopsy: It is a piece of tissue or organ taken from dead body for histological diagnosis, this medical procedure consist of a thorough examination of corpse to determine the cause and manner of death and to evaluate any disease or injury that may be present.
It is usually performed by a specialized medical doctor called a pathologist.

Light Microscopy

It is a type of microscope that commonly uses visible light and a system of lenses to generate magnified images of small objects.

Parts of light microscope

1- Eyepiece – Its standard magnification is 10x
2- Eyepiece tube – it's the eyepiece holder.
3- Objective lenses – These are the major lenses used for specimen visualization. They have a magnification power of 40x-100X. There are about 1- 4 objective lenses placed on one microscope, each lens has its own magnification power.
4- The Adjustment knobs –Fine adjustment knobs and coarse adjustment knobs.
5- Stage – This is the section on which the specimen is placed for viewing.
6- Aperture – This is a hole on the microscope stage, through which the transmitted light from the source reaches the stage.
7- Microscopic illuminator – This is the microscopes light source
8- Condenser – These are lenses that are used to collect and focus light from the illuminator into the specimen.
9-Diaphragm
10-The rack stop – It controls how far the stages should go preventing the objective lens from getting too close to the specimen slide which may damage the specimen.



Light Microscope

5-Headed LM has lighted pointer

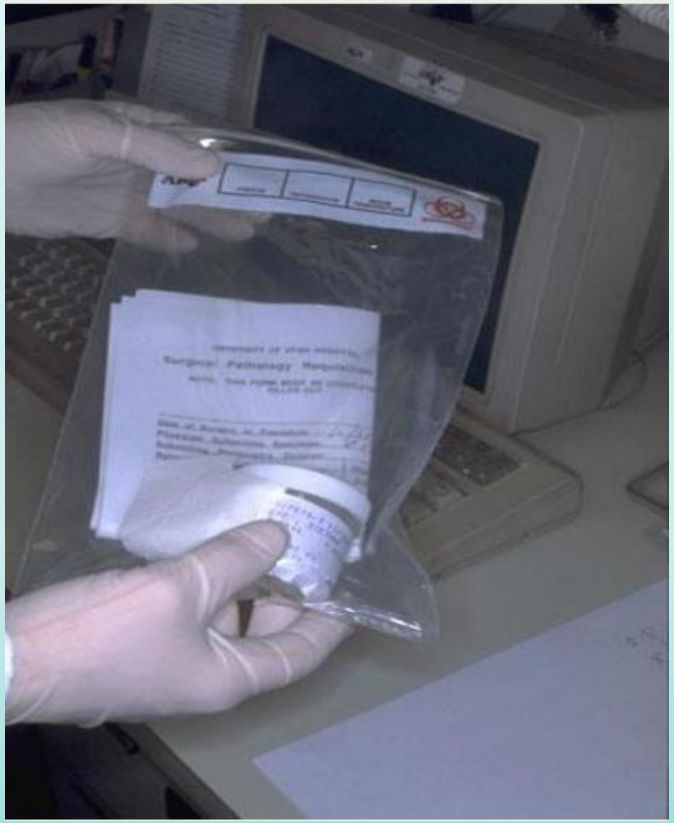
2-Headed LM
Has video camera and computer with frame grabber to view/print/save images

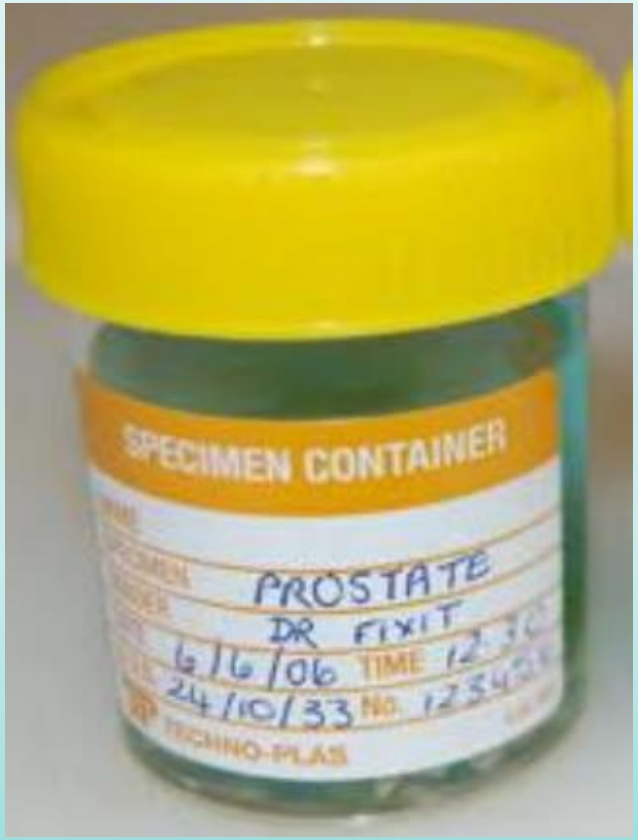
Protocols followed in Histo-techniques (tissue processing)

