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HISTOLOGICAL STAINING METHODS

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Annotation: When observing a tissue sample under the light microscope, it is often difficult to distinguish between different cells and tissue, as they are almost colorless. Therefore staining is used to create differential coloration, allowing clearer observation and analysis of cells. **Staining** is widely used in histopathology and diagnosis, as it allows for the identification of abnormalities in cell count and structure under the microscope. A huge range of stains is used in histology, from dyes and metals to labeled antibodies. Certain stains change the coloration of cells and tissues significantly, different from the color of the original dye complex, a phenomenon known as metachromasia. For staining, paraffin sections of tissue are normally used. They are rehydrated and then made translucent (cleared) using a clearing substance such as xylene, before being stained. The most common stains used in histology are outlined in this article.
Key words: Haematoxylin and eosin , stain , histology , histopathology .

Haematoxylin and eosin (H & E): Routine stain This is the most common histologic stain, used to differentiate different tissue structures. It also plays an important role in the diagnoses of various pathologies. Haematoxylin, is a naturally occurring dye found in Longwood tree wood in Central America. In the H&E stain, a mixture of oxidised hematoxylin known as hematein is used. Due to poor affinity of hematin with tissues, a mordant is incorporated in the H&E stain. Most commonly used mordants are salts of aluminium, iron and tungsten. This substance is known as hemalum. When applied to a tissue section, hemalum stains nuclei blue. The sample is then counterstained using a solution of eosin (either alcohol or water), which stains proteins and cytoplasm varying shades of pink. Eosin are xanthene dyes and have different types, but generally Eosin Y is commonly used.



The general procedure of the H & E stain is as follows:

- The section is rehydrated and then cleared using xylene
- It is then submerged in haematoxylin, the time in the stain varies according to the type, age of stain and on personal preferences.

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- Here there are 2 potential options - progressive or regressive. Regressive involves over staining the section, and then rinsing away the excess. Progressive involves using a less concentrated dye, and checking it at intervals until the section is sufficiently stained
- The section is rinsed in tap water and then submerged in an acid-alcohol solution
- It is submerged in tap water
- The section is immersed in eosin stain
- The excess stain is rinsed off with tap water
- The section is dehydrated with ethanol, and mounted using a resinous medium

Special stains

Special stains stain are used to identify and demonstrate particular structures and tissues which are not visualized by H&E stains. There are variety of special stains, a brief detail of some of them is given below.

Van Gieson

The van Gieson stain is a very common stain used to highlight the difference between **collagen** and other **connective tissue** such as muscle tissues. It is often used to identify the characteristic arrangement of fibers in different types of tumours. The stain uses a mixture of picric acid and acid fuchsin to penetrate the tissue sample, causing collagen to become **red**. Surrounding muscle tissues and blood cells are stained **yellow**.



Picric acid molecules are very small, and therefore penetrate all types of tissues. However, they are only fully retained in red blood cells and muscle, as these have a fine, close texture. **Acid fuchsin** has larger molecules, which penetrate collagen, displacing the picric acid molecules. This causes collagen to be stained differently from muscle and red blood cells,

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allowing differentiation. Nowadays, a substance called **ponceau S** is often used as a substitute for acid fuchsin, as it is slightly more efficient.

To conduct the van Gieson stain, the following steps are carried out:

- The section is rehydrated and then cleared using **xylene**
- The section is stained with **celestin blue-hematoxylin** sequence to colour the nuclei
- Excess is rinsed off using **water**
- The section is immersed in **Van Gieson's stain** for 1-5 minutes
- It is rinsed again with distilled **water**
- It is then dehydrated with **ethanol** and cleared using **xylene**
- The section is mounted using a **resinous medium**

Toluidine blue

Sometimes referred to as tolonium chloride, toluidine blue is a type of metachromatic dye, that is acidophilic, meaning that it stains **acidic tissues**. It is particularly attracted to nucleic acids, and is therefore used to stain tissues with high concentrations of **DNA and RNA**. When in contact with Toluidine Blue, nucleic acids become blue in colour, whilst mucins and cartilage become purple. It is used to identify mast cells granules, mucins, and cartilage.

The basic procedure to carry out a Toluidine Blue stain is as follows:

- The tissue section is hydrated using distilled **water**
- The section is immersed in **Toluidine Blue** for a few minutes
- Excess is washed off with distilled **water**
- It is then dehydrated with **ethanol** and cleared using **xylene**
- The section is mounted using a **resinous medium**

Alcian blue

Alcian blue is one of the alcian dyes introduced way back in 1948. The other alcian dyes include alcian yellow and alcian green. This is a family of **polyvalent basic dyes** contains 2-4 isothiouronium groups having positive charge.

Alcian blue is a phthalocyanine dye which shows specificity for substances such as **glycosaminoglycans** and **acid mucins**. To characterize the subtypes of acid mucins, the pH of the alcian blue solution is varied and adjusted. The alcian blue stain causes acid mucins and mucosubstances to appear **blue**, and nuclei to appear reddish **pink** when counterstain neutral red is used. Alcian blue dyes are water soluble, and appear blue as they contain copper. They attach to sulfate and carboxylated acid mucopolysaccharides and glycoproteins, and dye binding is purely electrostatic. This staining is performed to see the mucoid degeneration and to identify acid mucins which are released by various connective and epithelial tissue **tumors**. The procedure of staining is usually carried out as follows:

- The section is hydrated with distilled **water**
- It is submerged in **alcian blue** (time in the alcian blue can vary)

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- It is then washed in tap **water**, and rinsed in distilled water
- **Nuclear-fast red** is added as a counterstain for 5 minutes
- It is then dehydrated with **ethanol** and cleared using **xylene**
- The section is mounted using a **resinous medium**

Giemsa

This is a blood stain that can be used histopathologically. It was designed primarily to demonstrate malarial parasites. It was later used in histology because of its high quality staining capabilities of chromatin and nuclear membranes. It stains human and pathogenic cells differently, therefore, it is used in the **diagnosis** of many diseases as it stains human cells purple, and bacterial cells pink, so that they may be differentiated. It is also used to stain blood cells, so that their composition and structure may be observed.

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