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Pentachrome Staining: A Comprehensive Review of the Novel Method for Collagen and Sulfated Mucopolysaccharides

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Abstract: ***One of the most prevalent fibers in the extracellular matrix is collagen, which is also home to sulfated mucopolysaccharides. Sulfated mucopolysaccharides are also found in certain secretory glands and goblet cells. This article's goal is to provide a review of novel staining technique that can identify these two macromolecules in the same sample at the same time. Using the given procedure, tissues are stained in five primary colors: red for collagen, violet for sulfated mucopolysaccharides, yellow for red blood cells, orange for muscle, and green for nuclei. In this study, xylene deparaffinized tissue sections are used for the pentachrome staining. Xylene is expensive, dangerous, and will contaminate the work place. There's also the disposal issue. In some other studies, eco-friendly substitutes are successfully used for the deparaffinization of sections for H&E staining. Therefore, for the pentachrome staining also xylene free deparaffinization may be used. Recommendations for future research are made in order to overcome this knowledge gap.***

Keywords: collagen, sulfated mucopolysaccharides, pentachrome

1. Introduction

Even in cartilage, two tissue components that make up the extracellular matrix are collagen and sulfated mucopolysaccharides. Additionally, certain goblet cells and secretory glands contain sulfated mucopolysaccharides. Basic histology uses a variety of staining techniques. Collagen has historically been stained with various dyes, such as Sirius red or light green, which are parts of the Masson Trichrome staining process. But sulfated mucopolysaccharides are colored using dyes such as alcian blue or toluidine blue. Each sample exhibited five basic colors, each of which was linked to a distinct structure. Muscles were dyed orange, red blood cells were stained yellow, sulfated mucopolysaccharides were stained violet, and collagen fibers were stained red. Leukocytes, like lymphocytes and plasma cells, were dyed green because of their pronounced nuclei. Using inexpensive, nontoxic, and less biohazardous environmentally friendly chemicals is the main objective in all life science domains. In the home, washing dishes with liquid soap is a daily chore. To clean greasy kitchenware, this liquid detergent is used in kitchens. The clever idea to dewax the tissue sections by using liquid dishwashing detergent in place of xylene. Xylene is a vital component of a pathology lab. The histology laboratory's previous use of xylene serves as an example of a subpar substitute.

2. Principle of new pentachrome method

Three reagents are used in the new pentachrome approach to produce the five basic colors of collagen, sulfated mucopolysaccharides, muscles, leukocytes, and red blood cells: 6 percent nitric acid, toluidine blue solution, and picrosirus solution. Toluidine blue solution is typically used to stain cells and tissues blue. However, this new pentachrome approach does not stain sulfated mucopolysaccharides blue because it pre-treats them with 6% nitric acid, which changes most of the anionic groups to their protonated form. The majority of cell and tissue structures contain carboxylic groups, which can be protonated. This can prevent tolonium, the cation in toluidine blue solution, from binding. Sulfated mucopolysaccharides hence lose their initial blue staining color. Nitric acid therapy, however, does not cause sulphate ions to be protonated. Because of tolonium's intriguing property known as metachromasia—which causes its colour to shift from blue to violet when it attaches to materials with concentrated negative charges, such as glycosaminoglycans—sulfated mucopolysaccharides are consequently dyed violet. When it binds to substances or structures that contain a lot of anions, it is because of tolonium polymerization. The novel tolonium "polymer" is violet in hue. Sulfated mucopolysaccharides tend to concentrate a large number of the tolonium polymers because the 6 percent nitric acid treatment does not protonate sulphate ions in the sample. Because of this, only tissues containing sulfated mucopolysaccharides—such as cartilage, mast cells, and some secretory glands—will tint violet when stained with toluidine blue. Because they contain sulfated mucopolysaccharides, cartilage, goblet cells, and secretory glands are so stained violet. Collagen and red blood cells are stained in red and yellow, respectively. These colors are the result of the picrosirus solution, which contains two colorants: Sirius red and picric acid. Sirius red stains collagen, while picric acid stains cytoplasms. So collagen is stained in red, and red blood cells are stained in yellow. The green color of lymphocyte and plasma cells are due to the mixing of tolonium cation and picric acid. Tolonium cation binds to phosphate groups in the nucleus, providing some weak ortochromatic blue staining in the nucleus . Afterward, picric acid, which has a negative charge, binds to tolonium cation. Since tolonium has a blue color and picric acid has a yellow color, they mix together to create a green color.

