Hematoxylin Eosin Staining

Hematoxylin-eosin staining (HE) is a transparent staining used for the preparation of semi-thin sections.

Preparing solutions

Hematoxylin

- hematoxylin is supplied to the laboratory as a yellowish crystalline powder;
- in order for the hematoxylin solution to stain, it must be oxidised with oxidising agents (e.g. sodium or potassium iodate, ferrous ions). This converts it to hematein;
- the hematein must also be made into a coloured so-called hematein varnish using a mordant;
- the most common mordants include alum (e.g. potassium alum = potassium aluminium sulphate), according to which we distinguish types of haematoxylins;
- among the frequently used haematoxylins we mention e.g. Harris', Gill's, Heidenhein's

Eosin

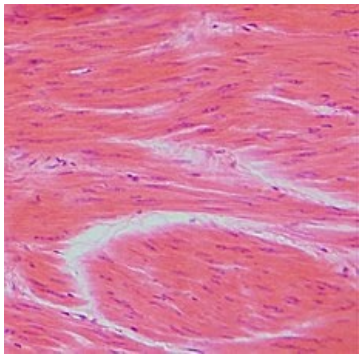
There are several types of eosin. They differ in solubility, chemical composition and also in colour:

1. **bromeosins** – eosin yellow, red
2. **iodeosines** – erytrosins

Staining

The tissue section on the slides are ready for staining, which consists of the following steps:

- deparaffinization ;
- hydration (washing in tap water);
- staining;
- dehydration;
- clearing;
- mounting;



Smooth muscle cells stained with hematoxylin-eosin

Deparaffinization and hydration

The cuts must be free of paraffin before staining in aqueous solutions. This is done in a descending **series of graded alcohols**.

Procedure

1	xylene	**5 min**
2	xylene	**5 min**
3	96 % ethanol	**3–5 min**
4	80 % ethanol	**3–5 min**
4	70 % ethanol	**3–5 min**
5	tap water	**5 min**

Staining

1. deparaffinized and hydrated sections are first stained with hematoxylin solution for **3-10 minutes**. The staining time depends on:
   - type of hematoxylin;
   - age of the solution;
   - type of tissue;
   - fixation.
2. washing in running water
3. a possible step is the so-called differentiation in acid ethanol, briefly and under the control of a microscope
4. washing in running water **5 minutes**
5. followed by eosin staining **1–3 minutes**
6. rinse in distilled water

If the cuts are recoloured, we briefly differentiate in 80% alcohol.

Dehydration

Drainage is carried out in **ascending series of graded alcohol (reverse descending)**.

- xylene 5 minutes,
- xylene 5 minutes.

At the same time, the sections become **clear** in the xylene. After clearing, they must be completely transparent, no whitish cloudy areas must remain, which is a sign that the sections have not been well dehydrated.

Mounting of stained sections

The mounting medium must be a transparent substance with a high refractive index (as close to glass as possible) that will not discolour the tissue.

Types of mounting media

Not mixable with water

Soluble in xylene. The sections must be properly drained and saturated with e.g. xylene.

- Ex: formerly Canadian balsam, cedar oil, today e.g. Solakryl.

Mixable with water

Water soluble, closing without prior dehydration.

- Ex.: glycerine, glycerine gelatin, levulose syrup

Method of execution

1. A drop of mounting medium is dropped onto the slide, the cover slip is carefully applied from an oblique angle to avoid air bubbles. Any bubbles can be squeezed out by applying slight pressure to the coverslip, e.g. with tweezers, etc.
2. The cut must not dry out during mounting.
3. The finished slide is placed in a thermostat (37 °C) where the mounting medium dries.

Links

Related articles

- Basic staining methods
- Histochemistry
- Burri staining
- Gram staining
- Chromosome staining
- AZAN

Literature

- VACEK, Zdeněk. *Histologie a histologická technika. Díl 2, Histologická technika.* 1. edition. Institut pro další vzdělávání pracovníků ve zdravotnictví, 1996. 184 pp. ISBN 80-7013-202-7.