TISSUE-PROCESSING

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Abstract: The present study is about how tissue is fixed before going to observe it under microscope for microscopic study of anatomy of cells and tissues of plant and animals. Tissues from the body are taken for diagnosis of disease. It is commonly performed by examining cells & tissue using sectioning and staining, followed by examination under a light microscope by pathologist. This whole process is called as Tissue Processing. Steps in tissue processing are Labeling, Grossing, Fixation, Dehydration, Impregnation, Embedding of tissue, Microtomy, Routine Staining, Mounting coverslip. Each step is described in further.

Keywords Tissue processing , Grossing, Fixation, Dehydration, Embedding, Microtomy, Staining.

Introduction

The technique of getting fixed tissue into paraffin is called tissue processing. Stabilized tissue must be adequately supported before they can be sectioned for microscopical examination. While they may be sectioned following range of preparatory freezing methods, tissues are more commonly taken through a series of reagents & finally infiltrated & embedded in a stable medium which provides necessary support for microtomy; this whole procedure is known as tissue processing. Tissue processing describes the steps required to take animal & human tissue from fixation to the state where it is completely infiltrated with a suitable wax i.e. paraffin wax & can be embedded & ready for section cutting on microtome. If tissue is completely fixed, processing problems are less likely to occur. The main steps in tissue processing are dehydration & clearing. First the water from the tissue must be removed by dehydration. This is usually done with a series of alcohols. Next step is called clearing & consist of removal of dehydrant with substance that will be miscible with embedding medium. Commonest clearing agent is Xylene. [1]

Finally the tissue is infiltrated with embedding agent, almost always paraffin. Embedding is a process in which the tissues or the specimens are enclosed in a mass of the embedding medium using a mould. Since the tissue blocks are very thin in thickness they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin, synthetic resins, gelatins etc. Embedding techniques using waxes & resins have been developed & perfected for different specific aim & different types of specimens. Paraffin is most suitable for embedding soft tissue & decalcified hard tissue for thin sections of 3-6um & is the most widely used embedding method. Celloidin is better option when working with large, harder & more fragile tissue. Before embedding the specimens requires time for fixation, decalcification, dehydration, clearing & impregnation or infiltration. [1]

STEPS:-

1] Labeling

2] Grossing

3] Fixation

4] Dehydration

5] Impregnation

6] Embedding of tissue

7] Microtomy

8] Routine Staining

9] Mounting coverslip

1] LABELING [1,2]

Specimen labeling is an important step before proceeding with the grossing procedures. This aids in facilitates in proper identification of the specimen. Most institutions have their own unique way of specimen identification giving each patient and tissue unique accession numbers which usually include the year the specimen was collected with varying prefixes for different types of specimens. The most important step in specimen handling is the correct identification of the specimen with unique numbers so that an accurate link between the specimen and the patient from whom the specimen was removed is maintained. Each specimen container should include the patient's name with age and a medical record number along with matching paperwork (e.g., a surgical request form). These labels must be consistent and should be on the container so that the labels cannot be separated from the specimen. The surgical request form provides the actual request for pathological services and the required relevant clinical history of the patient. Misidentification of any specimen can result in failure to make a proper diagnosis of one or more patients, incorrect treatment and possibly legal action.

2] GROSSING[1,2]

Grossing is an initial and most important step in surgical pathology for obtaining accurate diagnosis. In this step pathologists inspect the excised specimen with the bare eye to obtain diagnostic information. It refers to the examination and dissection of surgical specimens, along with preparation of sections from those tissues requiring processing. The gross room is the area where pathology specimens from the operating rooms are transferred for pathological review and analysis. Thus, it serves as the bridge between the surgeon and diagnostic surgical pathologist in that a correct diagnosis or treatment of a patient depends on the proper handling and processing of the specimens of tissue transferred to this area. The room should be large, well illuminated. It should be properly ventilated with an exhaust fan. It should contain shelves for specimen containers, ready access to formalin, large table for dissection of specimens and sink with provision for water. Other facilities ideally required are photographic facility, X-ray unit with view box and refrigerator. There are three main points for considerations during biopsy grossing triage they are-

1] Specimen's category (i.e., whether it is a cytology or a surgical pathology sample),

2] Determination of the priority for processing and securing appropriate specimen preservation for processing.

3] Effective triaging of the specimen requires knowledge, experience and common sense.

3] FIXATION[1,2]

Fixation is the foundation for the subsequent stages in the preparation of tissue sections, up to the making of diagnosis. Fixation is a complex series of chemical events which brings about changes in the various chemical constituents of cell like hardening however the cell morphology and structural detail is preserved. Unless the tissue is fixed soon after the removal from body it will undergo degenerative changes due to autolysis and putrefaction so that the morphology of individual cell will not be lost. Principle of fixation is to bring about cross linking of proteins which produces denaturation or coagulation of proteins so that the semifluid state is converted into semisolid state; so that it maintains everything in vivo in relation to each other. Thus semisolid state facilitates easy manipulation of tissue. The main aim of fixation is to preserve the tissue in life like manner as possible. There are so many fixatives which are as follows- Alcohol like ethyl alcohol, Acetone, Mercuric Chloride, Picric acid, Potassium dichromate, Osmium tetraoxide, Acetic acid, Glutaraldehyde etc. Some vapor fixative examples are Formaldehyde, Acetaldehyde, and Glutaraldehyde etc.

Washing out step- After use of certain fixative it is necessary that the tissue be thoroughly washed in running water to remove the fixative entirely.

