Course :Human Pathology

HISTOPATHOLOGY TECHNIQUES

Dr. K. Premkumar
Associate Professor
Dept of Biomedical Science
Bharathidasan University

istopathology

It is the branch of science which deals with the gross and microscopic study of tissue affected by disease

Tissue for study can be obtained from

- Biopsies
- Autopsies

issue rocessing

- Fixation
- Dehydration
- Embedding
- Sectioning
- Frozen Sections
- Clearing
- Staining

ixation

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

- Fixation should be carried out as soon as possible after removal of the tissues (in the case of surgical pathology) or soon after death (with autopsy) to prevent autolysis.

- A variety of fixatives are available for use, depending on the type of tissue present.

imple ixative- ormalin

- The Most commonly used fixative is Formalin
- It is prepared by mixing 40% Formaldehyde in 100w/v of distilled water/saline buffer.

Mechanism of Action:

- It forms cross links between amino acids of proteins thereby making them insoluble
- It fixes 4mm thick tissue in 8 hours

ther imple ixatives

- Glutaradehyde
- Osmium Tetraoxide
- Pottasium Dichromate
- Mercuric Chloride
- Picric Acid
- Zenker's Fluid
- Zenker's Formal
- Bouin's Fluid

ixation - factors affecting fixation

- There are a number of factors that will affect the fixation process:
- Buffering
- Penetration
- Volume
- Temperature
- Concentration
- Time interval

ehydration

- It is the process in which the water content in the tissue to be completely reduced passing the tissue through increasing concentrations of dehydrating agents
- The various dehydrating agents used are

a. Ethyl alcohol
b. Acetone
c. Isopropyl alcohol
d. Dioxane

- Tissues are dehydrated by using increasing strength of alcohol
- The duration for which tissues are kept in each strength of alcohol depends upon the size of tissue, fixative used and type of tissue.
- The volume of alcohol should be 50- 100 times that of tissue

ehydration rocedure

- The duration of the procedure can be noted
- 50% alcohol- 1 hour
- 70% alcohol- 1 hour
- 80% alcohol- 1 hour
- 90% alcohol- 1 hour
- Absolute alcohol- 1 hour
- Absolute alcohol- 1 hour
- Absolute alcohol- 1 hour
- Dehydration is done so that the wax i.e Paraffin wax which is used for impregnation, can be easily miscible as it is immiscible with water

ypes of ax

- 1. Paraffin wax
- 2. Paraplast
- 3. Gelatin
- 4. Cellodin

Paraffin wax is used routinely. It has hard consistency, so section of 3-4 micron thickness can be cut.

mbedding rocedure

- Select the mould, there should be sufficient room for the tissue with allowance for at least a 2 mm surrounding margin of wax.
- Fill the mould with paraffin wax.
- Using warm forceps select the tissue, taking care that it does not cool in the air; at the same time

- Chill the mould on the cold plate, orienting the tissue and firming it into the wax with warmed forceps. This ensures that the correct orientation is maintained and the tissue surface to be sectioned is kept in flat.
- Insert the identifying label or place the labeled embedding ring or cassette base onto the mould.
- Cool the block on the cold plate, or carefully submerge it under water when a thin skin has formed over the wax surface.
- Remove the block from the mould.
- Cross check block, label and worksheet

ection utting

- It is the procedure in which the blocks which have been prepared are cut or sectioned and thin strips of varying thickness are prepared
- The instrument by which this done is called as a Microtome

Types of Microtomes

a. Sliding
b. Rotary
c. Rocking
d. Freezing
e. Base Sledge

rozen ections

- 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.

rozen ections

- Sections are prepared quickly for histological examination by freezing the tissue. The section should be thin, and without water crystals. It is an important procedure for quick diagnosis.

rocedure

- Tissue must reach histopathology laboratory immediately.
- To avoid tissue being dried it should be kept in saline.
- The size of the tissue should be small thin, so that good smooth sections can be obtained and freezing is quick.
- Thickness of the tissue should be about 3mm to 4mm.The tissue can directly be taken to cryostat or can be fixed with 10% formalin or formol – alcohol

Contin..