1- Receipt & Identification.
2- Labeling of the specimen with numbering in pathology laboratory
3- Fixation.
4-Gross examination and dissectioning.
5- Washing and Dehydration.
6- Clearing.
7- Embedding.
8- Sectioning or cutting .
9-De-wax and rehydration
10-Staining.
11-Mounting and cover sliping

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 in pathology laboratory

The specimen are labelled by giving them a number that will identify each specimen for each patient.

3- Fixation :

It is a chemical process by which biological tissues are preserved from decay.

The purpose of fixation:

1- A fixative usually acts to disable intrinsic biomolecules- particularly proteolytic enzymes – which would otherwise digest or damage the sample.

2- Fixatives are toxic to most common microorganism (bacteria in particular) which might exist in a tissue sample or which might otherwise colonize the tissue.
In addition, many fixatives will chemically alter the fixed material to make it indigestible or toxic to opportunistic microorganisms.

3- A fixative will typically protect a sample from extrinsic damage that it alters the cells or tissues on a molecular level to increase their mechanical strength or stability. This increased strength and rigidity can help preserve the morphology (shape and structure) of the tissue being sampled and prevent damage during subsequent procedures
Fixation takes place by converting the fluid cytoplasm into an irreversible gel.

Most common fixative used in histopathology is formaldehyde solution (10% formaldehyde)

Other fixatives include Bouin's, Zenker's solutions and alcohol

The fixative should be 10 times more in volume than the specimen.

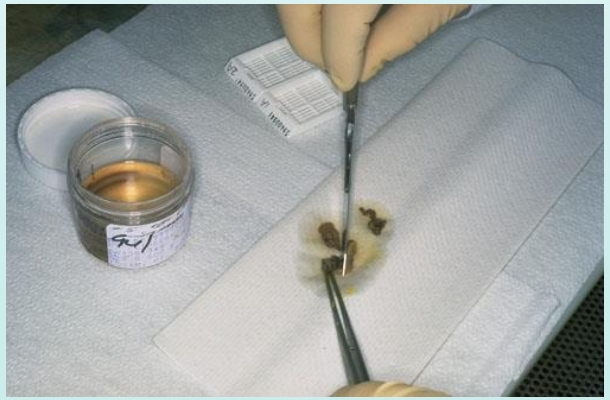
Specimens should be fixed for approximately 6 to 72 hours.

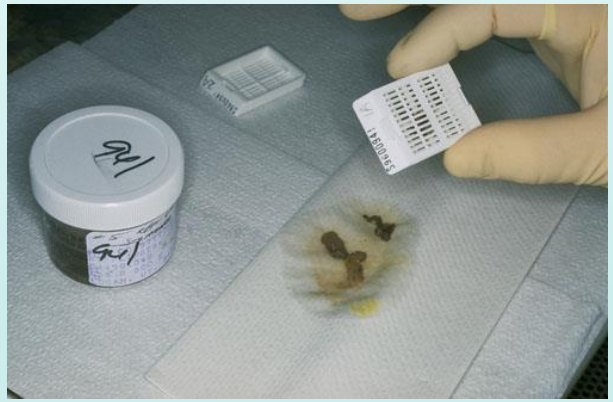
Overnight fixation (i.e. 8-12 hours) is generally indicated for 10 mm thick slices of tissues.

