AMALIY VA TIBBIYOT FANLARI
ILMIY JURNALI
ISSN: 2181-3469
Jild: 03 Nashr:12 2024 yil

SCIENCE BOX

HISTOLOGY AND TISSUE STAINING

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Abstract. *Medical histology is the study of tissues and organs at the microscopic level, achieved through processes such as sectioning, staining, and examining the sections under a microscope. Also known as microscopic anatomy and histochemistry, histology provides a detailed visualization of tissue structure, revealing any significant changes the tissue may have undergone. This field is essential for medical diagnostics, scientific research, autopsies, and forensic investigations. Tissue samples go through a sequence of steps—fixation, processing, embedding, sectioning, and staining—before being examined under a microscope, with the findings interpreted by a pathologist. The specific histological stains selected for a specimen depend on the primary question of investigation. When combined with a patient's medical history, advanced interpretation of histology slides can provide critical insights that impact treatment decisions and prognosis.*

Keywords: *Neutral Buffered Formalin, immunohistochemical response, the Giemsa stain, The Nissl stain.*

Issues of Concern

A foundational understanding of tissue preparation and staining is essential for interpreting pathology reports, whether from inpatient or outpatient biopsies. However, it’s not guaranteed that the interpreting pathologist has applied appropriate histological stains comprehensively. Omissions in staining can hinder accurate diagnosis and affect patient outcomes.

Structure

Histological techniques allow the visualization of four fundamental human tissue types: epithelial, connective, muscle, and nervous tissues. Although these tissues share some similarities, staining reveals unique structural details. Each stain highlights a specific feature or component within the tissue type. For instance, hematoxylin, a common basic dye, stains proteins blue, while eosin, another widely used dye, stains proteins pink. These two stains are often used together to define intracellular organelles and proteins. Additionally, some stains are developed to highlight specific proteins, which will be discussed further in this review. Special stains enhance visibility for certain proteins but may obscure other structures; thus, multiple slides from a single specimen are often prepared with different stains to collect comprehensive information.

Function

Most tissue stains are applied to samples removed from the body. However, in rare cases, specialized stains known as vital stains can be used directly on tissue still in the body. These stains are valuable for identifying specific tissue types and detecting abnormal tissue, making subsequent biopsies more targeted and accurate in capturing the affected area.

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Tissue Preparation

Before specific staining can take place, tissue samples must first go through several preparation steps, including fixation, processing, embedding, sectioning, and sometimes antigen retrieval. In modern histology labs, most of these steps are automated for efficiency and consistency.

- **Fixation**: This step uses chemical fixatives to preserve the tissue's natural structure and protect it from degradation by cross-linking proteins irreversibly. While there are various specialized fixatives, Neutral Buffered Formalin is a standard choice. Fixation is crucial because it retains the chemical composition of the tissue, hardening the sample and making it easier to section. Paraffin-formalin, another effective fixative, is ideal for immunostaining but requires special preparation at the time of fixation. Bouin's solution is commonly used for preserving delicate nuclei and glycogen in embryonic and brain tissues, although it does not preserve kidney tissues well and distorts mitochondrial structure.
- **Dehydration**: Dehydration is achieved by adding ethanol, which removes water from the tissue sample, further hardening it for light microscopy. Following ethanol application, xylene is used to clear away the ethanol, completing the dehydration process.
- Embedding: Embedding involves placing the sample in paraffin wax or plastic resin, which enhances the extraction of cellular structures. However, caution is required if immunostaining is planned, as paraffin can inhibit antibody penetration and potentially yield false results.
- Sectioning: Sectioning involves mounting the specimen onto a microtome and slicing it into thin sections, typically 4-5 micrometers thick. This thinness allows for effective staining and examination on a microscope slide.
- Antigen Retrieval: This step is necessary to expose antigens that may have been masked during fixation and embedding. Cross-linked proteins can sometimes conceal antigen sites, reducing immunohistochemical response. Antigen retrieval is performed using heat or proteolytic methods to break these cross-links and reveal epitopes and antigens. While there is a risk of denaturing both the fixative and antigens, effective antigen retrieval can enhance immunostaining results.

Histochemistry and Cytochemistry

- **Hematoxylin and Eosin (H&E)**: As a dual staining method, H&E staining uses hematoxylin as a basic dye to color acidic structures purple-blue (basophilic), such as DNA in cell nuclei, RNA in ribosomes, and rough endoplasmic reticulum. Eosin is applied afterward as a counterstain, targeting basic structures and coloring them pink-red (eosinophilic). The cytoplasm is an example of an eosinophilic structure.
- **Gram Stain**: This sequential staining method, developed to differentiate bacterial species, helps identify bacterial infections by staining bacterial cell walls. Bacterial samples are heat-fixed and stained in four steps: primary staining with crystal violet, secondary staining with Gram's iodine, decolorizing with alcohol or acetone, and counterstaining with safranin. Gram-positive bacteria, which have thick peptidoglycan layers, retain the violet stain and appear purple. In contrast, gram-negative bacteria, with thinner peptidoglycan layers and more lipids, lose the violet stain during decolorizing and appear pink.
- **Giemsa Stain**: Common in hematology, the Giemsa stain is effective for staining bone marrow, plasma cells, and mast cells and is widely used for detecting blood parasites. Giemsa staining can also help visualize chromosomal abnormalities by revealing dark and light nucleotide bands on chromosomes during mitosis, known as Giemsa-based banding.