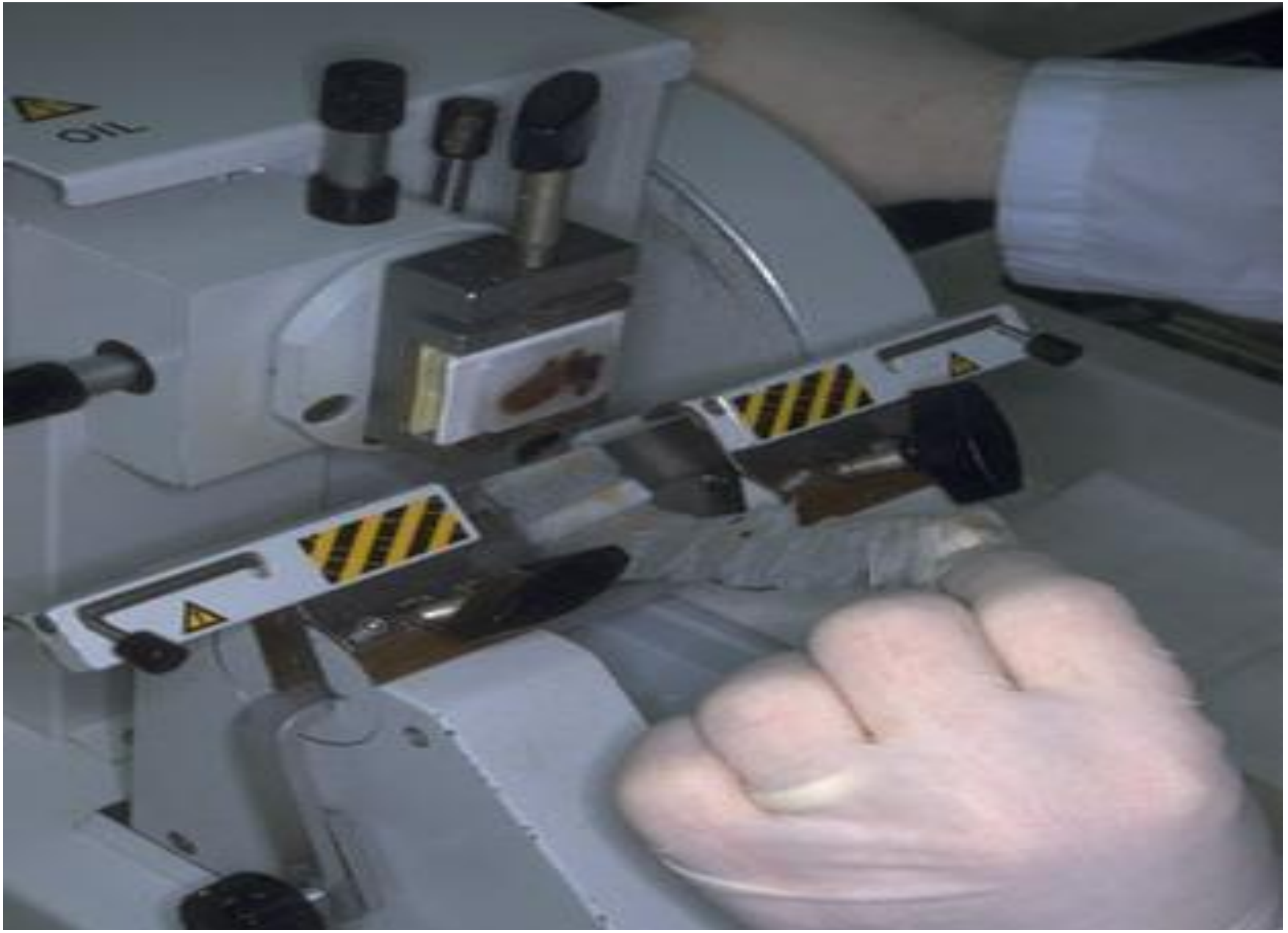
- Sucrose (20%) or a drop of water may be applied on the chuck. Optimum Cooling temperature (OCT) compounds or 20% sucrose gives good result.
- Other embedding media are available with cryostat.
- Completion of freezing is observed by the change of color of tissue which turns glossy white.
- Freezing should be done fast. This will prevent ice crystal formation. The morphology is better preserved and artifacts are less.

ectioning tissues in \otary . icrotome

- Turn on the water bath and check that the temp is 35-37ºC.
- Use fresh deionized water.
- Blocks to be sectioned are placed face down on an ice block or heat sink for 10 minutes.
- Place a fresh blade on the microtome.
- Insert the block into the microtome chuck so the wax block faces the blade and is aligned in the vertical plane. Set the dial to cut 4-10 µM sections.
- The blade should angled 4-6º.

- Face the block by cutting it down to the desired tissue plane and discard the paraffin ribbon.
- If the block is ribboning well then cut another four sections and pick them up with forceps or a fine paint brush and float them on the surface of the 37ºC water bath.
- Float the sections onto the surface of clean glass slides.

- If the block is not ribboning well then place it back on the ice block to cool off firm up the wax.
- If the specimens fragment when placed on the water bath then it may be too hot.
- Place the slides with paraffin sections in a 65°C oven for 20 minutes (so the wax just starts to melt) to bond the tissue to the glass.
- Slides can be stored overnight at room temperature


OIL

learing

The next step alcohol should be replaced by paraffin wax.

- As paraffin wax is not alcohol soluble, we replace alcohol with a substance in which wax is soluble.
- This step is call clearing.
- Clearing of tissue is achieved by any one of the following reagents:

a. Xylene
b. Chloroform
c. Benzene
d. Carbon tetrachloride
e. Toluene

leaning, \ehydration and taining rocedure

- Deparaffinization with xylene 1hour
- Hydration - 100% - 70% alcohol – each 5min
- Wash under water
- Stain with Haematoxylin for 15 min
- Wash with water
- Wash with 70% - 90% alcohol for each 10 min
- Stain with 1% Eosin for 2min
- Wash with water
- Dehydration with 90% - 100% alcohol
- Clearing with xylene
- Dry
- Mount

- The Nucleus stain Blue
- The cytoplasm stain pink