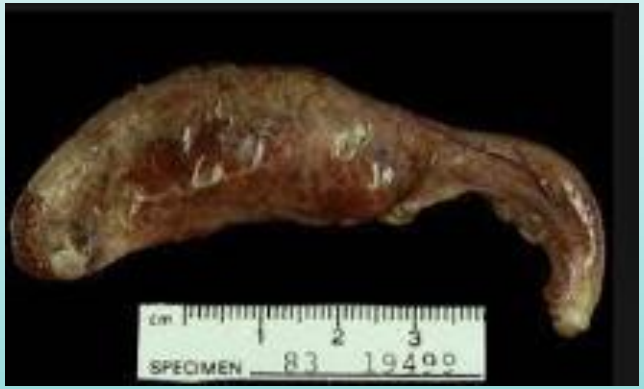
4-Gross examination and tissue dissection:

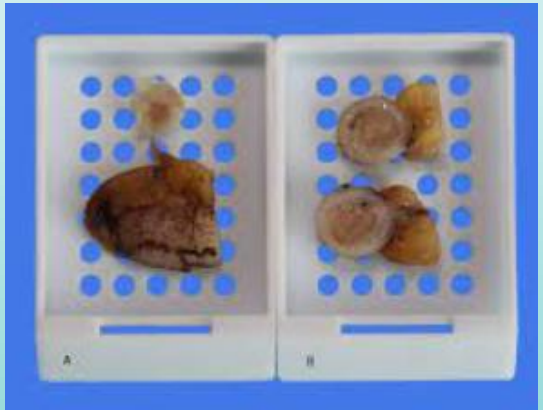
Each organ or tissue has a special procedure in dissectioning:

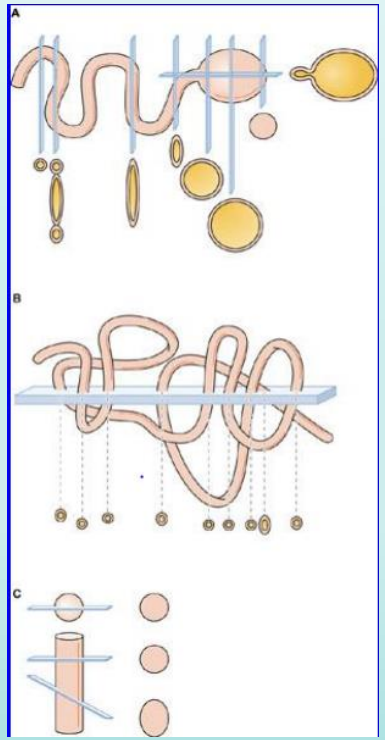
- Tissue dissection and then taking representative sections followed by labeling .
- The pieces taken will placed in tissue cassette .
- Transfer cassette either to automatic tissue processor or to jars (in case of manual tissue processing) to **complete fixation and then to start tissue processing**







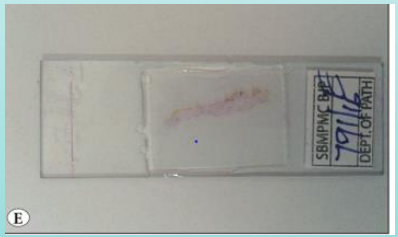




Representative sections taken

When a malignancy is suspected, then the specimen is often covered with ink in order to mark the margins of the specimen.

Different colored inks can be used to identify different areas if needed. When sections are made and processed, the ink will mark the actual margin on the slide.



