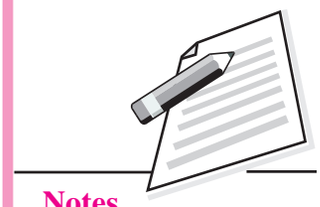


10

HEMATOXYLIN AND EOSIN STAINING

10.1 INTRODUCTION

The sections, as they are prepared, are colourless and different components cannot be appreciated. Staining them by different coloured dyes, having affinities of specific components of tissues, makes identification and study of their morphology possible. Hematoxylin and Eosin (H&E) is the most frequently used stain in histology.

OBJECTIVES

After reading this lesson, you will be able to:

- describe Hematoxylin and its preparation
- describe the properties of Hematoxylin
- explain Eosin and its preparation
- describe the method of staining.

10.2 HEMATOXYLIN

It is extracted from the bark of a tree", hematoxylom campechianum". The hematoxylin which we buy is extracted from this bloodwood tree. To obtain the bark of freshly logged tree is chipped off, then boil the chips in water. An orange red solution is obtained, which turns yellow, then black on cooling. The water is evaporated leaving crude hematoxylin. Further purification is done.

Solutions of the dye should be oxidized to retain its staining ability longer. The dye may be oxidized by exposure to the natural light for 3-4 months. Chemical

Notes

oxidation is achieved by using either sodium iodate or mercuric oxide. The chemical oxidation converts the dye almost instantaneously but the product does not have shelf life. Sodium iodate is most commonly used oxidizing agent (0.2 gm oxidizes 1.0 gm hematoxylin).

Hematoxylin is neither a dye nor it has coloring properties. For nuclear staining it is necessary to oxidize the hematoxylin to hematin which is a weak anionic purple dye. Anionic hematin will have no affinity for the nucleic acids of nuclei. Hence a metallic salt or mordant is combined with hematoxylin so that a positive charge to the dye is obtained by virtue of the metal action. Thus the cationic dye –metal complex will bind to the anionic nuclear chromatin. Various mordants are ammonium or potassium alum ferric salt, chrom alum and phosphotungstic acid. The tissue component most frequently demonstrated is nuclear chromatin using an alum mordant in the H&E staining method.

The combination of hematoxylin and mordant is called a hematoxylin lake. The aluminium lake formed with ammonium alum is particularly useful for staining nuclei. Hematoxylin recipes using these mordants are called alum hematoxylin.

10.3 PROPERTIES OF HEMATOXYLIN

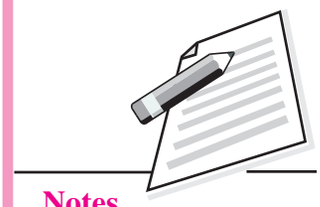
1. Hematoxylin has no staining property
2. Hematin with mordant such as ammonium or potassium alum forms lake which functions as cationic dye and stains anionic tissue components.
3. Hematin in an aqueous solution can be acidic or an alkaline dye depending on pH.
4. Hematin has affinity for several tissues with an appropriate mordant.

Progessive staining - When tissue is left in the stain just long enough to reach the proper end point. The slides have to be examined at different interval to find out when the staining is optimum.

Regressive staining - In this method the tissue is overstained and then destained (differentiate) until the proper endpoint is reached.

Harris hematoxylin is a regressive stain; the overstaining is removed by acid - alcohol. The removal of **this excess dye is called differentiation.**

The hematoxylin alum gives a reddish hue to the tissues because of acidic pH. To convert this colour to the final blue, alkaline pH is required. This process is called “blueing”. It is done either by tap water or by ammonium hydroxide.



Preparation of Harris's hematoxylin

Ingredients :

Hematoxylin	5gm
Absolute alcohol	50ml
Ammonium alum	100gm
Distilled water	1000ml
Mercuric oxide	2.5gm
Glacial acetic acid	40ml

Method - Dissolve the hematoxylin in absolute alcohol and ammonium alum in hot water. Mix the two solutions and heat to boiling. Remove from flame, and add mercuric oxide and cool rapidly. Glacial acetic acid if added gives brisk nuclear staining, but life of the solution is reduced. Hence if acetic acid is to be added, it should be added in working solution.

