Tissue Processing

Tissues from the body taken for diagnosis of disease processes must be processed in the histology laboratory to produce microscopic slides that are viewed under the microscope by pathologists. The techniques for processing the tissues, whether biopsies, larger specimens removed at surgery, or tissues from autopsy, are described below. The persons who do the tissue processing and make the glass microscopic slides are histotechnologists.

Specimen Accessioning

Tissue specimens received in the surgical pathology laboratory have a request form that lists the patient information and history along with a description of the site of origin. The specimens are identified by giving a number for each specimen for each patient.

Gross Examination

Tissues removed from the body for diagnosis arrive in the Pathology department and are examined by a pathologist, pathology assistant, or pathology resident. Tissue specimen are sent to the department in a fixative (usually formalin)

Gross examination consists of (a) describing the specimen and (b) placing the entire specimen(if it is small , e g .needle biopsies) or taking small section from important areas into small plastic cassettes .These cassettes hold the tissue while being processed to a paraffin block. Initially, the cassettes are placed into a fixative.

Fixation

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. There is no perfect fixative, though formaldehyde(formalin) comes the closest and is used most commonly..

Formalin- Tissue is fixed by cross-linkages formed in the proteins, particularly between lysine residues. This cross-linkage does not harm the structure of proteins greatly, so that antigenicity is not lost. Therefore, **formalin** is good for immunoperoxidase techniques. Formalin penetrates tissue well, but is relatively slow. The standard solution is 10% **neutral buffered formalin**. A buffer prevents acidity that would promote autolysis. Neutral buffered formalin is the most commonly used routine fixative.

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 3-5 routinely. The technique of getting fixed tissue into paraffin is called tissue processing. The main steps in this process are dehydration, clearing, paraffin wax impregnation and embedding.

1. **Dehydration**: Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. First, the water from the tissues must be removed by dehydration. This is usually done with a series of alcohols, say 70% to 95% to 100%. Sometimes the first step is a mixture of formalin and alcohol.
2. **Clearing**: 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.

3. **Impregnation with paraffin wax**: Finally, the tissue is infiltrated with the embedding agent, almost always paraffin.

Automated tissue processor: 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.

4. **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.

Sectioning

Once the tissues have been embedded, they must be cut into very thin sections 3-5 microns in thickness that can be placed on a slide. This is done with a **microtome**. The microtome is nothing more than a knife with a mechanism for advancing a paraffin block standard distances across it.

Once sections are cut, they are floated on a warm water bath that helps remove wrinkles. Then they are picked up on a glass microscopic slide.

Staining

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 serial changes in xylene followed by alcohol to water. There are no stains that can be done on tissues containing 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 for tissue sections is **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.

Frozen Sections

At times during performance of surgical procedures, it is necessary to get a rapid diagnosis of a pathologic process. The surgeon may want to know if the margins of his resection for a malignant neoplasm are clear before closing, or an unexpected disease process may be found and require diagnosis to decide what to do next, or it may be necessary to determine if the appropriate tissue has been obtained for further workup of a disease process. This is accomplished through use of a frozen section. The piece(s) of tissue to be studied are snap frozen in a cold liquid or cold environment **(-20 to -70 Celsius)**. Freezing makes the tissue solid enough to section with a microtome.

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.