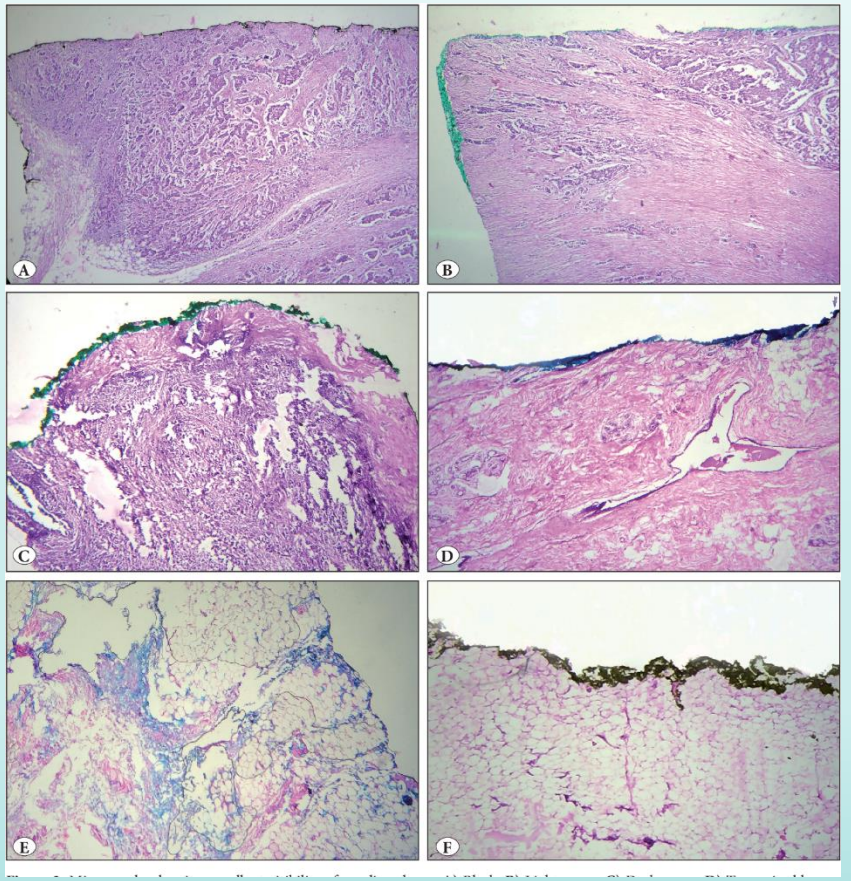


Figure 1: A) Acrylic colours and paint brushes. **B)** Inking of gross specimen by acrylic colours. **C)** Contamination of processing fluid by acrylic yellow colour. **D)** Visibility of acrylic colours on blocks. **E)** Visibility of acrylic colours on naked eye examination of slide.

Once the tissue has been fixed, it must be processed into a form in which it can be made into thin microscopic sections.

The usual way this is done is with paraffin. Tissues embedded in paraffin, which is similar in density to tissue, can be sectioned at anywhere from 3 to 10 microns, usually 3-5 routinely.

The technique of getting fixed tissue into paraffin is called tissue processing.

The main steps in this process are dehydration and clearing.

Tissue processing is achieved either by

Manual tissue processing Or automated tissue processing

5- Dehydration

Removal of water from the tissues because water is not miscible with wax.

Wet fixed tissues cannot be directly infiltrated with paraffin.
First, the water from the tissues must be removed by dehydration.
This is usually done by a series of alcohols, 70% to 95% to 100%.

6- Clearing :

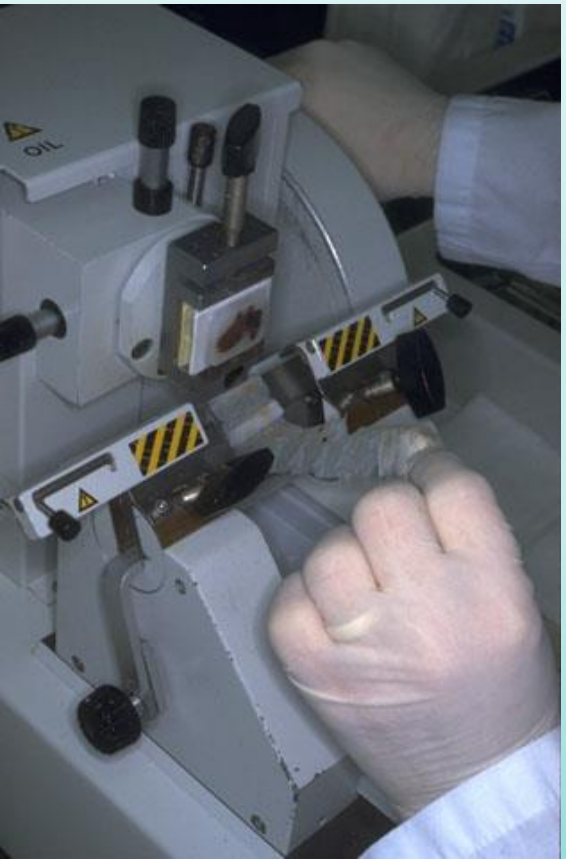
The next step is called "clearing" and consists of removal of the dehydrant (alcohol) with a substance that will be miscible with the embedding medium (paraffin).
The commonest clearing agent is xylene , it is cheap and rapid in action, though tends to harden tissue on prolonged application.

