# HISTOCHEMICAL USES OF HAEMATOXYLIN - A REVIEW

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**ABSTRACT**

The natural dye, haematoxylin obtained from the logwood, haematoxylon campechianum is the most important and most used dye in histology, histochemistry, histopathology and in cytology. It is especially used in histopathology and cytology for the diagnosis of malignant and non malignant lesions. It can be used as a primary stain and as a counter stain where it will differentiate acidophilic materials from basophilic materials and stain non cellular substances such as fibrin, crystals and pigments in various shades depending on the nature of the mordant used and the second stain. Haematoxylin has been used extensively in the demonstration of certain parasites, lipids, carbohydrates, nucleic acids, metals, connective tissue fibers and in immunohistochemistry. Haematoxylin has also been used in the demonstration of several intracellular substances such as mitochondria, chromosomes, chromatin, nucleoli, centrioles, nuclear membrane, ground cytoplasm, cross striations of muscle fibres and chromatin granules in several staining techniques. Haematoxylin is therefore an indispensable dye in histochemistry and histopathology.

**Key words**: *Haematoxylin, histochemistry, staining, mordant*

## 1. INTRODUCTION

Haematoxylin is the most important and most used dye in the histopathology and histochemistry laboratories [1]. It is especially used in Erhlich's haematoxylin and eosin technique for the demonstration of general tissue structures where it stains tissue in shades of blue, pink and red enabling recognition of malignant and non malignant cells as well as several intracellular and extracellular substances necessary for diagnosis of disease conditions in tissues and cells [1]. The colour of the dye can be altered by mixing it with other agents such as iron alum (black), potash alum (blue) and salts of tin (red) [2]. Haematoxylin which has been used extensively as a nuclear counter stain and for the staining of specific intracellular and extracellular substances has also been used in the textile industries, although to a lesser extent. Haematoxylin is obtained from the logwood, haematoxylon campechianum [3]. The crude product is obtained from the milled heartwood by hot water or steam, purified by ether extraction, dried and recrystallized from water [4,5,6]. Alternatively, the aqueous extract is precipitated from solution with urea[7]. Haematoxylin has also been prepared synthetically [8], but it is not widely available. Haematoxylin has no staining properties, unless it is oxidized in a process called ripening to yield haematein, which is then combined with certain chemicals called mordants [1]. The process of oxidizing haematoxylin to haematein [5,9] (Fig.1), which is the active staining ingredient can be achieved naturally and artificially. In the natural process, prepared haematoxylin solution is exposed to air and sunlight for 6 to 8 weeks before it is used [10]. The advantage of the natural process of oxidation is that the solution lasts longer because oxidation is slow and gradual. In the artificial process of oxidation, oxidising agents such as sodium iodate, hydrogen peroxide, mercuric oxide and potassium permanganate are added to the solution of haematoxylin. These effect instant oxidation of haematoxylin to haematein and the solution may be used immediately, although with a shorter life span because dye solution is quickly over oxidized. Haematoxylin cannot stain a tissue unless a mordant is incorporated into the dye [11,12]. Mordants are metallic salts, which act as a bridge between the stain and tissue enabling staining to take place [1]. The colour of the staining reaction depends on the constituents of the staining solution and the type of mordant used. Some staining solutions, which contain aluminium alum and potassium alum as the mordant, give a blue nuclear staining while those which contain iron give a black staining reaction. Other metallic salts which have been combined with haematoxylin in special staining techniques are chrome alum for the staining of lipoproteins [13], myelin, phospholipids and cytoplasmic granules in B cells of the anterior pituitary and pancreatic islet [14], molybdenum for the staining of collagen[15,16] and neural tissue[17]. Copper haematoxylin has been used to stain fatty acids [13], myelin sheaths and mitochondria [18]. Lead-haematoxylin solution has been used for the staining of axis cylinders, although staining may be up to 6 weeks.