4] DEHYDRATION[1,2]

Water is present in tissues in free and bound (molecular) forms. Tissues are processed to the embedding medium by removing some or all of the free water. During this procedure various cellular components are dissolved by dehydrating fluids.

Dehydration is effected as follows:-

* Dilution dehydration the most commonly used method. Specimens are transferred through increasing concentrations of hydrophilic or water miscible fluids which dilute and eventually replace free water in the tissues.

*Chemical dehydration, where the dehydrant , acidified dimethoxypropane or diethoxypropane, is hydrolyzed by free water present in tissues to form acetone and methanol 43-50 in an endothermic reaction.

Choice of dehydrant is determined by the nature of the task, the embedding medium, processing method and economic factors.

There are so many dehydrating agents like Alcohols:- Ethanol, Methanol, Isopropanol, tertiary butanols ; Glycol ethers:- 2-Ethoxyethanol, ethylene glycol monoethyl ether, cellosolve or oxitol, Dioxane, 1,4 diethylene dioxide, Polyethylene glycols (PEG); Other dehydrating agent like Phenol, Acetone, Tetrahydrofuran ,2,2 dimethoxypropane (DMP) and 2,2 diethoxypropane (DEP) are used for chemical dehydration of tissues.

5] IMPREGNATION

After clearing, tissues are transferred to molten paraffin wax for filtration and impregnation. [1,2]During this process clearing agent diffuses out and molten wax is infiltrated in the tissue gets deposited. This process is called impregnation. [3]

6] EMBEDDING OF TISSUE

Embedding is a process by which tissue are surrounded by a medium such as wax, agar or gelatin, will provide sufficient external support during sectioning. [1,2] Embedding technique using waxes and resins have been developed and preferred for different specific aims and different types of specimen. Paraffin is most widely used method which is suitable for embedding soft tissues and decalcified hard tissues for thin sections 3-6 µm. Celloidin is better option when working with large, harder and fragile tissues. Hard embedding materials such as glycol methacrylate, Methyl methacrylate (MMA) are chosen for undecalcified hard tissue embedding suitable for heavy duty sectioning or ground sectioning. [4]

Before embedding, the specimens require a lengthy time for fixation, decalcification, dehydration, clearing, impregnation or infiltration. Each step is interdependent and failure in one of these will directly affects both the ease of sectioning and the quality of the sections. Thus, it is important that all steps of processing be carried out by patient, careful and responsible technician.[5]

7] MICROTOMY/ SECTIONING[1,2]

We must be familiar with the control on the rotary microtome before you can proceed with sectioning. The wheel of microtome should be in the locked position. To section, turn the handle of microtome, which advances the block towards the knife. On each rotation of the wheel, the block is moved forward the number of microns that have been set for sectioning thickness. Turn the wheel in a smooth slow motion to ensure getting the best sections. When sections begin to come off the block, the first several may be incomplete. These can be brushed away with a small hair paintbrush. No other instrument should be used, since these would damage the knife edge.

Using two hair paintbrushes to hold the two ends of each piece of ribbon, transfer pieces of ribbon to the water on the slide. It is best if the ribbons are touching one another their length so that they are rafted together.

Next step is put slide on a slide warming tray that is set at a temperature that feels very warm to touch but not hot enough to burn. As the water warms on slide, the sections will expand, greatly increasing the length of the ribbons. When the ribbon look completely expanded remove the slide from the warming tray. Next step is staining. Before staining, slides must be dry. If any water remains prior to staining, the sections will not stick properly to the slide.

8] ROUTINE STAINING

During the processing of the slides, the general scheme is that first the paraffin is removed from the sections using xylene or toluene, since the paraffin prevents staining of the tissues. The slides are then processed down to water, since most staining solutions are water based.[8] Following staining the slides are dehydrated again through a graded series of alcohol, cleared in xylene or toluene and coverslip are applied using a plastic mounting medium that is miscible with clearing agent. For clearing agent it is better to use toluene than xylene since it causes less shrinkage. For alcohol it is better to use ethanol than methanol since it is gentler on tissue and is nontoxic. For up to nine slides, coplin jars are used.[9] This ensures that slide touches another slide. Hematoxylin and eosin are most commonly used general nuclear and cytoplasmic stains. Hematoxylin stains acidic components. Eosin has an affinity for cytoplasmic elements. Its yellowish red color makes it an ideal counter stain to hematoxylin. [9,10]

9] MOUNTING COVERSLIP

Using mounting medium coverslip is put over the slide. Hold one end of coverslip with forceps and lower the other end onto the slide, holding it in place with a dissecting needle. Slowly lower the rest of coverslip. If an air bubble is introduced it will usually move to edge as we lower the coverslip. Once the coverslip is in place very slight pressure on the top of coverslip using the end of pencil will push out any bubbles on the edges. If too much mounting medium is used it is oozing out under from under coverslip, don't touch it. After it dries, excess mountant can be removed with razor blade carefully. These slides should be left at room temperature for several days before observing them under the microscope. When slides are thoroughly dry they can be stored in slide box. This is the whole process of tissue processing.[1,11]

CONCLUSION

The technique of getting fixed tissue into paraffin is known as tissue processing. Stabilized tissue must be adequately supported before they can be sectioned for microscopical examination**.** In tissue processing each step is interdependent & failure in one of these will directly affect quality of the sections. Thus, it is important that all the steps of tissue processing be carried by a careful & responsible technician.

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