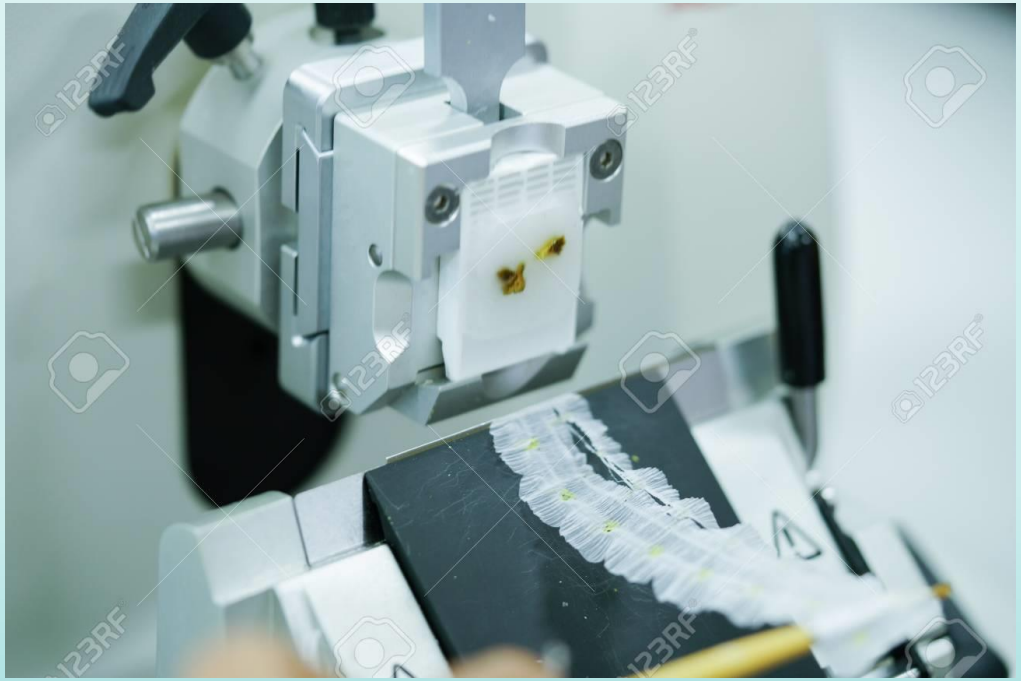
7- Embedding:

Tissues that come off the tissue processor are still in the cassettes and must be manually put into the blocks by a technician who must pick the tissues out of the cassette and pour molten paraffin over them.
This "embedding" process is very important, because the tissues must be aligned, or oriented, properly in the block of paraffin.

8- Cutting or sectioning :

Once the tissues have been embedded, they must be cut into sections that can be placed on a slide. This is done with a **microtome.** The microtome has a very sharp knife with a mechanism for advancing a paraffin block standard distances across it





Adhesion of the tissue section to slide:

This is usually done by thinly smearing with egg albumin and picking the section up of the water by the slide inclined in a 45 degree angle.

Picking sections up from water bath.

Unstained section on glass slide.

9- Drying or De-waxing in the oven and Rehydration:

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 xylenes (or substitutes) to alcohols to water.

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

De-waxing done at temp. 65 C, this temperature depends on the melting point of wax.

The slides then should be transferred to a jar containing a fat solvent (xylene or toluene).