## 2. USES OF HAEMATOXYLIN

**Carbohydrates** Connective tissue mucin can be stained with Mayer's mucihaematein while an alum haematoxylin may be used to quench nuclear fluorescence, which would give a false positive result if not quenched when staining amyloid with thioflavine T fluorescence stain [17]. Haematoxylin has also been used as a nuclear stain in some

techniques when demonstrating carbohydrates. These techniques include Best's carmine for glycogen [18], Periodic acid Schiff for neutral mucopolysaccharide [21], Mucicarmine for mucin [22] and Congo red for amyloid [23].

**Lipids** Baker's acid haematein method is used for the demonstration of phospholipids [24], while the alum haematoxylins are used as nuclear counter stains when Oil red O is used for the staining of lipids [25].

**Connective tissue fibers** Verhoeff's haematoxylin [26] has been used for the staining of elastic fibers black. Weigert's haematoxylin [27] and Celestine blue haemalum sequence are used as nuclear stains when demonstrating connective tissue fibers particularly when subsequent staining reagents are acidic, such as van Gieson stain [28] which will remove alum haematoxylin from tissue.

**Nervous tissue** The Weigert-Pal [29] and Loyez [30] haematoxylin methods are used for staining myelin. The phosphotungstic acid haematoxylin [31] which is used for the staining of astrocytes, myoglia and fibroglia contains haematoxylin as the active constituent.

**Microfilaria and Amoeba** The differential diagnosis of microfilaria based on nuclear arrangement is enhanced when their nuclei are stained with hot haemalum. Amoeba in sections are also well demonstrated with haemalum [32].

**Intracellular substances** The Gomori's chrome alum haematoxylin phloxine has been used for the staining of alpha and beta cells of the pancreas [33]. The pituitary alpha and beta cells are well demonstrated with the trichrome stains, majority of which contain haematoxylin as the nuclear stain[34]. Heidenhain's iron haematoxylin is an excellent cytological stain, which stains chromosomes, chromatin, nucleoli, mitochondria, centriole and cross striations of muscle fibers in shades of black and grey [35].

**Cytology** Haematoxylin has been found extremely useful in cytology where it is used for differentiating malignant cells from non-malignant cells. Cytohormonal, sex chromatin body and nutritional influences on epithelial cells studied in the Papanicolaou technique [36] depend essentially on the staining character of haematoxylin [37].

**Fibrin** Celestine blue haemalum is used as a nuclear stain in Martius Scarlet Blue [34], and the Fuchsin-Millers method for fibrin.

**Metals** Aqueous solutions of haematoxylin have been used for the identification of lead, copper and iron (haemosiderin) deposits in tissues [38].

**Photomicrography** Being able to stain many cytoplasmic and nuclear structures in shades of black and grey, the Heidenhain's haematoxylin [35] is particularly an excellent stain for photomicrography.

**Immunohistochemistry S**ome immunohistochemical techniques for the demonstration of tissue antigens have been counter stained with haematoxylin in order to give a good contrast between the object demonstrated and the surrounding tissue [39,40].

## 3. BLUEING

Most haematoxylin solutions, which stain regressively, require differentiation [41], i.e. treatment with an acid reagent to remove excess stain from tissue section. The tissue section is subsequently treated with an alkaline solution or vapour to neutralise the acid and restore a blue colour to the tissue [42]. This process is called blueing and it can be achieved by treating tissue section with one of the following alkaline vapours or solutions.

1. Ammonia vapour for a few seconds
2. 5% ammonium hydroxide for 2 minutes
3. Running tap water for 10 minutes
4. 2% potassium hydroxide for 2 minutes
5. Scott's [43] tap water substitute (TWS) for 2 minutes.

TWS is prepared by dissolving 3.5g sodium bicarbonate and 20g magnesium sulphate in 1 litre of distilled water. A grain of thymol is added to serve as a preservative. It must be emphasized that the higher the pH of a blueing solution, the faster the speed at which blueing takes place but with a risk of tendency of sections to fall off slides.

