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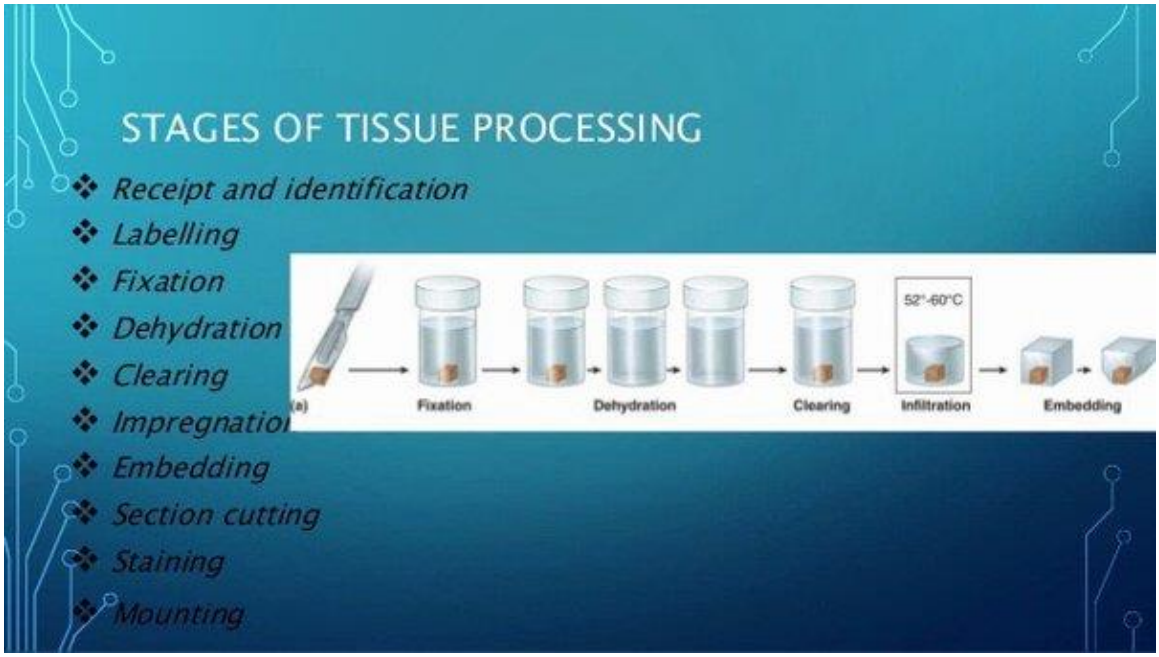
M.Sc. Pathology

Tissue processing: A procedure which needs to take place after gross examination between tissue fixation and the embedding and then sectioning of paraffin blocks is called tissue processing.



•There are main 2 steps in preparation of slide:

1. Sectioning of tissue

2. Staining of tissue

•1.Sectioning of tissue is 2 Types

•A. Frozen sectioning

•B. Paraffin sectioning

•2.**Staining of tissue are 2 types**

•A. **Routine stain**: a. Hematoxylin and Eosin staining (H and E stain)

•b. Papanicolaou staining (Pap's stain)

•B. **Special stain**: PAS stain ,AFB stain, Grocotts stain ,Argentaffin and argyrophilic stain,Amyloid stain,Reticulin stain,Trichome stain ,PTAH stain ,Pearl stain ,Fontona masson stain ,Vonkossa stain ,Oil-red-o stain ,Mucin stain ,Giemsa stain ,Elastic stain ,Myelin stain.

A. Frozen sectioning: is a rapid way to fix and mount histology section using a refrigerator device called cryostat. A modified rotatory microtome housed in a refrigerated cabinet. The temperature can be controlled with in -15ºc to -20ºc.The microtome is remotely operated from outside.

Freezing technique:

1. Dry ice(Carbon di- oxide mixed with acetone with high pressure)

2. Liquid nitrogen (Iso pentene cooled in liquid nitrogen)

3. Cold contact (Refrigerated test tube)

4. Commercial sprays.

5. Thermomodulate attachment.

Uses:

1. **Urgent diagnosis** :To detect whether a breast nodule is benign or malignant to decide lumpectomy or mastectomy.

2. To prevent dissolution or destruction of substance need to be examined .Example are

a. Demonstration of fat in liposarcoma by Sudan black B.

b. Enzyme ATPase, NADPH in muscle biopsy for enzyme histochemistry.

3. For immunofluorescence studies like renal and skin biopsies to demonstrate deposit of antibodies, complements, fibrin and another antigen.

4. For silver and gold impregnation method in neurohistopathology.

5. To see excision margin whether it is free or invaded by tumor.

6. Diagnosis of hirusprung disease or congenital megacolon in neonates and children.

B. **Paraffin sectioning**: Tissues from the body taken for diagnosis of disease processes must be processed in histopathology laboratory to produce microscopic slides that are viewed under microscope by pathologist. The person who do the tissue processing and make the glass microscopic slide are histotechnologist.