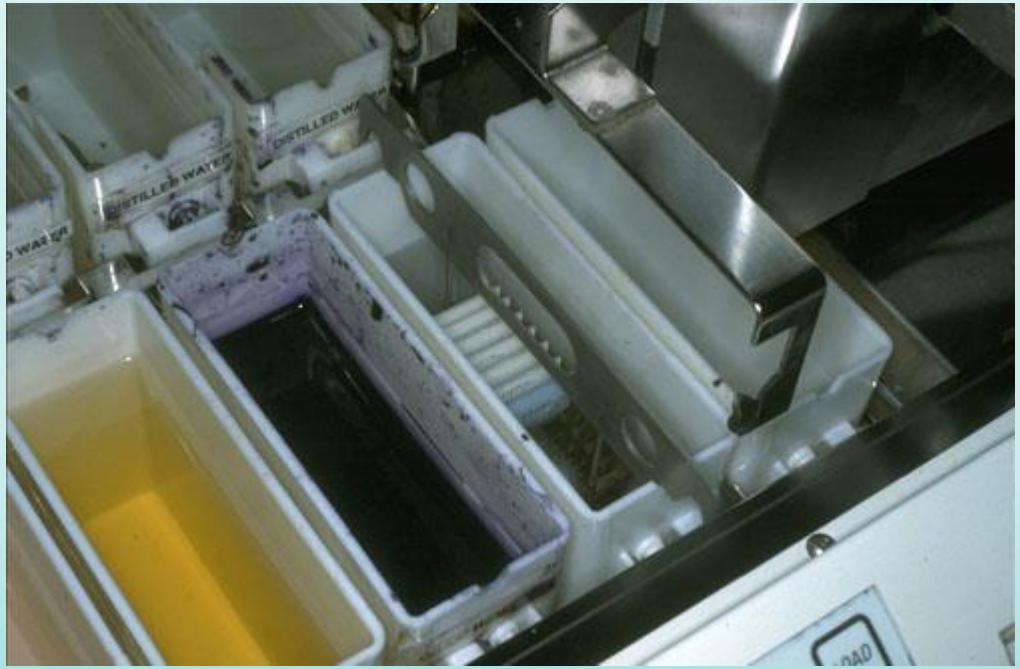
While **Rehydrationdone** by opposite to the above step of dehydration

10- Staining:

The staining process makes use of a variety of dyes that have been chosen for their ability to stain various cellular components of tissue.

The routine stain is that of **hematoxylin and eosin (H and E).**

Other stains are referred to as "special stains" because they are employed in specific situations according to the diagnostic need.

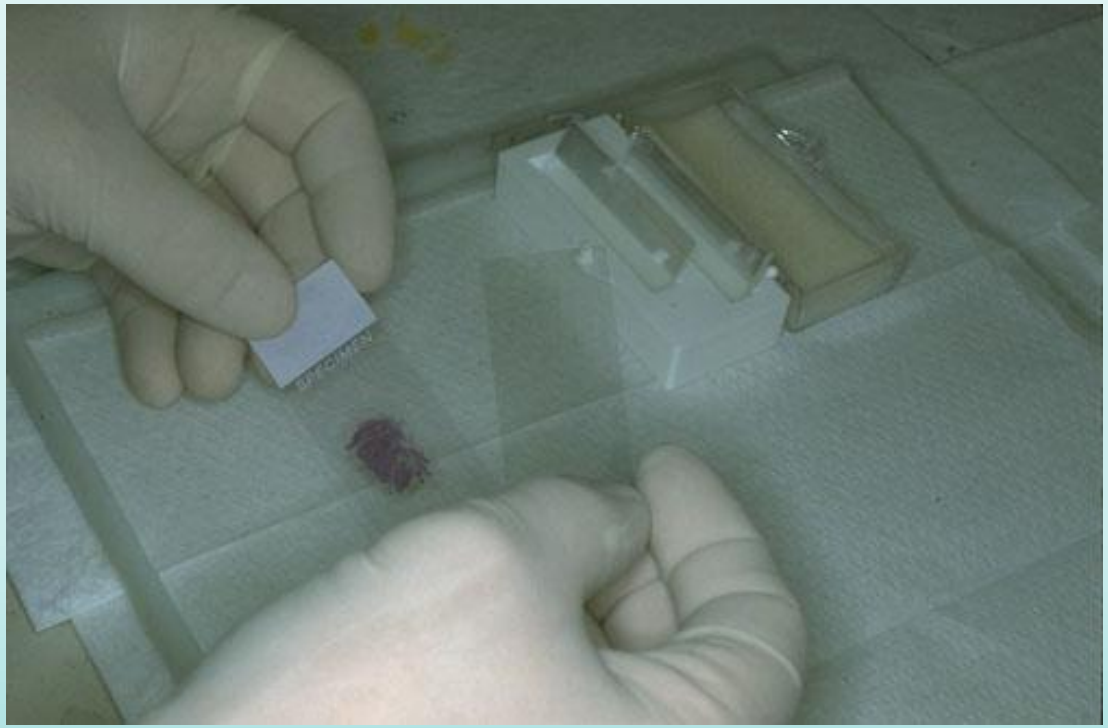


Slides being stained on automated stainer.