## 4. ALUM HAEMATOXYLIN

Alum haematoxylin solutions contain potassium alum or ammonium alum as the mordant. They include Ehrlich's [43], Mayer's [31], Cole's [44], Harris [36], Delafield's [45], Iyiola and Avwioro's [55] and Carazzi's [54] haematoxylin. Alum haematoxylins are used when the counter stain does not contain an acid. Acidic counter stains such as van Gieson [27] rapidly remove alum haematoxylin from sections; therefore, they are not used on tissues, which have been stained with an alum haematoxylin.

**Ehrlich's haematoxylin** Ehrlich's haematoxylin [44] is a regressive stain requiring differentiation with 1% acid alcohol. It has a staining time of 5-30 minutes depending on the extent of oxidation of haematoxylin and previous treatment of tissue such as fixation. When counterstained with eosin, Ehrlich's haematoxylin is used for the demonstration of general tissue structures where they stain various tissue structures in shades of blue, pink and red. It also stains mucopolysaccharides and cement lines of bone [47]. The glycerine content helps to stabilize the stain and prevent over oxidation. It also slows down the rate of evaporation. The acetic acid in Ehrlich's haematoxylin reduces the pH and sharpens nuclear staining.

**Harris haematoxylin** Harris haematoxylin [37] is a powerful nuclear stain, which may be used regressively and progressively. In view of its improved selectivity of nuclear staining, it is generally used in exfoliative cytology for the demonstration of malignant and non malignant cells[1]. Staining time is 2-5 minutes. Harris haematoxylin contains mercuric oxide, which oxidizes haematoxylin to haematein (Fig.1) making it possible for the solution to be used almost immediately.

**Mayer's haemalum** Mayer's haemalum [32] is a more powerful stain than Ehrlich's haematoxylin and a precise nuclear stain which is used progressively, although, it may be used regressively with a staining time of 5-10 minutes. Mayer's haemalum [32], unlike Ehrlich's haematoxylin [42] does not stain mucopolysaccharides. Therefore, it is used as a nuclear counter stain for the demonstration of glycogen [48], amyloid [23] and Mucicarmine [22]. Nuclei of microfilaria and amoebae in sections and smears are well demonstrated with this stain. Mayer's haemalum [22] is also used in the Celestine blue-haemalum nuclear stain. Mayer's haemalum contains sodium iodate, which oxidizes haematoxylin to haematein; therefore, the stain may be used immediately after preparation. Chloral hydrate in Mayer's haematoxylin acts as a preservative while the citric acid sharpens nuclear staining. Potassium alum or ammonium alum is the mordant in Mayer's haematoxylin [1].

**Cole's haematoxylin** Cole's haematoxylin [45] can be as a progressive stain and it can also be used regressively as a routine stain similar to Ehrlich's haematoxylin with a staining time of about 10 minutes. Cole's haematoxylin [45] may be used in place of Mayer's haemalum [22] in the Celestine blue haemalum nuclear stain. It contains iodine, which oxidizes haematoxylin to haematein making it possible for the solution to be used immediately [1].

## 5. IRON HAEMATOXYLIN

The mordant in these solutions are ferric chloride or ferric ammonium sulphate [49]. These ferric compounds in addition to being mordants [1] also oxidize haematoxylin to haematein (Fig.1) causing over oxidation of prepared and stored haematoxylin. For the latter reason, iron haematoxylin solutions are prepared just before use, but simple alcoholic and aqueous solutions of haematoxylin must be prepared and kept for 4-6 weeks as stock solutions to enable ripening or oxidation before use. The solutions of haematoxylin and the iron alum are either mixed immediately before use as in Weigert's [27] and Verhoeff's [26] haematoxylins or tissue sections are mordanted in the iron alum before application of the haematoxylin solution as in Heidenhain's haematoxylin [35]. Iron haematoxylins are used when an acidic counter stain such as van Gieson [28] is to be applied to a section because iron haematoxylins are not quickly decolourised by acidic stains.

