TECHNIQUES USED IN SURGICAL PATHOLOGY SERVICES

Surgical Pathology is that part of Anatomic Pathology concerned with the study of tissue and organ samples removed from patients, either by biopsy or through a surgical procedure, in an attempt to obtain diagnosis of a lesion or disease. The pathologist is therefore able to advise the attending physician as to the nature of the disease, the prognosis, and the need for additional sampling or exploration.
Cytopathology is the study and evaluation of cells present in smears, fine needle aspirates and body fluids. Analysis of nuclear and cytoplasmic characteristics permit diagnosis of various disease processes.

The value of tissue sample in variable lesions:
1-Determine a tissue diagnosis where clinical diagnosis is doubt e.g. liver core biopsy in cases of cirrhosis of unknown etiology.
2-Ascertain whether benign or malignant e.g. gastric ulcer biopsy.
3-Ascertain extent of spread of disease.
4- In itself, the biopsy with excision of the lesion may be a form of local treatment e.g. excision biopsy of rodent ulcer.
N.B. biopsy is a form of special investigation and should interpreted in the light of the clinical picture.

Methods for tissue sampling:

1- Excision biopsy: The whole lesion removal.
2- Incision biopsy: Part of the lesion is sampled.
3- Core needle biopsy.
4- Endoscopic biopsy.
5- Punch biopsy.
6- Frozen section: Examination of fresh samples.

The Routine Specimen manipulation:

a. Labeling: Each specimen must be labeled with the patient's name, hospital number, source of the tissue and site and side of the body.
b. Specimen Fixation: Universal precautions are to be exercised in handling and transporting all surgical pathology specimens. ***Proper and timely fixation is a critical step in tissue preparation for diagnosis.***

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

- Aldehydes include formaldehyde (formalin)
- Mercurials
- Alcohols

- Oxidizing agents
- Picrates

c-Tissue Processing:

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 6-8 routinely. The technique of getting fixed tissue into paraffin is called tissue processing. The main steps in this process are dehydration and clearing.

- First, the water from the tissues must be removed by dehydration. Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. This is usually done with a series of alcohols, 70% , 95% to 100%.
- The next step is called "clearing" and consists of removal of the dehydrant with a substance that will be miscible with the embedding medium (paraffin). The commonest clearing agent is xylene.
- Finally, the tissue is infiltrated with the paraffin.
- The above processes are almost always automated for the large volumes of routine tissues processed. Automation consists of an instrument that moves the tissues around through the various agents on a preset time scale.
- Tissues that come off the tissue processor are still in the cassettes and must be manually put into the blocks by a technician. This "embedding" process is very important, because the tissues must be aligned, or oriented, properly in the block of paraffin.
- 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 & E). Other stains are referred to as "special stains" because they are employed in specific situations according to the diagnostic need.

Ancilliary diagnostic techniques

Are techniques that aid in the diagnosis when routine methods fail to provide the answer

- **Cyto/histochemistry:** Is the light microscopic study of the chemistry of cells and tissues after treating them with special reagents

- **Immuno cyto/histochemistry:** Immunohistochemistry (IH) is a technique for identifying cellular or tissue constituents (antigens) which may be normal tissue constituents or produced as a result of a pathologic process. IH techniques are based on the specificity antibody-antigen binding. The site of antibody binding is identified either by direct labeling of the antibody, or by use of a secondary labeling method.

- **Molecular pathology& Cytogenetics:**

Molecular pathology entails the study of the biochemical based changes in a single nucleotide in genomic *DNA* resulting in a defective gene product, which may produce a *lesion* in some disorders as congenital diseases and cancer. The method used is Polymerase Chain Reaction Technology (PCR), which allows the analysis of DNA or RNA from any specimen by means of amplifying the length of DNA understudy a million times, and therefore can detect even minute amounts

Cytogenetics: Involves the study of abnormal chromosomes and genes responsible for certain diseases by a process of karyotyping.