11- Mounting the slide and cover sliping :

With Canada balsam or DPX, then covered with a cover slid to fix the slide, which is then given a label with a number.

Mounting the slide



Coverslipping

Frozen section technique:

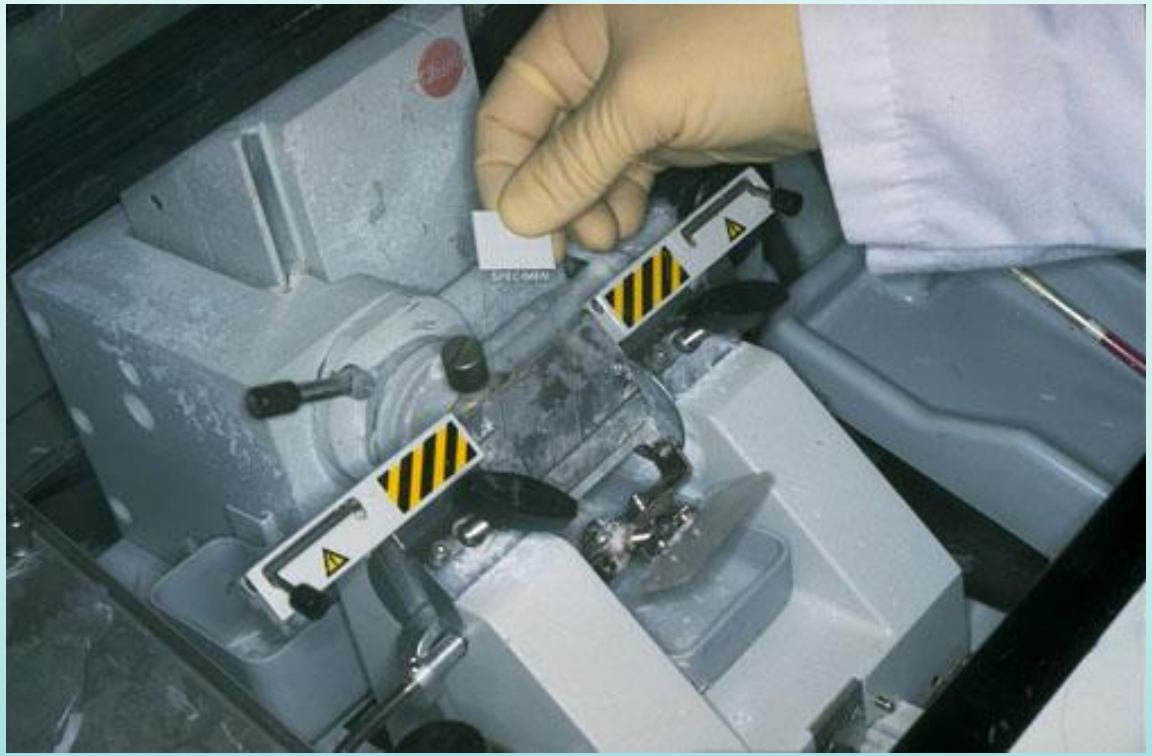
This is used for on the spot diagnosis, identification of lipid or certain enzymes. Usually fresh unfixed tissue is utilized.

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 Celsius.

The tissue sections are cut and picked up on a glass slide. The sections are then ready for staining.The section is cut at thickness of 5-10 microns.

A rapid H. and E. stain is used for these sections.



Cutting a frozen section.

Staining a frozen section.

