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An Overview of Specific Aspects of Histopathology

Nicholas Kadriyan*

Department of Pathology, Aix-Marseille University, Marseille, France

***Corresponding author**: Nicholas Kadriyan, Department of Pathology, Aix-Marseille University, Marseille, France, E-mail: nicholas@nkadriyan.fr

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Description

Staining

The use of staining provides attention to important components of the tissue while also increasing contrast. Hematoxylin is a typical stain dye used in this technique that gives the nuclei a bluish colour, whereas eosin (another stain dye used in histology) gives the cell's nucleus a pinkish colour. However, different staining procedures are utilized for different cells and components. Staining is a popular medical procedure for locating sick or tumorous cells or other abnormal cells in sample tissues. Various different staining techniques, such as differential staining, double staining, and multiple staining, are utilized in some instances. Fixation is a term used in histology to describe the use of chemicals to preserve the natural tissue structure and keep the cell structure from degrading. When a light microscope is being utilized to conduct the investigation, neutral buffered formalin is usually used. Fixatives aid in the preservation of tissues and cells by cross-linking proteins in an irreversible process. While the technique preserves the cell structure for histology research, it has been discovered that it destroys and denatures proteins, rendering them useless.

The DNA, miRNA, and mRNA tissues are denatured by formalin fixation, and extracting these components for histological purposes may result in erroneous results. The fixation phase preserves the tissue's molecular formula, hardens cells or tissues for sectioning, and prevents breakdown. However, fixatives alter tissue penetration and antigen exposures, which can be beneficial or harmful. These fixatives are given to the prepared tissue in two ways: perfusion and immersion. Diffusion is employed to inject these fixatives into the animals' bodies. Perfusion is a time-consuming method in which only one fixative can be used at a time. Fixatives come in a variety of forms, although formaldehyde fixatives are the most frequent.

The goal of this process is to eliminate water from the selected tissues in order to solidify them and make cutting tiny slices of slides easier, thinner for light microscopes and thicker for electron microscopes. The dehydration procedure uses ethanol to eliminate water from the tissues. To remove the alcohol and paraffin wax, as well as the infiltrating agent, the process is repeated using a hydrophobic clearing solvent like xylene. Resins are utilized to aid in the cutting of thin tissue slices.

Embedding: The method of embedding is done using paraffin wax in staining to facilitate the extraction of cellular components. Plastic resin or wax, or a mixture of fixatives, are utilized to ensure good morphology in complicated biological tissues. However, prolonged heating of these fixatives may cause deterioration of cell and tissue structures, which could cause issues during the hybridization procedure due to the unstable RNA. In the same way, paraffin wax infiltration prevents antibody, chemical, and other fixatives from penetrating. To overcome this limitation, tissue freezing after embedding, wax removal after staining and the application of PFA fixatives provide a consistent solution for improved morphology.

Sectioning: The production of 'ribbon' like microtomes of a tissue for mounting on a microscope slide for examination is referred to as sectioning in histology. Successions of thin slices of tissues of the appropriate thickness are cut and produced using the paraffin method in this case.

Antigens retrieval: Following fixation and embedding, this step focuses on recovering antigens that have been masked. When formalin fixatives are employed in conjunction with other aldehyde fixatives, the cross-linking of proteins causes antigen sites to be covered, resulting in decreased immunohistochemistry staining. Antigen retrieval is used to break down protein cross-links to reveal epitopes and antigens that have been fixed and embedded in formalin and paraffin. The ultimate goal is to increase the intensity of the antibodies staining. Heat-induced and proteolytic retrieval methods are the most often utilized antigen retrieval procedures. To avoid denaturement of tissue structures and epitopes, the proteolysis digestion process should use the smallest dosage and time possible. Protein denaturalization occurs as a result of the heat technique, and antigens are lost in some situations. Similarly, heating may cause the chemical changes induced during the fixing period to reverse. Microwaves and other heating devices cause chemical reactions in the protein structure. A combination of enzymatic and heat retrieval procedures, on the other hand, results in effective staining intensity.