Special Stains Used in Histopathological Techniques: A Brief View

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Abstract

The routine staining which is also called as H & E staining (Hematoxylin and eosin) is the most widely used stain in the laboratories or histopathology laboratory. In this procedure the nuclear protein stains purple and the cytoplasm and any other substances stains as red or orange. It plays a very crucial role in medical diagnosis or research where the specimen or tissue is been sent for processing. The staining procedure is performed by trained professionals and after which the role of highly trained pathologists and researchers come, which under the microscope view the specific structures, cells and even microorganisms to provide a diagnosis for the specific disease. The special stains are those which come under the special staining technique or alternative method for the H & E stains, where H & E staining does not provide the proper identification or appreciation of certain normal and abnormal tissue or structure which the pathologist or researchers needs. This article throws light on the Special stains which are been used in histopathological procedures in which these abnormal or normal tissues can be properly appreciated. The article also focuses on the procedure, principle and uses of these special stains in their respective field.

Keywords: *H & E stain, Special stain, Histopathology, Pathologist.*

Introduction

Special stains are those which come into the action where the given Hematoxylin and eosin staining does not provide much information regarding the tissue and structure of cells. Some of the particular tissue structure does not stain well with H & E stains like the reticular fibers in the liver which need a special staining technique, this special staining technique is performed for a better appreciation of normal and abnormal substance which is present in the cell and tissue.[1] These stains are used to identify specific microorganisms like bacteria or parasites, carbohydrates, lipids, mucosubstances which include both neutral and acidic, nucleic acid, and minerals along with connective tissues. The special stains include classification which makes it a convenient choice for different diagnosing procedures.[2]

Classification The classification includes:

I. Stains for amyloid

II. Stains for carbohydrates

III. Stains for microorganisms

IV. Lipid stains

V. Connective tissue stains

VI. Nucleic acid stains

VII. Stains for pigments and minerals

- Carbohydrate Staining- The carbohydrates are of two types of simple carbohydrates which include monosaccharides, oligosaccharides, and polysaccharides, whereas glycoconjugates consist of proteoglycans, mucins,and others glycoprotein.

A carbohydrate staining includes many techniques in which some of the most widely used method have been described:-

Periodic acid Schiff (PAS) stain- The dyes used in this technique involve 1%periodic acid and Schiff's reagent. The substance which shows positive result to PAS has purple magenta color and nuclei are seen blue. The substances which show positive PAS are

glycogen, neutral mucoprotein, glycoprotein, all fungi, cerebrosides, basement membrane, and phosphorylated sugar.[3]

This stain is used in the diagnosis of several medical conditions like mucins in adenocarcinoma of the large intestine, demonstration of fungi, glycogen storage disorder, Ewing's sarcoma, rhabdomyosarcoma, Whipple's disease, etc.

Alcian Blue Method: The reagents used are Alcian blue, aluminum sulfate, and nuclear fast red.

The results of this method show the blue color appearance of Hyaluronic acid, sialomucin, proteoglycans, and the nucleus is red. Neutral mucins are not reactive to Alcian blue.[4]

Combined Alcian blue-PAS method: The principle of this method is to differentiate the acid mucin from neutral mucin and demonstrate the presence of mucin. In this method only neutral mucin will be stained in magenta color appearance.

Mucicarmine Technique: The positively charged carmine and Mucicarmine bonds with negatively charged acid mucin in result acidic mucin stain strongly whereas neutral mucin does not stain. The reagents involved carmine, aluminum hydroxide, and 50% alcohol and aluminum chloride with Mayer's Hematoxylin. It is used in GIT disorders and to identify the acidic mucin also to identify fungi-Cryptococcus neoformans, etc.[5]

Nucleic Acids Staining: There are 2 nucleic acids which are DNA which is present in the nucleus & RNA that is present in the cytoplasm. They consist of deoxyribose or ribose, phosphate, and a nitrogenous base. The demonstration of nucleic acids depends upon the following factors which include the production of the aldehydes from sugar and reaction of dyes with the phosphate groups. There is no histochemical method to demonstrate the nitrogenous base. Several mostly used techniques to demonstrate the DNA includes-