A) Cyto/histochemistry

Is the light microscopic study of the chemistry of cells and tissues after treating them with special reagents. Each tissue type has characteristic features and preponderance of one particular substance characteristic of the tissue or cell

1- Mucin stains:

- Alcian blue - The dye stains acid mucopolysaccharides and glycosaminoglycans,
- PAS (peroidic acid-Schiff) - Stains glycogen, mucins as well as fungi, but tissue can be pre-digested with diastase to remove glycogen.
- Mucicarmine - Very specific for epithelial mucins.

2- Fat stains:

The oil red O (ORO) stain can identify neutral lipids and fatty acids in smears and tissues. The ORO is a rapid and simple stain. It can be useful in identifying fat emboli in lung tissue or clot sections of peripheral blood.

3- Connective tissue stains:

The trichrome stain helps to highlight the supporting collagenous stroma in sections from a variety of organs. This helps to determine the pattern of tissue injury. Trichrome will also aid in identifying normal structures, such as connective tissue capsules of organs, the lamina propria of gastrointestinal tract, and the bronchovascular structures in lung.

B) Immunohistochemistry

- Immunohistochemistry is the localization of **PROTEINS** in tissue sections by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as enzyme labels have been introduced e.g peroxidase and alkaline phosphatase .

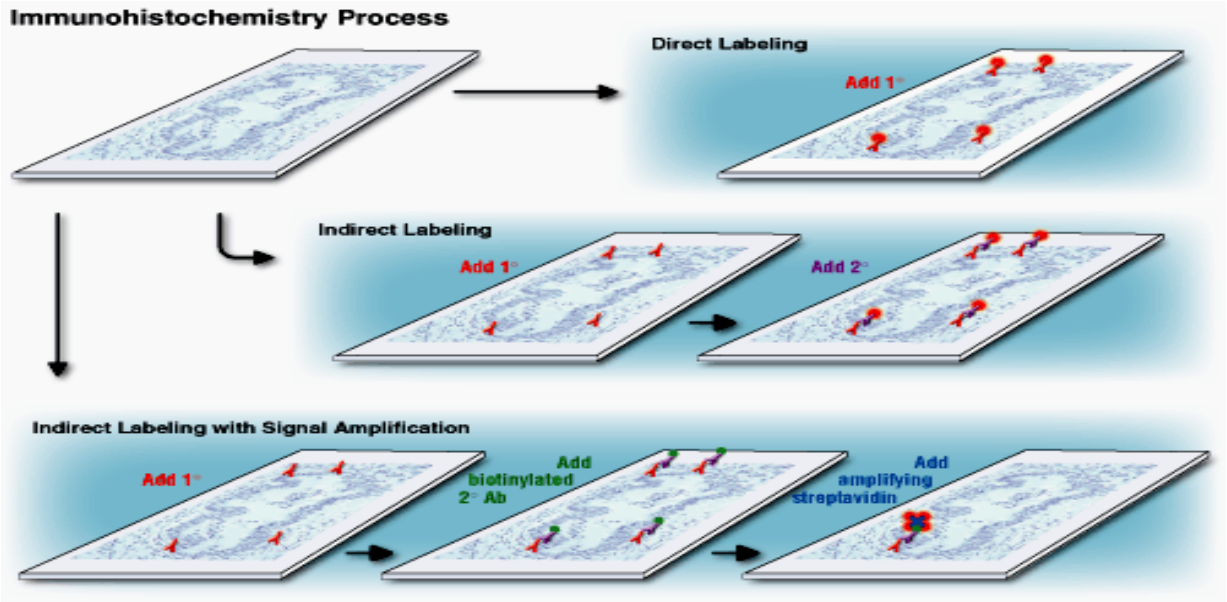
- Since immunohistochemistry involves specific antigen-antibody reaction , it has become a crucial technique and widely used in many medical research laboratories as well as clinical diagnostics.

