

Histopathology Techniques

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- **Autopsy:** seeing with **one's own eyes** (Used in human medicine)
 - postmortem study of self (same species)
- **Necropsy:** seeing a carcass (Used in veterinary medicine)
 - study of the dead
- **Biopsy:** is examination of biological samples like fluid, tissue, etc., collected **from living animals.**

Histopathology Techniques

- Tissue Processing and Staining

- Method of Biopsy Taking:

1. Incisional biopsy
2. Excisional biopsy
3. Punch biopsy
4. Core needle biopsy

1. Incisional biopsy:

- It is performed when **removal of entire lesion is impossible.**
- Often performed **prior to** major surgical procedure.
- It is strictly a **diagnostic nature.**

2. Excisional necropsy:

- In this technique, the **entire lesion is removed**, usually with a rim of normal tissue.
- It is performed when the lesion is **smaller in size.**
- The procedure serves the **diagnostic and therapeutic function.**

3. Punch Biopsy:

- It is done by **biopsy forceps**.
- It is performed in the lesion of **uterine cervix, oral cavity, esophagus, stomach, intestine and bronchus**.

4. Core Needle Biopsy:

- It is done with special type of wide bore biopsy needle.
- It permits a **percutaneous approach** to internal structures

Some General Rules for the biopsy Procedure

- The larger the lesion, the numerous the biopsies that should be taken from it because of the fact that the diagnostic areas may be present only focally.
- Necropsy should be taken from the periphery that includes normal and diseased tissue.

- **Crushing or squeezing of the tissue** with forceps should be carefully avoided.
- Once the biopsy is obtained, it should be placed **immediately into container** with adequate volume of fixative.

Handling of Specimen

- Specimen should be transported in **glass, plastic or metal container** or in a plastic bag in 10%formalin.
 - If **formalin is not available** at hand, place the specimen in refrigerator at 4oC to slow down autolysis.
- The container should have an opening larger enough so that the tissue can be removed easily after it has **hardened** by fixation.

General Principle of Gross Examination:

- ✓ Proper identification and orientation of the specimen.
- ✓ **Unlabeled specimen** should never be processed.
- ✓ A properly completed histopathology requisition form containing **animal's ID, age, sex, breed relevant clinical data**, surgical findings, nature of operation and name of tissue submitted.
- ✓ Careful search and examination of all the tissue submitted in order.

- ✓ Place the specimen on cutting board in an anatomic position and **record the following information:**
 - a. Types of specimen
 - b. Structure included.
 - c. Dimensions
 - c. Weight
 - e. Shape
 - d. Colour
 - g. Consistency
 - h. Surgical margin, whether included or not involved by tumour.

- ✓ Measurements are usually given in **centimeter** unless the specimen is **very small in which mm** can be used.
- ✓ **Endometrial and prostatic tissue** should be measured by **aggregate pieces in volume.**

Sampling for Histopathological Examination

- Tissue submitted for histopathology **must not be more than 3 mm thick** and **not larger than the diameter of slides** used.
- Most specimens from **solid tissues** are cut in the form of **pieces** measuring **10 to 15 mm** on the slides and **2 to 3 mm in thickness**.
- Discrete areas of **calcification or ossification** should be taken out and **should be decalcified in nitric acid/HCl**.
- **Small fragments of tissue** must be **wrapped in thin paper**.

Histological Technique

- Histological technique deals with the **preparation of tissue for microscopic examination.**
- The aim of good histological technique to **preserve microscopic anatomy of tissue.**
- This is achieved by passing through a series of process.
 - These processes are:
 1. Fixation
 2. Dehydration
 3. Cleaning
 4. Embedding
 5. Cutting
 6. Staining

Fixation

- This is the process by which the constituents of cells and tissue are **fixed in a physical and a chemical state** so that they will withstand subsequent treatment with various reagents **with minimum loss of architecture.**
- This is achieved by **exposing the tissue to chemical compounds, call fixatives**

- *Mechanism of action of fixatives:*
- Most fixatives act by precipitating proteins
- **No fixative will penetrate** a piece of tissue thicker than 1 cm.
- For **dealing with specimen thicker than this**, following methods are recommended:
 - Solid organ: **Cut slices** as necessary as but not thicker than 5mm.
 - Hollow organ: Either **open or fill with fixative** or pack lightly with wool soaked in fixative.
 - Large specimen: **Inject fixative** along the vessels or bronchi as in case of lung so that it reaches all parts of the organ

Properties of an Ideal Fixative:

1. Prevents autolysis and bacterial decomposition.
2. Preserves tissue in their natural state and fix all components.
3. Make the cellular components insoluble to reagent used in tissue processing.
4. Preserves tissue volume.
5. Avoid excessive hardness of tissue.
6. Allows enhanced staining of tissue.
7. Should be non-toxic and non-allergic for user.
8. Should not be very expensive.