**Heidenhain's iron haematoxylin** Heidenhain's iron haematoxylin [50] is a regressive cytological stain which stains tissue jet black, and by careful selective differentiation, many tissue and cell components can be revealed in shades of black and grey. This makes it useful for photomicrography. In the technique, iron alum is also used as a differentiating agent and as an oxidising agent, which oxidises haematoxylin to haematein (Fig.1), the active staining component. Being a cytological stain, tissue sections must be very thin to enable easy demonstration of cell constituents. Staining time is 30-45 minutes at $56^0$C. Heidenhain's iron haematoxylin will demonstrate mitochondria, chromatin, chromosomes, nucleoli, centrioles, nuclear membrane, cross-striations of muscle fibers and myelin [1]. Red blood cells are stained black. Heidenhain's iron haematoxylin [50] is usually not counterstained but it may be counterstained with a connective tissue stain such as van Gieson [28].

**Weigert's iron haematoxylin** Weigert's iron haematoxylin [27] is used for the staining of cell nuclei when subsequent staining reagents contain acid such as in van Gieson [28] stain which will decolourise nuclear staining if stained previously with a solution of haematoxylin which contains potassium alum or ammonium alum as the mordant. The Weigert's haematoxylin, which is 1% alcoholic haematoxylin, is stored separately from the mordant, which is acidified ferric chloride. Equal volumes are mixed immediately before use. The resulting colour should be purplish black with a staining time of 20-30 minutes. Weigert's iron haematoxylin is used for the staining of cell nuclei when demonstrating collagen and muscle with the van Gieson stain and the trichrome connective tissue stains [51].

**Verhoeff's iron haematoxylin** Verhoeff's iron haematoxylin [26] is an elastic tissue stain. The constituents, 5% alcoholic haematoxylin, 10% ferric chloride and strong iodine are prepared separately and mixed immediately before use. This is because prepared Verhoeff's haematoxylin does not keep because of its rapid over oxidation. The ferric chloride is acting as a mordant [1] and it is also used as a differentiator [40]. Staining time is 25-60 minutes.

**Celestine blue-haemalum** Celestine blue [52] is an oxazine dye that is used as a nuclear stain in place of iron haematoxylins. Celestine blue haemalum sequence utilises two mordants incorporated into two different stains. Celestine blue is combined with the mordant ferric ammonium alum (iron alum) and used in sequence with Mayer's haematoxylin [32] (Mayer's haematoxylin contains ammonium alum or potassium alum as the mordant) to give a very precise and powerful nuclear stain, which resists decolourisation when subsequently treated with acid stains. Cole's haematoxylin [45] can be used in place of Mayer's haematoxylin [32]. A disadvantage of Celestine blue is that it stains cellulose nitrate very strongly and it is very difficult to remove. Therefore, it is not suitable for cellulose nitrate embedded materials.

## 6. PHOSPHOTUNGSTIC ACID HAEMATOXYLIN (PTAH)[31]

In many laboratories, PTAH [31] has now become a routine stain for nervous tissue owing to its ability to stain astrocytes, fibroglia, myoglia, muscle striations, collagen, reticulin, fibrin, etc in shades of blue and red [1]. PTAH is a progressive stain with a staining time of 1-16 hours at room temperature or 1-2 hours at $60^0$C. Tissue sections may be treated with Mallory bleach to suppress staining of myelin. The bleaching process involves treating sections with potassium permanganate and oxalic acid. The dehydrating alcohols rapidly remove the red staining from the sections; therefore, dehydration in alcohol should be very rapid.

## 7. CONSTITUENTS OF THE HAEMATOXYLIN STAINS

### EHRLICH'S ALUM HAEMATOXYLIN [44]

Staining time 10-15 minutes

| Constituents | |
|---|---|
| Haematoxylin | 6g |
| Absolute alcohol | 300ml |
| Distilled water | 300ml |
| Glycerol | 300ml |
| Glacial acetic acid | 30ml |

Add excess potassium or ammonium alum until solution is saturated. Dissolve haematoxylin in the alcohol and add other reagents in the order given. The alum should be added until solution is saturated. The prepared solution should be covered with a loose cotton wool or gauze and exposed to light for 4 to 6 weeks to enable it oxidise or ripen. Solution lasts more than a year.

