HEMATOXYLIN AND EOSIN STAINING IN HISTOLOGY

1. *Introduction*

Hematoxylin and eosin (H & E) is the most widely used histological stain. It is simple to use, easy to automate and demonstrates different tissue structures clearly. Hematoxylin stains the cell nuclei blue-black, showing clear intranuclear detail, whilst eosin stains cell cytoplasm and most connective tissue fibers in varying shades and intensities of pink, orange and red. Automated staining machines and commercially prepared hematoxylin and eosin solutions are commonly used in today's laboratories for routine staining.

2. *Manual Preparation of Hematoxylin and Eosin*

a. Harris's Haematoxylin Stain

It is widely used as a nuclear stain in histopathology, exfoliative cytology & sex chromatin staining because of a powerful, sharp & selective staining of nuclear structure.

Harris's Haematoxylin Preparation

Haematoxylin	1g
Absolute alcohol	10ml
Ammonium or Potassium alum	20g
Distilled water	200ml
Mercuric oxide	0.5 g
Glacial acid	8ml

Procedure

- Dissolve the haematoxylin in the alcohol.
- Add to the alum previously dissolved in hot water & bring quickly to the boil.
- Add the mercuric oxide when the solution turns dark purple, cool rapidly & filter before use.
- After cooling, add 8 ml glacial acetic acid to the solution to sharpen nuclear staining.
- The stains remain stable for several months.

a) Eosin Stain

1% Eosin Solution Preparation

Eosin	1g	OR	Eosin	1g
Distilled water or tap water (for water-soluble eosin)	100ml		Absolute alcohol (for alcohol soluble)	100ml

Procedure

- Dissolve eosin in 100 ml of distilled water or tap water or absolute alcohol.

3. *H & E Staining Procedure*

Reagents required

- ✓ Harris's Haematoxylin
- ✓ 1% Eosin solution
- ✓ 1% Acid alcohol (1% hydrochloric acid in 70% ethanol)

Manual Procedure

➢ Bringing section to water

- Dewax sections in xylene 1,2,3 for 2 minutes each.
- Rehydrate with absolute alcohol, 90% ethyl alcohol and 70% ethyl alcohol for 2 mins each.
- Wash with tap water.

➢ Staining with Haematoxylin

- Stain in Harris' haematoxylin for 5 - 10 mins.
- Wash well in running tap water for 2 - 3 mins.

➢ Differentiating

- Remove excess stain in 1% acid alcohol for a few seconds.
- Examine microscopically to ensure that only nuclei are stained.

➢ Bluing

- Blue in alkaline running tap water for 5 - 10 mins.

➢ Counterstaining

- Counterstain in 1% eosin for 1 - 3 mins.
- Wash in running tap water.

➢ Dehydrating

- Dehydrate in fresh 70% ethyl alcohol, 90% ethyl alcohol and absolute alcohol for 2 mins each. (or)
- Drain the excess stain and blot dry with blotting paper and place on hot plate at for a few seconds.

➢ Clearing

- Clear in fresh Xylene 4,5 and 6 for 2 mins each.

➢ Mounting

- Glass coverslips of standard sizes are used, depending on the size of the section.
- Place the coverslip on a piece of blotting paper.
- Place a small drop of DPX on the middle of the coverslip.
- Remove the slide from the xylene and wipe the surplus xylene from the back of the slide.
- The slide is quickly inverted over the coverslip with one end place on the blotting paper.
- The other end lowered until the DPX makes contact with the slide.
- The DPX will spread between coverslip and the slide.
- Then the slide, with the coverslip attached, is again quickly inverted.
- Any air bubbles is squeezed out and the coverslip guided into place with a dissecting needle.
- The DPX may take some hours to set, when handling the section.
- If too much or too little DPX, the process can be repeated by placing the section in xylene, gentle sliding off the coverslip and remounting.

➢ Labelling

- On label tape, write the following information:
  (1) Tissue type

(2) Staining method
(3) Date
(4) Your initial

4. Result interpretation of H&E staining slide

Results interpretation of the correct coloration of tissue structure under microscope

Nuclei	----------	Blue to blue-black
Red blood cells	----------	Red
Muscle and elastic fibers	-------	Deep pink
Collagen	----------	Light pink
Cytoplasm	----------	Shades of pink

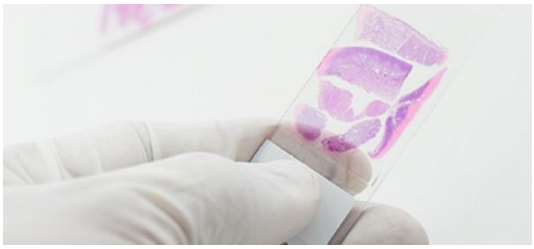

Figure 1. H & E stained slide

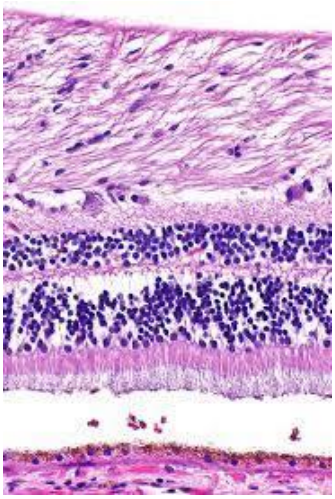

Figure 2. H & E stained slide under microscope

Reference

- University of Medical Technology, Department of Medical Technology, Histopathology Practical Manual Handbook
- https://www.leicabiosystems.com/knowledge-pathway/he-staining-overview-a-guide-to-best-practices/