- **Feulgen Stain:** In this staining procedure the solutions used are 1ml HCL acid, Schiff's reagent in which aldehydes are demonstrated, and bisulfate solution. The result of this staining gives Red-purple color to DNA & Green color to the cytoplasm. The standard control method for the Feulgen technique is the NAH method. (Naphthoic acid hydrazide). The blue thionin Feulgen technique is mostly used in the research for studying the nuclear morphology and ploidy of cancer cells. NOTE: The RNA can't be demonstrated by this stain as ribose purine bond is unaffected by hydrolysis 1M HCL.6,7
- **Methyl Green pyronin Method:** In this staining the reagents used is Methyl green in which the pure methyl green is specified for DNA and the impure dye contains methyl violet which can be removed by washing with chloroform. The NH2 of the dye reacts with the phosphate of DNA. The other reagent which is used is pyronin that binds to negatively charged constituent of tissue and also binds with mucins and cartilage, apart from RNA. The results show Green –Blue color to DNA whereas RNA shows Red color.

Staining of Lipids-3 forms of lipids is simple, compound, and derivative which includes such as fats, fatty acids, or cholesterol. The most widely used method for staining of lipids are Sudan black B stain and oil red o stain.[8]

Sudan Black B Method: This method is used for staining the lipoprotein,neutral triglycerides and it is very useful in hematological disorder. The result which it gives is fat is seen blue or black color and nuclei is seen red color.

Stains For Amyloid: Amyloid is an extracellular, amorphous, and eosinophilic material. It is composed of a protein in an anti-parallel beta-pleated sheet of configuration. Many times Hematoxylin and eosin is been confused with hyaline and fibrinoid substances. Earlier the stain which was used for amyloid was Iodine which was introduced by Virchow. The staining method widely used for amyloid is Congo red stain and Methyl crystal violet method.[9,10]

Congo red Stain: The mechanism principle is that the amyloidis extracellular, eosinophilic, and homogenous which can damage the surrounding tissue,hence when it is stained with Congo red the amyloid shows apple green color i.e. birefringence. The reagent includes 0.5%Congo red and 0.2% potassium hydroxide in 50% & 80% alcohol. It is used in renal amyloidosis, medullary carcinoma of thyroid, Alzheimer's disease, etc. The result gives nuclei blue color and amyloid, eosinophilic granules show red to pink in color.

Methyl/Crystal violet Method: The reagents used are 95% alcohol, crystal, 0.2% acetic acid, and 1% aqueous ammonium oxalate. The procedure includes

first the solution is prepared by using 2-gram crystal or methyl violet, alcohol, and 1% aqueous ammonium oxalate. After that the section is brought to water level then stain in crystal solution for five minutes after that washing and differentiating is done in 0.2% acetic acid and at last washes and mount in DPX. The result shows purple color is seen in amyloid whereas blue is seen in the background.[11,12]

Stains For Microorganisms: There are different types of staining method for different types of microorganisms which include bacteria, spirochetes, fungi. Different staining process includes Ziehl Nielsen stain, wade file Faraco stain, Warthin starry stain, Giemsa stain, and Gram staining. Among these widely used staining process is been described.

ZN stain process- In this method mycobacterium bacillus is stained. Mycobacteria cannot be demonstrated by gram's staining process as they possess a capsule that contains long-chain fatty acid i.e. Mycolic acid which makes it hydrophobic and it can be stained by carbol fuchsin. The Mycobacteria shows PAS-positive as their cell wall contains carbohydrates. The result of ZN stains shows red color to mycobacterium and background appears pale blue.[12]

Gram staining of bacteria- Includes:

Modified Brown- Brenn Method: This method includes the reagents like crystal violet stain, ethyl alcohol, gram's iodine solution, acetone-xylene solution, basic fuchsine, and picric acid, 0.1% in acetone. The result of this staining process gives Gram +ve bacteria shows blue color whereas Gram –ve bacteria shows red and the nuclei are also shows red color and yellow color presents other tissue elements.

Staining for spirochetes Warthin starry method is mostly used for spirochetes.