### HARRIS ALUM HAEMATOXYLIN [37]

Staining time 5 minutes

| Constituents | |
|---|---|
| Haematoxylin | 2.5g |
| Absolute alcohol | 50ml |
| Ammonium or potassium alum | 50g |
| Distilled water | 500ml |
| Mercuric oxide | 1.5g |
| Glacial acetic acid | 20ml |

Dissolve the haematoxylin in absolute alcohol, and the alum in distilled water. Where necessary, heat may be applied. Then, mix the two solutions. Boil solution in a large flask, add mercuric chloride and mix. Cool immediately in cold water and add glacial acetic acid.
The stain can be used immediately. It lasts for about three months.

**MAYER'S HAEMALUM** [32]
Staining time as a progressive stain is 5-10 minutes, while as a regressive stain is 40-60 minutes.

| **Constituents** | |
|---|---|
| Haematoxylin | 1g |
| Distilled water | 1000ml |
| Potassium alum or Ammonium alum | 50g |
| Sodium iodate | 0.2g |
| Citric acid | 1g |
| Chloral hydrate | 50g |

Reagents are added in the order given making sure that each addition dissolves before the next is added. Heat may be applied where necessary. The stain may be used immediately and lasts 3-4 months.

**COLE'S HAEMATOXYLIN** [45]
Staining time 5 minutes

| **Constituents** | |
|---|---|
| Haematoxylin | 1.5g |
| 1% iodine in 95% alcohol | 50ml |
| Saturated aqueous ammonium or potassium alum | 700ml |
| Distilled water | 250ml |

Heat distilled water until it boils and dissolve haematoxylin in it. Then add iodine and alum. Cool and filter.
Stain lasts about 3 months.

**GILL'S HAEMATOXYLIN** [53]

| **Constituents** | |
|---|---|
| Distilled water | 730ml |
| Ethylene glycol | 250ml |
| Haematoxylin | 2g |
| Sodium iodate | 0.2g |
| Aluminium sulphate | 17.6g |
| Glacial acetic acid | 20ml |

Combine the reagents in the order given and mix for 1 hour at room temperature. The stain can be used immediately.

**CARAZZI'S HAEMATOXYLIN** [54]

| **Constituents** | |
|---|---|
| Haematoxylin | 0.5g |
| Potassium iodate | 0.01g |
| Potassium alum | 25g |
| Glycerol | 100ml |
| Distilled water | 400ml |

Add the haematoxylin to the glycerol. Dissolve the potassium iodate in about 25ml of the water and prepare the alum using the remainder. Mix the haematoxylin and alum solutions and then carefully add the potassium iodate.

**IYIOLA AND AVWIORO'S ALUM HAEMATOXYLIN** [55]

| | |
|---|---|
| Heamatoxylin | 1g |
| Citric acid | 1g |
| Ammonium alum | 50g |
| Glycerine | 50ml |
| Distilled water | 1000ml |
| Sodium iodate | 0.15g |

The reagents are added to about 500ml of distilled water, mixed and made up to 1000ml with distilled water. The mixture is boiled, removed from flame and sodium iodate added immediately and mixed gently. The mixture which will turn deep red should be cooled rapidly in running water.

**HEAMATOXYLIN AND EOSIN FOR GENERAL TISSUE STRUCTURE** [1]
**Solutions required** (Erhlich's haematoxylin, 1% HCl in 70% alcohol, 1% eosin)

**METHOD**

1. Dewax and hydrate
1. Stain in Erhlich's haematoxylin 15 minutes
2. Rinse in water
3. Differentiate in 1% HCl in 70% alcohol 1 minute
4. Rinse in water
5. Blue in tap water 10 minutes
6. or in Scott's tap water substitute 2 minutes
7. Counter stain with 1% eosin 1 minute
8. Rinse in water
9. Dehydrate, clear and mount

**Results**

| | |
|---|---|
| Nuclei | Blue to blue-black |
| karyosomes | Dark blue |
| Cytoplasm | Pink |
| Collagen and osteoid tissue | Light pink |
| Cartilage, cement lines of bone, calcified bone | Shades of blue |
| Red blood cells, eosinophil granules, Paneth cell granules, keratin | Red |