Decalcification of bone :

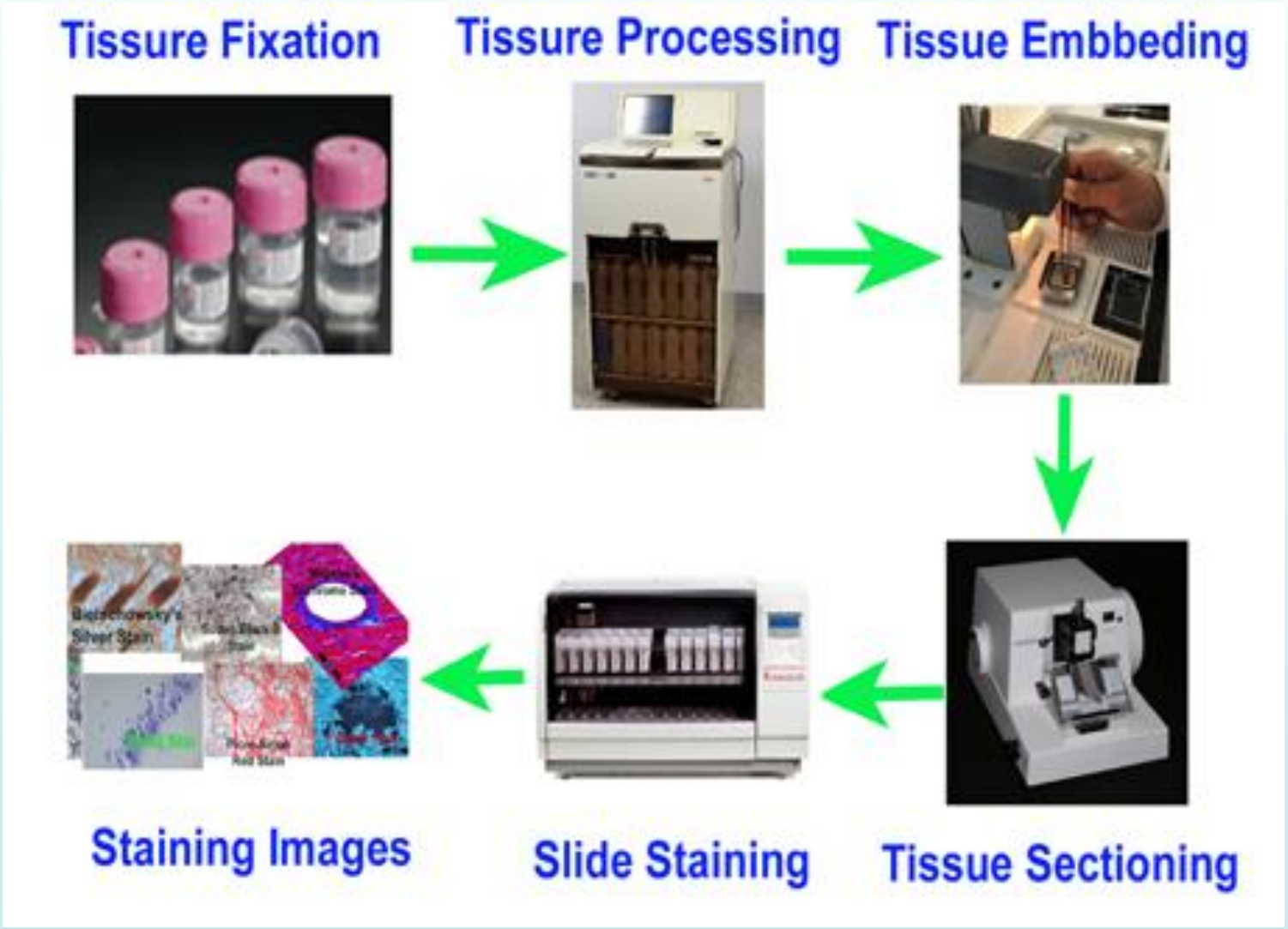
It is sometimes necessary to remove deposits of calcium from tissue e.g. calcified arteries, bones teeth, etc.

This should be done immediately after fixation

fixatives containing mercuric chloride are said to cause swelling of soft tissue during decalcification.

If a large piece is submitted, cut into small pieces with a saw (not exceeding 5mm. in thickness)

Suspend the tissue in decalcification fluid ,the best one is (**nitric acid 5-10%).**


Tissure Fixation
Tissure Processing
Tissue Embbeding
Tissue Sectioning
Slide Staining
Staining Images

Thank you