Warthin starry method: The reagents involved acetate buffer pH- 3.6 & 1% silver nitrate. This staining process gives the result as spirochetes-Black & Background – golden- yellow.[13]

Fungal stains Mostly used technique is GMS (Gomori methenamine silver nitrate): The reagents involves are 4%chromic acid, 1% sodium bisulfate, 5% sodium thiosulfate and 0.21% silver nitrate and light green solution and the results gives black color appearance of pneumocystis and fungi. The melanin also appears black. The dark grey appearance shows the presence of mucin and glycogen whereas pale green color appears in the background. [14]

The Giemsa stain is used for parasites:

Connective Tissue Stains: The connective tissues provide a matrix that connects and binds the cells and organs, which gives support to the body. Connective tissue consists of a proper one which includes loose and dense connective tissue like adipose, irregular, etc. The other one is the specialized connective tissue which includes bone, blood, and cartilage.

The Stains used for collagen fibers includes many techniques in which Masson's trichrome technique is mostly used.[15]

Masson's Trichrome Technique: The reagents used in this technique are Weigert's iron Hematoxylin, acid fuchsin, glacial acetic acid, methyl blue, and phosphomolybdic acid. The fixatives which are recommended are bruin's, Zenke's formal mercury, and zinc formalin.

The technique demonstrates the collagen and muscle in normal tissue, identifying the increase in collagenous tissue, to differentiate between collagen and muscle in tumors. It can indicate the fibrotic change in cirrhosis of the liver and also fibrotic changes in pyelonephritis. It gives blue or black color to nuclei, RBC, and muscle shows red color, and the cytoplasm appears in yellow.

Stains for pigments and minerals: There are different pigments including endogenous, exogenous, and artifact pigments. Different staining process includes:

Perl's Stain: This stain helps in identifying the ferric ions. When the ferric ions come in contact with ferrocyanide it gives Prussian blue which is a blue pigment. The result showsthe blue color of iron and nuclei appears in red whereas the background is pink in color.

Modified Fouchet's Technique-It is used for bile pigments. The reagents involved are Fouchet's reagent which includes 25% aq trichloracetic acid, 60% aq ferric chloride, distilled water, and Sirius red stain. The procedures follow to bring the sections to distilled water and oxidized in Fouchet's for five minutes then rinse well in distilled water and stain with citrus red for at least 30 minutes then dehydrate, clear and mount. The result

gives green color appearance to bile pigments, collagen shows red and background is yellow in this respective staining process.[16]

There are several staining tests for the demonstration of melanin which include Schmorl's ferrioferricyanide reduction test, enzyme method, IHC, and Masson Fontana silver method & Fluorescent method.

Masson Fontana Method: It is used for the identification of melanin and also argentaffin granules. In the diagnosis of malignant melanoma is also used. The principle of this process is as the melanin is insoluble in organic solvents and it is a non-lipid, non-hematogenous pigment that dissolves in 1M sodium hydroxide. The ammoniac silver nitrate solutions are reduced to black metallic silver by melanin and the result shows the black color appearance of melanin, and argentaffin granules whereas nuclei appear red.[17]

A note on IHC: The immunohistochemistry technique is the most useful and widely used staining process as it is used in the diagnosis of cancer or treatment of cancer, for differential diagnosis and has its application use in the research works. The method combines the immunological, histochemical and histological process foridentifying the specific tissue by using the definite antigen/antibody reaction which is a tag by an evident label. This process allows visualizing the specific cellular components which is been distributed and localized within a cell or tissues. The interaction of antigen-antibody is visualized by different types of markers like an enzyme, radioactive element, Fluorescent dye, or colloidal gold. The antibody is made up of two types of protein chain which includes heavy chain and light chain. Five types of immunoglobulin classes are found in serum which include IgG, IgM, IgE, IgD, & IgA. They all are distinguishing based onthe Heavy chain they contain. However every technique has its advantages and disadvantages & its disadvantages include such as the cost of the technique is high and it is not permanent, etc.[18]

Conclusion

Special stains are widely used in the different processes for the enhanced appreciation or detection of normal and abnormal substances, though the H & E stain is a routine test for histopathological diagnosis. The special stains give a more accurate result for the proper diagnosing of the respective disease and also it enhances the beneficiate in the research works. The pathologist and researchers should have a broad and clear cut idea regarding the special staining process for better outcomes. The use of special stains has revolutionized medical diagnosis in the Histopathological field and their application will surely lead the future.

Ethical Permission: Not required

Conflict of Interests: None

Funding: None

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