- Amount of fixative fluid:

- This should be approximately 10-20 times the volume of the specimen.
- Fixative should surround the specimen on all sides.

Factor affecting fixation:

- Size and thickness of piece of tissue.
- Tissue covered by large amount of mucous fix slowly.
- The same applies to tissue covered by blood or organ containing very large amount of blood.
- Fatty and lipomatous tissue fix slowly.
- Fixation is accelerated by agitation.
- Fixation is accelerated by maintaining temperature around 60oc.

❖ Classification of Fixatives

A. Tissue fixatives

- a. Buffered formalin
- b. Buffered gluteraldehyde
- c. Zenker's formal saline
- d. Bowen's fluid

B. Cytological fixatives

- a. Ethanol
- b. Methanol
- c. Ether

C. Histochemical fixatives

- a. Formal saline
- b. Cold acetone
- c. Absolute alcohol

Tissue Processing

- Tissue processing is a long procedure and required **24 hours**. Tissue processing can be done by **manually** or **mechanically**.
- It is done in **stages**. It can be subdivided into; **dehydration, clearing, impregnating** and **embedding**.
- It is important that all specimens are **properly labeled** before processing is started.
- For labeling, **pen containing ordinary ink** should not be used. Printed or graphite pencil written, are satisfactory.

A. Dehydration:

- Tissues are dehydrated by using **increasing strength of alcohol**; e.g. 50%, 70%, 90% and 100%.
- 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.

B. Clearing:

- 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 of the following reagents:
 - Xylene, Chloroform, Benzene, Carbon tetrachloride and Toluene
- **Xylene** is commonly used.
- **Small piece of tissue** are cleaned in **0.5 – 1 hour**; whereas **larger** (5cm or more thick) are cleaned **in 2-4 hours**.

C. Impregnation with Wax:

- This is allowed to occur at melting point temperature of paraffin wax, which is **54-60°C**.
- Volume of wax should be about **25-30 times** the volume of tissues.
- The duration of impregnation depends on **size** and **types of tissues** and the **clearing agents** employed.
- Total duration of **4 hours** is sufficient for routine impregnation.
- **Types of Wax** employed for Impregnation: can be Paraffin wax or Water soluble wax
- **Paraffin wax** is used routinely. It has hard consistency, so section of 3-4 micron thickness can be cut.

D. Blocking:

- Impregnated tissues are placed in a mould with their labels and then fresh melted wax is poured in it and allowed to settle and solidify.
- Once the block has cooled sufficiently to form a surface skin it should be immersed in cold water to cool it rapidly.
- After the block has completely cooled it is cut into individual **blocks** and each is **trimmed**.
- **Labels** are made to adhere on the surface of the block.

Staining

- **Staining** is a process by which we give colour to a section.
- There are hundreds of stains available.
- *Generally the stains are classified as:*
 - Acid stains
 - Basic stains
 - Neutral stains

Classification of Stains:

- Acid Dyes:

- In an acid dye the **basic component is coloured** and the acid component is colourless.
- Acid dyes stain basic components
 - e.g. eosin stains **cytoplasm red**.

- Basic Dyes:

- In a basic dye **the acid component is coloured** and the basic component is colourless.
- Basic dyes stain acidic components
 - e.g. **basic fuchsin stains nucleus blue**.

- *Neutral Dyes:*

- When an acid dye is combined with a basic dye a neutral dye is formed.
- As it contains both coloured radicals, it gives different colours to cytoplasm and nucleus simultaneously.
- This is the basis of Leishman stain.

- **Procedure of staining**

- Every stain is to be used according to a specified method.
- Staining can be done either manually or in an automatic stainer.
- **Haematoxylin and Eosin** staining: It is the most common used routine stain

Special Stains:

1.PAS (Periodic Acid Schiff) stain: This stain demonstrates glycogen

2.Stains for micro-organism:

- a. Gram-stain:
- b. Ziehl_Neelsen stain: This stain detect acid fast bacilli.
- c. PAS stain: It is used for fungi, amoeba and Tricomonas.
- d. Modified Giemsa (2% Giemsa in water):For Helicobacter pylori.

3.Congo-red: It is used for identification of amyloid.

4.Sudan-Black: It is used for fat staining.

5.Masson's Trichrome: It is used for differentiation of connective tissue

Summary



1. Grossing
2. Fixation
3. Tissue Processing
 - Dehydration
 - Clearing
 - Impregnation
4. Embedding
5. Sectioning
6. Staining
7. Mounting