

Practical-6

staining methods



Practical-6-A

Stains used for demonstrating the general relationship of tissue to each other:

- **Indirect staining-** stained needs a mordant to work.
- **Direct stains-** the stains work without adding a mordant.
- **Simple stain-** when staining solution contains one dye.
- **Compound stain-** the staining solution is composed of more than one or more dye.

- **A progressive stain-** when the different elements in the tissue are colored in sequence and at the correct time differential coloration of tissue are achieved.

A regressive stain- when the tissue is over stained and then differentiated (washed out) by removing excess stain from the unwanted parts.

- **Selective stain-** the staining dyes are more than one by substance in the same color; but easy to identify the substance you want to demonstrate either by morphology or site.

- **Specific stain-** when the stain acts only on a certain constituents of the cell or tissue and has a little or no effect upon other elements.

- **Cytological Stains-** which are used to demonstrate of minute structure in the nucleus and the cytoplasm of cells tissue.

**WITHIN THIS COURSE WE ARE GOING TO EXPLAIN AND APPLY FEW OF THEM,
these represent:**

General purpose method

Selective method

Specific method

The Haematoxyline and Eosin:

The haematoxyline and eosin stains are probably the most widely used histological stains. Its popularity is based on its comparative simplicity and ability to demonstrate clearly an enormous number of different tissues structure.

The staining procedure:

- Removal of wax by xylene.
- Removal of xylene by alcohol.
- Removal of pigments and removal of colors.
- Application of staining solutions.
- Dehydration taking through ascending grades of alcohol.
- Clear with xylene.
- Mounting or cover slipping.
- Labeling and examine microscopically.

Other factors which affect staining:

1. Solvents (alcoholic or aqueous solutions).
2. Low or high temperature during reaction.
3. Simple or multiple combinations of dyes.
4. The covering power of the dye.
5. The time (period) the dye acting.
6. The type of the tissue and the fixative used.
7. The type and thickness of the section .
8. The temperature applied during drying paraffin section on the slides.
9. The makeup of the dye.

Haematoxylin Solutions

The three main alum haematoxylin solutions employed are:

- Ehrlich's haematoxylin
- Harris's haematoxylin
- Mayer's haematoxylin.

Haematoxylin and Eosin staining protocol- is used frequently in histology to examine thin sections of tissue.

Haematoxylin stains cell nuclei blue, while eosin stains cytoplasm, connective tissue and other extracellular substances pink or red.

Structures that stain readily with eosin are termed eosinophilic.

- Eosin is most often used as a counter stain to haematoxylin in H&E (Haematoxylin and Eosin) staining.
- For **prevention of mold growth** in aqueous solutions, thymol is sometimes added. A small concentration (0.5 percent) of acetic acid usually gives a **deeper red stain to the tissue**.

Prepare the stock solutions first, and then create the working solution as needed.

Eosin Stock Solution:

Eosin Y, 1 g
Distilled water, 100 ml
Mix to dissolve.

Phloxine Stock Solution:

Phloxine B, 1 g
Distilled water, 100 ml
Mix to dissolve.

Eosin-Phloxine B Working Solution:

Eosin stock solution, 100 ml
Phloxine stock solution, 10 ml
Ethanol (95%), 780 ml
Glacial acetic acid, 4 ml
Mix well.

THANK YOU
FOR
LISTENING...