3. Xylene- free deparaffinization

There is a chance of xylene exposure during tissue processing, deparaffinizing tissue sections, cover slippage, cleaning tissue processors, and recycling in a laboratory. When tissue is treated and the slices are dewaxed before staining, methanol exposure occurs. In an attempt to stop using xylene in laboratory settings, a variety of substitute chemicals have been employed, including limonene reagents, aliphatic hydrocarbons, aromatic hydrocarbons, vegetable oils, olive oil, and mineral oil substitutes. These substances were used in place of xylene as a clearing agent during routine processing, even though xylene is handled and exposed to the greatest extent during the dewaxing of the tissue sections. Using inexpensive, nontoxic, and less biohazardous environmentally friendly chemicals is the main objective in all life science domains. In the home, washing dishes with liquid soap is a daily chore. To clean greasy kitchenware, this liquid detergent is used in kitchens. The clever idea to dewax the tissue sections by using liquid dishwashing detergent in place of xylene. Xylene is a vital component of a pathology lab. The histology laboratory's previous use of xylene serves as an example of a subpar substitute. Standardised methods for the disposal of xylene in pathology labs are lacking in underdeveloped countries, as are ways to track exposure. Therefore, any technique that reduces the amount of xylene used by using non-biohazardous substitutes, shortens the staining time, and does not compromise the staining quality will be very valuable for both diagnostic purposes and maintaining a healthy laboratory environment. This will minimise the risk to the laboratory personnel. As a result, the study uses diluted liquid dishwashing solution as a deparaffinizing agent for the H&E staining procedure as a less hazardous, more accessible, and cost-effective method of enhancing the working conditions in a histology lab. The liquid dishwashing solution is composed of cocamidopropyl betaine, sodium laureth sulphate, sodium dodecyl benzene sulfonate, and nonionic surfactants.Anionic surfactants, which are commonly found in shampoos and detergent soaps, are these chemicals.

4. Conclusion and future aspects

The newly reported pentachrome approach is quick and easy to use, and it produces five different coloured stains for five different tissue architectures. Future research on the potential applications of this technique, including as a teaching aid for histology, a fundamental histology approach, or even in histopathological and cytopathological studies, could be fascinating. It is evident from this review of the literature that there are many gaps in our knowledge of pentachrome staining of tissue sections. Excellent positive results were obtained using the xylene-free H and E staining method utilising a simple diluted dish soap solution, which is comparable to the traditional H and E method. It yields a sharp nuclear and cytoplasmic staining as well as a high-quality staining with enough clarity. Its other benefits include being nontoxic, affordable, nonflammable, nonhazardous, easy to handle, and having no disposal issues. It also takes less time to stain. Therefore, for the pentachrome staining also xylene free deparaffinization may be used. Recommendations for future research are made in order to overcome this knowledge gap.

References

[1] Leslie PG, Hiatt J. Color Textbook of Histology. 3rd edition. Philadelphia: WB Saunders Company; 2001.
[2] Kierszenbaum AL, Tres LL. Histology and Cell Biology: An Introduction to Pathology. 3rd edition. Philadelphia: Elsevier Saunders; 2012.
[3] Ross MH, Pawlina W. Histología. 5th edition. Buenos Aires: Panamericana; 2007.
[4] Kiernan JA. Histological and histochemical methods. Oxford, UK: Butterworth Heinemann; 2008.
[5] Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 6th edition. Philadelphia: Churchill Livingstone; 2008.
[6] Cook DJ. Cellular Pathology: An Introduction to Techniques and Applications. 2nd edition. Oxford, UK: Scion Publishing Limited; 2006.
[7] John D. bancrofti, Marilyn Gamble: “Theory and Practice of Histological Techniques” 5th edition; Churchill Livingstone;85
[8] Falkholm L, Grant C A, Magnusson A, Moller E. “Xylene-Free Method for Histological Preparation: A Multicentre Evaluation”. Lab Invest 2001; 81:1213-21
[9] Falkholm L, Grant C A, Magnusson A, Moller E. “Xylene-Free Method for Histological Preparation: A Multicentre Evaluation”. Lab Invest 2001; 81:1213-21
[10] Buesa R J, Peshkov M V. “Histology without Xylene” Ann Diagn Pathol 2009;13:246-56
[11] Buesa R J, Peshkov M V. “Histology without Xylene” Ann Diagn Pathol 2009;13:246-56
[12] Kevin Doello, “A New Pentachrome Method for the Simultaneous Staining of Collagen and Sulphated Mucopolysaccharides”

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