**RAPID HAEMATOXYLIN AND EOSIN METHOD FOR GENERAL TISSUE STRUCTURE** [1]
**Solutions required** (Mayer's or Harris haematoxylin, 1% HCl in 70% alcohol, Scott's tap water substitute or ammonia vapour, 1% alcoholic eosin)

**METHOD**

1. Dewax and hydrate
2. Stain in Harris or Mayer's haematoxylin - 2 to 5 minutes
3. Rinse in water
4. Differentiate in 1% HCl in 70% alcohol - 1 minute
5. Rinse in water
6. Blue in ammonia vapour for 5 to 10 seconds
or in Scott's tap water substitute - 2 minutes
7. Counter stain with 1% eosin - 1 minute
8. Transfer to 70% alcohol eosin - 1 minute
9. Complete dehydration in absolute alcohol, clear and mount

**Results**

| | |
|---|---|
| Nuclei | Blue |
| Cytoplasm | Pink |
| Red blood cells, eosinophil granules, Paneth cell granules, other eosinophlic substances | Red |

**HEIDENHAIN'S IRON HAEMATOXYLIN** [35]
Staining time 30-45 minutes at $60^0$C or 12-24 hours at room temperature.

**Constituents**

| **5% Iron alum (mordant and differentiator)** | |
|---|---|
| Ferric ammonium sulphate | 5g |
| Distilled water | 100ml |

| **0.5% haematoxylin in 10% alcohol** | |
|---|---|
| Haematoxylin | 0.5g |
| Absolute alcohol | 10ml |
| Distilled water | 90ml |

Dissolve haematoxylin in alcohol before adding water.
The solution should be allowed to ripe for 4-6 weeks before use.

**OR**

| | |
|---|---|
| 5% ripened alcoholic haematoxylin | 10ml |
| Distilled water | 90ml |

This solution can be used immediately.

**METHOD**

1. Dewax and take sections to 90% alcohol through absolute alcohol
2. Mordant in 5% iron alum solution -30-45 minutes at $60^0$C or 12-24 hours at room temperature
3. Rinse very briefly in water
4. Stain in 0.5% haematoxylin - for the same time and temperature as in 5% alum
5. Rinse briefly in water
6. Differentiate in 2-5% iron alum. (2% iron alum is easier to control)

**OR**

Saturated alcoholic picric acid diluted 2 in 3 (6%) is slower, easier to control and differentiates muscle striations better.

7. Wash in running water to remove iron alum - 5 minutes
8. Dehydrate, clear and mount.

| **Results** | |
|---|---|
| Mitochondria, chromosomes, chromatin, nucleoli, centrioles, nuclear membrane, ground cytoplasm, cross striations of muscle fibres | Shades of grey and black depending on degree of differentiation |

**WEIGERT'S IRON HAEMATOXYLIN** [27]
Staining time 20-30 minutes

**Constituents:** Solutions A and B

| **Solution A** |
|---|
| 1% alcoholic haematoxylin (not less than 5 days old to enable ripening) |

| **Solution B** | |
|---|---|
| 30% aqueous ferric chloride | 4ml |
| Distilled water | 100ml |
| Hydrochloric acid | 7ml |

Mix equal volumes of solutions A and B just before use.
The mixed solution lasts about 24 hours depending on the age of the haematoxylin.

| **1% acid alcohol** | |
|---|---|
| Absolute alcohol | 70ml |
| Distilled water | 29ml |
| Hydrochloric acid | 1ml |

**METHOD**

1. Dewax and hydrate
2. Stain in equal volumes of solutions A and B -20 minutes
3. Rinse in water
4. Differentiate in 1% acid alcohol.
5. Wash and blue in running tap water -10 minutes
6. Counter stain according to substances to be demonstrated.
7. Dehydrate, clear and mount

| **Results** | |
|---|---|
| Nuclei | Brown to black |
| Other structures | According to counter stain |

**VERHOEFF'S HAEMATOXYLIN** [26]

**Constituents**

| **Stock Solutions** | |
|---|---|
| 5% alcoholic haematoxylin | 10ml |
| 10% ferric chloride | 4ml |
| Strong iodine | 4ml |

Strong iodine is prepared by dissolving 4g potassium iodide in 100ml distilled water. 2g iodine is then added to the solution.