- **Periodic Acid Schiff (PAS) Reaction**. The Periodic Acid Schiff (PAS) Reaction stain is used to identify structures rich in carbohydrates, such as the intestinal brush border, renal tubular cells, mucus, and reticular fibers in connective tissue. Glycogen, glycoproteins, glycolipids, and mucins appear red or magenta after staining. In this process, periodic acid, a strong oxidizing agent, transforms hydroxyl groups on adjacent sugar molecules into aldehydes. The Schiff reagent then binds to the aldehydes, producing a red-magenta color for visual examination.
- **Masson's Trichrome**. Masson's Trichrome stain is a multicolor staining technique, widely recognized for its ability to stain collagen fibers blue, despite the presence of red counterstains. It is commonly used to detect fibrosis in the heart and lungs, chronic kidney disease, and muscular dystrophy.
- **Congo Red**. Congo Red is a water-soluble blue dye that forms a red solution in a pH range of 3.0-5.0. The dye binds through hydrophobic interactions, particularly within amyloid fibers, which stain red to orange, making Congo Red useful in diagnosing amyloidosis. Under polarized light, Congo Red-stained tissue with high amyloid content exhibits bright "apple green" birefringence.
- **Prussian Blue**. The Prussian Blue stain identifies iron deposits in tissue. The staining process involves treating the tissue with hydrochloric acid, which reacts with ferric ions to produce a bright blue pigment. This stain is valuable in diagnosing iron overload conditions, such as hemochromatosis or hemosiderosis, as it highlights iron accumulation in liver tissue, particularly around peri-portal hepatocytes or along sinusoidal linings. High iron stores in bone marrow may indicate ineffective red blood cell production, as seen in anemia of chronic disease, while a negative result may suggest iron deficiency anemia.
- **Mucicarmine**. Mucicarmine stain targets mucin, a secretion produced by epithelial and connective tissue cells. When aluminum binds with carmine, it forms a positively charged complex that attaches to mucin, turning it red for easy identification. This stain is especially useful in identifying carcinomas and inflammatory conditions with high mucin production. It can also help locate primary tumors by highlighting mucus-secreting epithelium at sites where mucin-producing cells are not typically present, and it stains the capsule of the fungus Cryptococcus.
- **Sudan Black**. Sudan Black stains lipid-rich structures like triglycerides and lipoproteins in a dark black or brown color. To avoid removing lipids, tissue samples are not dehydrated with alcohol. This stain is helpful in diagnosing conditions like atherosclerosis by highlighting atherosclerotic plaques and identifying leukodystrophy through macrophage staining in brain white matter post-mortem.
- **Oil Red O**. Similar to Sudan Black, Oil Red O is commonly used to stain hydrophobic fats and lipids, which are traditionally difficult to visualize. This stain is especially useful for examining atherosclerotic plaques and lipid accumulation in liver and muscle tissues.
- **Silver Stain**. Silver stains encompass various staining techniques used to study accumulation-related neurological diseases. Methods such as Bielschowsky, Gallyas, Bodian, and Campbell-Switzer attach silver ions or salt complexes to target tissue, which are then reduced in situ to form silver particles. Fluorolabeling can enhance this process by generating nanoparticles that produce specific colors based on particle size, ranging from yellow to black. Silver staining is widely known for detecting amyloid beta-protein in Alzheimer's disease and Pick bodies in Pick's disease, where amyloid plaques darken depending on their concentration.
- **Nissl Stain**. The Nissl stain, also known as Cresyl Violet stain, uses a basic aniline dye to examine neurons in the brain and spinal cord. This stain colors the neuropil a purplish-blue, with ribosome-

rich Nissl substance appearing dark blue. It is particularly useful in neuropathology because it highlights the neuronal cytoplasm without staining other cell structures, like astrocytes.

- **Papanicolaou Stain**. The Papanicolaou stain, or Pap smear, is a multichromatic staining technique widely used for detecting cervical cancer. It involves collecting cells from gynecological smears, sputum, or fine needle aspiration, then applying five dyes: Hematoxylin for nuclei, Orange G for keratin, eosin for superficial structures, Light Green SF for cytoplasm, and Bismarck Brown. For cervical screenings, this stain reveals epithelial cells in the cervix's transitional zone, allowing for precancerous and cancerous analysis, often supplemented with immunostaining using the biomarker p16INK4a to detect dysplasia.

Light Microscopy

The light microscope, also known as the optical microscope, is suitable for observing both living and non-living specimens. Although its maximum magnification is lower than that of an electron microscope, reaching up to 1500 times, it is widely used with common stains like the Gram stain, H&E, and Giemsa. Unlike electron microscopes, the light microscope uses visible light for illumination rather than electron beams.

Electron Microscopy

The electron microscope is ideal for examining intracellular structures that are not visible with light microscopy, providing valuable insight into cellular abnormalities. Electron microscopes can magnify up to 100 to 300 times more than the highest light microscopy magnification. For effective imaging, tissue sections need to be ultra-thin to allow for electron penetration. Stains containing heavy metal salts are particularly useful in electron microscopy, as they produce the phase contrast needed to reveal fine structural details. Transmission electron microscopes are typically used for examining internal cell structures, while scanning electron microscopes are preferred for surface analysis.

Pathophysiology

Special stains not only reveal structural alterations in tissues but also provide insights into functional changes relevant to diagnosis. They can indicate abnormal deposits, such as iron or protein (in conditions like amyloidosis or paraproteinemia), abnormal glycogen or carbohydrate accumulations, and atypical fat deposits. Specialized staining techniques help detect a range of cellular physiology abnormalities.

Clinical Significance

Histological staining and examination are of significant clinical importance in diagnosing and treating diseases across nearly all areas of medicine. Histological analysis is the gold standard for diagnosing numerous pathologies, with staining as a fundamental part of the process. Through histochemical analysis, pathologists can diagnose conditions, assess disease severity, and offer prognostic information, guiding treatment decisions.

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