Advantages:

- Remarkable sensitivity and specificity.
- Applicability to routinely processed material even if stored to long time.
- It is compatible with most o the fixatives currently in use and in cytological preparations and even previously stained sections.

Methods:

- There are numerous immunohistochemistry methods that may be used to localize antigens.
- The selection of a suitable method should be based on parameters such as the type of specimen under investigation and the degree of sensitivity required.



***1- Direct* Method**

- Direct method is **one step** staining method, and involves a labeled antibody) reacting directly with the antigen in tissue sections.
- This technique utilizes only one antibody and the procedure is short and quick. However, it is **insensitive** due to little signal amplification and rarely used since the introduction of indirect method.

2- Indirect Method:

- Indirect method involves an **unlabeled primary antibody** (first layer) which react with tissue antigen, and **a labeled secondary antibody** (second layer) react with primary antibody .

- This method is more sensitive due to **signal amplification** through several secondary antibody reactions with different antigenic sites on the primary antibody.
- The second layer antibody is labeled with an enzyme such as peroxidase, alkaline phosphatase or glucose oxidase.

3- PAP Method (peroxidase anti-peroxidase method):

- PAP method is a further development of the indirect technique and it involves a third layer which is a rabbit antibody to peroxidase, coupled with peroxidase to make a very stable peroxidase anti-peroxidase complex.
- The sensitivity is about 100 to 1000 times higher.

4- Avidin-Biotin Complex (ABC) Method:

- ABC method is standard IHC method and one of widely used technique for immunhistochemical staining.
- **Avidin**, a large glycoprotein, can be labeled with peroxidase and has a very high affinity for biotin. **Biotin,** a low molecular weight vitamin, can be conjugated to a variety of biological molecules such as antibodies.
- The technique involves three layers. The first layer is unlabeled primary antibody. The second layer is biotinylated secondary antibody. The third layer is a complex of avidin-biotin peroxidase. The peroxidase is then developed by the DAB or other substrates to produce different colorimetric end products.

5- Labeled Strept Avidin Biotin (LSAB) Method:

- Streptavidin, derived from streptococcus avidini, is a recent innovation for substitution of avidin.
- LSAB is technically similar to standard ABC method, but The third layer is Enzyme-Streptavidin conjugates to replace the complex of avidin-biotin peroxidase. The enzyme is then visualized by application of the substrate chromogen solutions to produce different colorimetric end products.
- A recent report suggests that LSAB method is about 5 to 10 times more sensitive than standard ABC method.

Summary

Immunohistochemistry is a method of detecting the presence of specific proteins in cells or tissues and consists of the following steps:

1- Primary antibody binds with the specific antigen.

2- The antigen antibody complex is bound by enzyme conjugated secondary antibody.

3- In the presence o the substrate and chromogen, the enzyme form colored deposit a sites of antigen antibody binding.

Examples of diagnostic applications of immunohistochemistry:

Immunohistochemistry has assumed an increasingly prominent role in diagnostic breast pathology as it now frequently used in the evaluation of many epithelial proliferations of the breast.

Common applications include the use of:

- Myoepithelial markers (actin) to evaluate for stromal invasion.
- E-cadherin to distinguish between ductal and lobular neoplasia. Suppresion of the **E-cadherin** gene are frequently found in invasive **lobular carcinoma** (ILC) but never in invasive ductal carcinoma.
- High molecular weight cytokeratins to differentiate usual ductal hyperplasia (+ve) from ductal carcinoma in situ (-ve).
- Cytokeratin stains to detect metastases in sentinel lymph nodes.

Immunohistochemistry: a prognostic as well as diagnostic tool:

- The assessment of proliferating cell populations has been used to aid in the differentiation of benign from malignant neoplasms.
- The assessment of proliferation markers and oncogenic determinants holds information regarding prognosis.
- For example: Ki-67, p53 protein, bcl-2, are useful in understanding the biology of certain neoplasms and may carry prognostic information that influences clinical management.
- ER and PR and Her2 neu in breast carcinoma.