Preparation of Mayer's hematoxylin

Ingredients :

Hematoxylin	1.0gm
Distilled water	1000ml
Ammonium alum	50gm
Sodium iodate	0.2gm
Citric acid (reduces pH)	1.0gm
Chloral hydrate (preservative)	50gm

Method - Hematoxylin is dissolved in distilled water using gentle heat. Then alum is added and dissolved. Then sodium iodate, citric acid and chloral hydrate are added respectively.

10.4 EOSIN

Eosin is used as the counterstain that stains the cytoplasm rose coloured. The intensity of the eosin is individual choice. The most widely used eosin is "eosin Y". The "Y" stands for yellowish. It is available in either water soluble or alcohol soluble form. Most laboratories use the water soluble form of eosin Y in an alcohol-water solution which is described here.

Eosin Y (water soluble)	1.0gm
Distilled water	80ml

Notes

95% alcohol	320ml
Glacial acetic acid	0.4ml

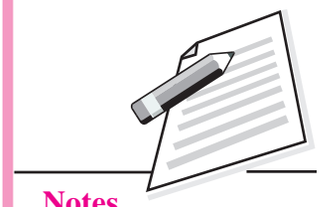
Preparation - Dissolve eosin in water and then add this to 95% alcohol (one part eosin solution with 4 parts alcohol). To the final mixture add a few drops of acetic acid (0.4ml). The acetic acid increases the staining intensity of eosin. When ready to use, the stain should be cloudy; if clear, add a few drops of the acetic acid. The solution should be standardized by staining the control slides.

10.5 METHOD OF STAINING

1. Deparaffinize sections in xylene, 10-20 minutes. Filter Hematoxylin.
2. Rehydrate sections:
   100% alcohol for 1-2 minutes
   95% alcohol for 1-2 minutes
3. Rinse in tap water
4. Rinse in distilled water
5. Stain with Hematoxylin for 3-5 minutes
6. Wash in tap water
7. Differentiate section with 1% HCl in 70% alcohol 1-2 dips and check under microscope. If necessary, return slides to HCl for further differentiation.
8. Wash slides in running tape water for 15 minutes
9. Stain slides in Eosin for 1-4 minutes
10. Dehydration and Differentiation:
    95% alcohol 5-6 dips
    100% alcohol 5-6 dips
11. Clear slides in xylene 2 times
12. Mount slides with mounting media (Permount or DPX)

Note

1. At no stage of staining the section should be dry
2. H&E is a regressive stain in which a tissue is over-stained and then excess dye is removed to obtain desired intensity of stain
3. Filter Hematoxylin each time before staining
4. Change most of alcohol and xylene each time before staining



INTEXT QUESTIONS 10.1

1. Most commonly used stain in histology is .....
2. ..... is the most commonly used oxidising agent
3. Tissue component commonly demonstrated is ..... by hematoxylin.
4. Combination of hematoxylin and mordant is called .....
5. In H & E staining ..... staining technique is followed
6. Process of removing excess dye is called .....
7. Converting red hue to blue colour by use of alkaline pH is called .....
8. ..... is used as counter stain which stains the cytoplasm rose colour

WHAT HAVE YOU LEARNT

- Staining with different coloured dyes makes identification and study of morphology possible
- Haemotoxylin and Eosin is the most commonly used stain in histology
- Sodium iodate is most commonly used oxidising agent
- Nuclear chromatin is usally demonstrated using H & E staining method
- Combination of hematoxylin & mordant is called hematoxylin lake
- Haematoxylin has no staining property, hematin has affinity for several tissues with an appropriate mordant
- Regressive staining is used in H & E staining
- The process of removing excess dye is called differentiation
- Process of converting red colour of tissue using alkaline pH to blue colour is called blueing

TERMINAL QUESTIONS

1. Explain the properties of hematoxylin
2. Explain preparation of hematoxylin and Eosin
3. Describe briefly H & E staining

Notes

ANSWERS TO INTEXT QUESTIONS

10.1

1. Hematoxylin & Eosin
2. Sodium iodate
3. Nuclear chromatin
4. Hematoxylin lake
5. Regressive
6. Differentiation
7. Blueing
8. Eosin