Paraffin sectioning can be done 2 ways:

1. Automated tissue processioning (specimen numbers high)

2. Manual tissue processioning (specimen numbers low or high)

•Tissues that come to the histopathology laboratory are

a. Biopsy specimen like endoscopy, colonoscopy, cervical biopsy.

b. Resected disease organ like gall bladder, uterus, breast, intestine, bone, cartilage, soft tissue etc.

c. Resected organ or tissue after chemotherapy or radiotherapy.

d. Forming cell block from FNAC.

e. Autopsy

f. Animal like mouse,

Specimen accessioning: Tissue specimen received in the surgical pathology has a request form, that list the patient information and history along with a description of the site of origin

.The specimen is accessioned by giving them a number that will identify each specimen for each patient and maintaining a registrar book.

•Gross examination: Tissue removed from the body for diagnosis arrives in pathology department and are examined by a pathologist, pathology assistant. When a malignancy is suspected, then the specimen is covered with ink in order to mark the margins of specimen. Different colored inks can be used to identify the different areas if needed.

When section are made and processed, the ink will mark the actual margin on the slide.

Gross examination consists of describing the specimen and placing all or parts of it by cutting a sharp knife in case of soft tissue or by a saw in case of hard tissue like bones or cartilage into small plastic cassette which hold the tissue . Initially the cassette is placed in a fixative.

Fixation: Fixative is a chemical substance which preserve the architecture of tissue near normal by killing cells before enzymatic degradation by cellular enzymes.

Fixation done in a fixative for 12 hours.

Factors that affecting fixative:

a. Buffering

b. Penetration

c. Volume

d. Temperature

e. Concentration

f. Time interval

Fixative general usages for soft tissue:

a.Formalin 10% : All routine surgical pathology and autopsy tissue.

b. **Zenkers fixative:** For reticuloendothelial tissue including lymph node, spleen, thymus and bone marrow.

c. **Bouins fixative:** For testis , GI tract, endocrine tissue.

d. **Glutaraldehyde**: For electron microscopy.

e. **Carnoys fixative**: For early fixation within 6 hours.

f. **Universal fixative**: For DNA preservation.

Fixative uses for hard tissue:

Decalcifier: Some tissue contain calcium deposits which are extremely firm and which will not section properly with paraffin embedding owing to the difference in densities between calcium and paraffin.

Decalcifiers are used to remove Ca ion from bony tissue are:

a. Strong mineral acid nitric acid and hydrochloric acid

b. Organic acid such as acetic acid and formic acid.

c. EDTA

d. Electrolysis.

End point of decalcification:

a. Decalfication is completed if specimen is floating

b. Bending of a specimen is possible if decalcification is completed.

c. Inserting a pin or nail is possible if decalcification is completed.

Manual tissue processioning:

a. **Dehydration:** First the water from the the tissue must be removed by dehydration. This is usually done by 1hour 30 minutes each series of alcohol from 70% to 80% to 95%.

b. **Clearing:** Consist of removal of dehydrate with a substance that will miscible with the embedding media. The commonest clearing agent is Toluene. Tissue placed in toluene 1 and toluene 2 each for 30 minutes. Others clearing agent are Xylene, chloroform, Paraffin, methyl benzoite, methyl salicylate, citrus fruits.

c. **Infiltrating:** Tissue is then infiltrating with embedding agent like molten paraffin wax in a becker placed in a hot air woven for 6-8 hours which replaces toluene. Others infiltrating media are paraplust, paraplust plus, gelatin, collidins. Epoxy resin for electron microscopy and acrylic resin for immunohistochemistry are used.

Automated tissue processioning: The above process (dehydration, clearing and infiltrating) are almost always automated for large volumes of routine tissue processed. Automation consists of an instrument that moves the tissues around through the various agents on a present time scale.

Embedding : After tissue have been dehydrated, cleared and infiltrating with embedding material like paraffin ,agar ,gelatin, which is then hardened. This is achieved by placing tissue in a metalic angle or leuckharts molds and cooling in case of paraffin and heating in case of epoxy resin for electron microscopy and acrylic resin for immunohistochemistry. In case of automated tissue processor tissues are still in the cassettes and pick the tissue out of the cassettes and pour molten paraffin over them.

Sectioning: The blocks are then chilled in a try of ice because the cold wax makes a clean cut compared to paraffin wax cut at room temperature. The paraffin block is cut by using a rotatory microtome with 4-5 micro-mitre thickness.

Cryo sectioning :In case of frozen section fixed or unfixed frozen tissues sliced by using Labeling : For indefinite storage of block, a identification number are written by india ink on a small piece of paper and attach this in one side of block.

Water Bathing: After sectioning the sectioned tissue placed in warm water bath to float that help to remove the wrinckles.

Making slide and Numbering: Sections tissue pick up on a glass microscopic slides from water bath and placed in a Hot air woven for 15 minutes to help the sections adhere to the slides. The slide is then numbered by non-removable ink.

Staining: Clearing: Rinse in xylol for 10 dips to remove wax. Then rinse in graded alcohol (70%, 80%, 95%) for 5dips each to remove xylol.

•Procedures of Heamatoxylin and eosin stain

•Procedure of papanicolaou stain

Drying: After staining, slides are drying by bloating paper.

Cover-slipping: The stained section on the slide must be covered by a thin piece of plastic or glass with DPX to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the tissue section for years to come.

Drying: Then the slide dried in hot air woven for 5 minutes.

Microscopic examination: Stained slide then examined by pathologist. Pathologist should write the report in a register book.

Signing out: The last step including entering the case into computer database and printing the labels. Then lebel the slide and deliver the book of the slide to assigned pathologist. The book can hold up to 20 slides with 1 book per case.