The solutions are prepared and kept separately as stock. They are mixed in the order and volumes given above immediately before use.

Prepared solution does not last more than a few hours and at most one day depending on the age of the haematoxylin solution.

**METHOD**

1. Dewax and hydrate.
2. Stain in freshly prepared haematoxylin solution until section is uniformly black -15-60 min
3. Rinse in water
4. Differentiate in 2% ferric chloride until elastic fibres remain black -1-2 min
5. Wash in tap water -5-10 min
6. Wash off iodine staining of background with 95% alcohol -5 min
7. OR in 2.5% sodium thiosulphate -1-2 min
8. Counter stain with 1% eosin -1 min
   OR van Gieson -2 min
9. Dehydrate, clear and mount.

| **Results** | |
|---|---|
| Elastic fibres | Black |
| Nuclei | Brown |
| Cytoplasm and other connective tissue | According to counter stain |
| If counter stained with van Gieson | |
| Collagen | Red |
| Muscle fibres, red blood cells | Yellow |
| Elastic fibres | Black |

**CELESTINE BLUE-HAEMALUM** [52]

**Celestine blue solution**

| **Constituents** | |
|---|---|
| Celestine blue B | 0.5g |
| Ferric ammonium sulphate (iron alum) | 5g |
| Glycerine | 14ml |
| Distilled water | 100ml |

Dissolve ferric ammonium sulphate in the water, add Celestine blue and boil for 3 to 5 minutes. Cool, filter and add glycerine. The stain lasts for about 6-8 months.

**METHOD**

1. Dewax and hydrate
2. Stain in Celestine blue solution -5 minutes
3. Rinse in water
4. Stain in Cole's or Mayer's haematoxylin - 5 minutes
5. Wash in running tap water
6. Differentiate and stain other structures by the desired technique.

**MALLORY'S PHOSPHOTUNGSTIC ACID HAEMATOXYLIN (PTAH)** [31]

Staining time 3-24 hours

| **Constituents** | |
|---|---|
| Haematein | 1g |
| Phosphotungstic acid | 20g |
| Distilled water | 1000ml |

The haematoxylin and phosphotungstic acid dissolved separately in distilled water applying heat if necessary and mix the two solutions. Then make it up to 1000ml with distilled water. Stain is ready for use after 24 hours.
If haematoxylin is used in place of haematein, then the stain should be oxidized with 0.177g potassium permanganate and used after 24 hours. Alternatively, stain may be exposed to light and warmth for 5 to 6 weeks to allow for natural oxidation before use.

**METHOD**

1. Dewax and hydrate
2. Oxidise in 0.25% potassium permanganate - 5 minutes.
3. Rinse in distilled water.
4. Bleach in 5% oxalic acid - 5 minutes.
5. Wash well in tap water.
6. Stain in PTAH solution at room temperature - 3-24 hours.
7. Dehydrate very rapidly through 95% alcohol and absolute alcohol because alcohol removes the red staining rapidly.
8. Clear in xylene and mount.

| **Results** | |
|---|---|
| Nuclei, centrioles, fibrin, cross striations of muscle fibres, red blood cells, fibroglia fibres, myoglia, astrocytes | Shades of blue |
| Collagen, reticulin fibres, ground fibres, ground substance of bone, cartilage | Yellow to brick red |

## 8. CONCLUSION

Haematoxylin is the most important and most used dye in the medical laboratory being able to differentiate malignant cells from non malignant cells makes it an excellent tool in the diagnosis of diseases affecting tissues. Its ability to stain several intracellular and extracellular substances in shades of blue to black also makes very useful in histochemistry and histology.

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