MAYERS'S HEMATOXYLIN SOLUTION

Principle

Mayer's haematoxylin is one of the types of haematoxylin normally used in haematoxylin-eosin stains. Its mode of staining is progressive, i.e. the longer it remains in the staining solution the more staining is achieved in the tissue.

Specifically, Mayer's hematoxylin has a very specific action on the nucleus when a progressive process is used, particularly in situations where a nuclear stain is needed to emphasize a cytoplasmic component, which has been demonstrated by a special stain and where acid/alcohol differentiation can destroy or discolor the stained cytoplasmic component. It is used as a nuclear stain in the demonstration of glycogen in various histochemical enzymatic techniques.

Mayer's haematoxylin is usually used progressively, although it can also be used retroactively. In a progressive staining, the sample is stained to the point of interest to the operator, checking it under the microscope or once it has been standardised, simply by the staining time.

After staining the colour obtained is burgundy-red, instead of the expected and classic blue-violet of the preparations stained with hemateine. At acidic pH, hemateine is red, but at slightly basic pH its colour changes to blue-violet. This phenomenon is called "blueing" and can be achieved by covering the preparation with slightly alkaline solutions or, more commonly, with cheap and convenient tap water.

At this point, the preparation would show, roughly speaking, a large number of cell nuclei and it would be very difficult to distinguish the cells to which they would belong. It becomes necessary to use a contrast dye to stain the structures not marked by the hematein. Eosin is the most common, as it stains cytoplasms, cell matrices and erythrocytes among others, providing a whole range of pink and red colour tones.

Material

Paraffinic cuts, frozen cuts, clinical cytological material

Reagents

Code	Description
251299	Eosin Tellowish (C.I. 45380) for clinical diagnosis (*)
256879	Eosin Yellowish alcoholic solution 1% for clinical diagnosis (*)
251301	Eosin Yellowish hydroalcoholic solution 1% for clinical diagnosis (*)
255298	Carazzi's Hematoxylin solution for clinical diagnosis (*)
253949	Harris Hematoxylin solution for clinical diagnosis (*)
256991	Harris Hematoxylin modified solution for clinical diagnosis (*)
252081	Phloxine B (C.I. 45410) for clinical diagnosis
251008	Acetic Acid glacial for clinical diagnosis
251769	Xylene, mixture of isomers for clinical diagnosis (*)
192695	Ethanol 70% v/v (BP) pharma grade

251085	Ethanol 96% v/v for clinical diagnosis (*)
251086	Ethanol absolute for clinical diagnosis (*)
253681	Eukitt ®, mounting medium for clinical diagnosis

Procedure

1. *Solution 1: Dissolve 1.0 g of yellowish eosin in water and dilute to 100.0 ml with distilled water.*
2. *Solution 2: dissolve 1.0 g of Floxin in water and dilute to 100.0 ml with water.*

Dewaxing of samples:

3. Soak the preparation in Xylene Container 1 for 5 minutes.
4. Soak the preparation in Xylene Contendor 2 for 5 minutes.
5. Soak the preparation in Xylene Contendor 3 for 5 minutes.

Preparation hydration

6. Soak the preparation in Container 1 of Absolute Ethanol for 7 minutes.
7. Soak the preparation in 90% Ethanol for 7 minutes.
8. Soak the preparation in 70% Ethanol for 7 minutes.
9. Soak the preparation in distilled water for 7 minutes.

Haematoxylin/Eosin Staining [1][3]

10. With the dropper, dose the required amount of sample onto the preparationto cover the preparation (4-5 drops) and let it act for 10 - 15 minutes, depending on the size of the sample.
11. Wash well in Container 1 of Tap Water for 5 minutes
12. Immerse the preparation in 70% ethanol for 10 seconds
13. Soak in tap water until it turns blue for 10 - 15 minutes.
14. Allow to drain for a few seconds and immerse the preparation in Eosin-Floxin Reagent for 10 minutes

15. Wash with tap water for 1 - 5 minutes

Dehydration of preparation, rinsing and assembly

16. Immerse the preparation in 70% ethanol for 5 seconds.
17. Soak the preparation in 90% Ethanol for 5 seconds.
18. Immerse the preparation in Container 2 Absolute Ethanol for 1 minute.
19. Soak the preparation in Container 1 Absolute Ethanol for 5 minutes,
20. Immerse the preparation in Xylene Container 3 for 5 seconds.
21. Allow to dry for 5 minutes.
22. Soak the preparation in Xylene container 2 for 5 minutes.
23. Soak the preparation in Xylene container 1 for 5 minutes.
24. Allow to dry for a few minutes.
25. Assemble with a coverslip and mounting medium. Allow to dry over time to ensure that the mounting medium is completely solidified, and the coverslip is firmly fixed to the slide.
26. Observe the staining carried out under the microscope. The objectives used should be l0x and 40x.

Results

Nuclei	Blue
Cytoplasm and the extracellular matrix	Different shades of pink to red

Technical note

The microscope used should correspond to the requirements of a clinical diagnostic laboratory. If an automatic staining device is used, the operating instructions of the appliance manufacturer and the software must be observed.

Sample preparation

All samples should be treated according to the state of the technology. All samples must be unambiguously labeled.

Diagnostics

Diagnosis should be established only by authorized and qualified persons. Each application should involve appropriate controls to rule out erroneous results.

Storage

The staining solution should be stored at room temperature

Expiration

The product stored at the indicated temperature and in a tightly closed container is usable until the expiration date indicated on the package.

Notes on use

To avoid errors, the staining must be carried out by specialized personnel. For professional use only. The national directives on safety at work and quality assurance must be complied with.

Advise on disposal of waste

Solutions used and expired solutions should be disposed of as hazardous waste and local waste disposal regulations must be observed. If further questions are asked about disposal, they may be processed through E-Mail: info.es@itwreagents.com. Inside the EU are valid the requirements based on Council Directive 67/548/EEC on the approximation of the laws, regulations and laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances in the relevant version.

Classification of hazardous substances

Observe the classification of dangerous substances on the label and the information on the safety data sheet.

Manufacturer

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(*) Producto sanitario para Diagnóstico In Vitro

IVD CE