

## UNIT 5

### AUTOPSY

Performed by pathologists to diagnose the cause of death.

Diagnosis could not be researched during treatment.

Pathology of organs due to some diseases.

### Purpose of Autopsy

- ❖ To know exact cause of deaths
- ❖ Find out circumstances of death
- ❖ Find out the time passed after death
- ❖ To find out nature of death natural (or) suicide
- ❖ Weapon (or) poison used
- ❖ Person received any treatment before death

### Pathological techniques

- ❖ Hematoxylin and eosin method

Composition- Hematoxylin

Absolute alcohol

Distilled H<sub>2</sub>O

Glycerol

Glacial acetic acid

Potassium alum

**Preparation:** Hematoxylin should be dissolved in the alcohol in which it is readily soluble and the other components added in the given order above.

**Ripening:** After preparation of the stain must be kept in a bottle in a warm. During ripening oxidation of the haematoxylin to haematein takes place.

### Staining techniques

Tissue section embedded in Paraffin wax



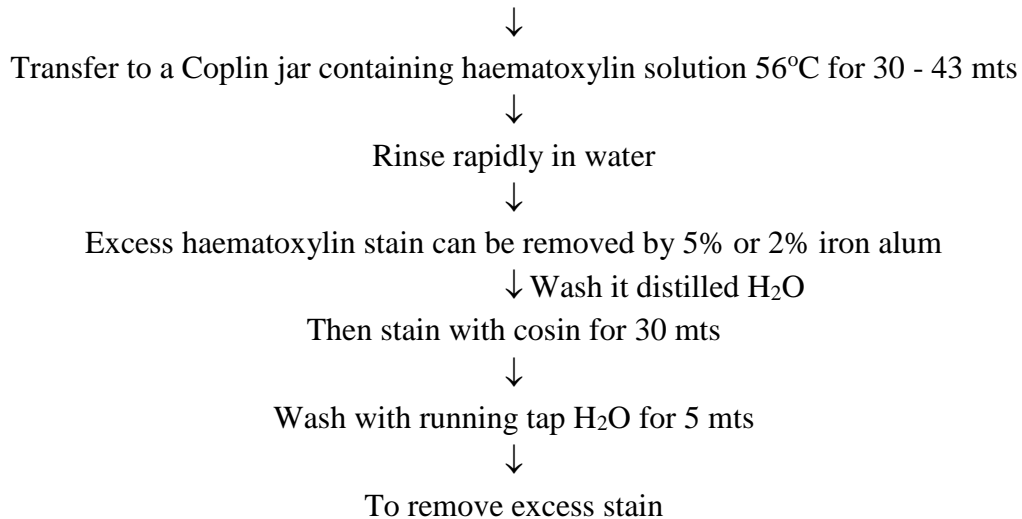
Treated with alcohol



Transfer to a Coplin jar containing iron alum solution 56°C for 30 – 45 mts.



Rinse rapidly with H<sub>2</sub>O



### Observation

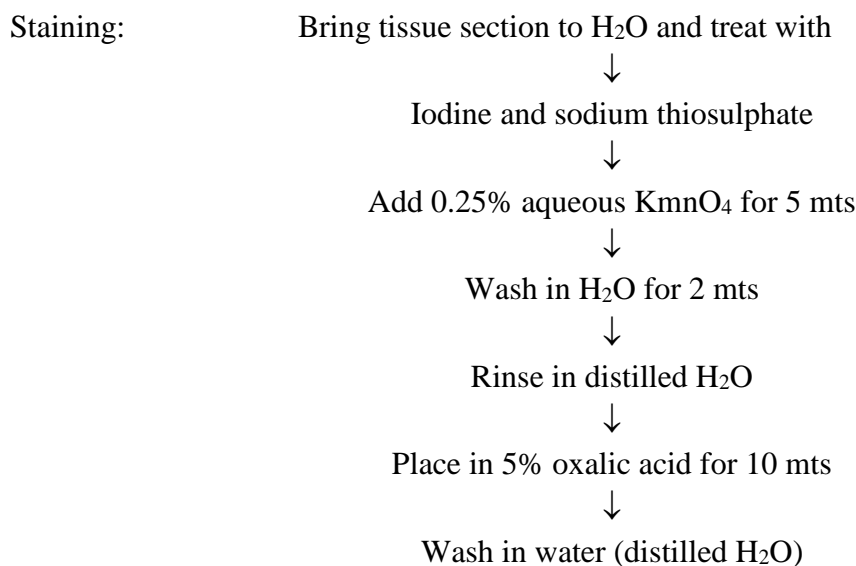
Mitochondria	Chromosomes
Nuclear membrane	Centrioles
Muscle fibres	Yolk
	Chromatin

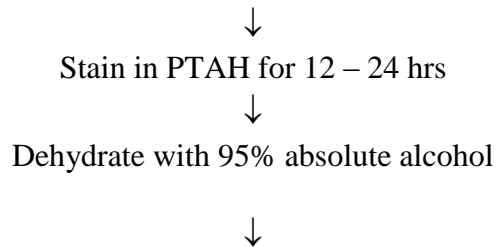
### Phosphotungstic acid haematoxylin (PTAH)

Composition – Haematein, Phosphotungstic acid, Distilled H<sub>2</sub>O

Mallory's haematoxylin is a stain used with tissues of the nervous system.

Preparation: Dissolve the hematein and the phosphotungstic acid separately in distilled H<sub>2</sub>O using gentle heating and followed by cooling.



**Observation**

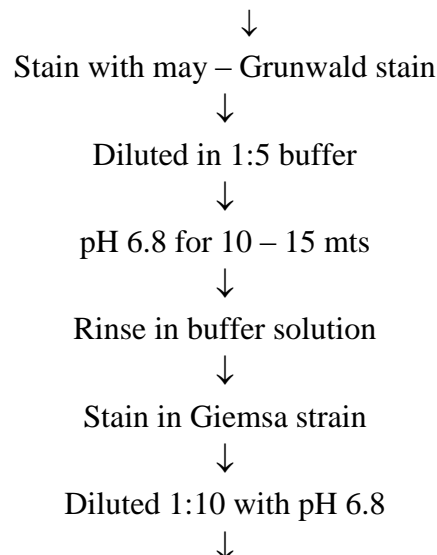
Nuclear to remove excess red staining  
 Centroles  
 Neuroglia fibers  
 Muscle fibres  
 Fibrin (Blue)  
 Fibrin (yellow) → brick red color

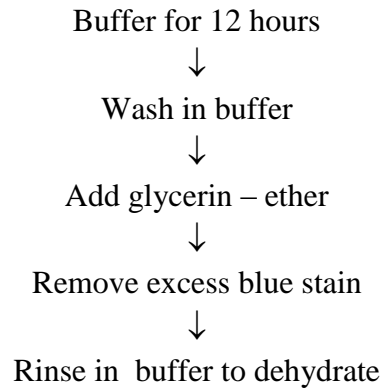
## 3. Toluidine blue staining

- Slides should be placed in 90% alcohol for a second
- Transfer to a absolute alcohol for a second
- Transfer to xylol and agitate the slide until the section is clear (2 sec)
- Transfer to 90% alcohol for a second
- Transfer to slide rack and flood with toluidine blue for 60 secs.
- Rapidly rinse in H<sub>2</sub>O, transfer to a paid of filter paper and blot family.
- Flood with absolute alcohol and blot firmly
- Flood with xylol and blot family.

**Results**

- Nuclear and bacteria – blue
- Observe bone marrow smear
- Specimen → treated with pH 6.8 buffer for 30 mts at 37°C





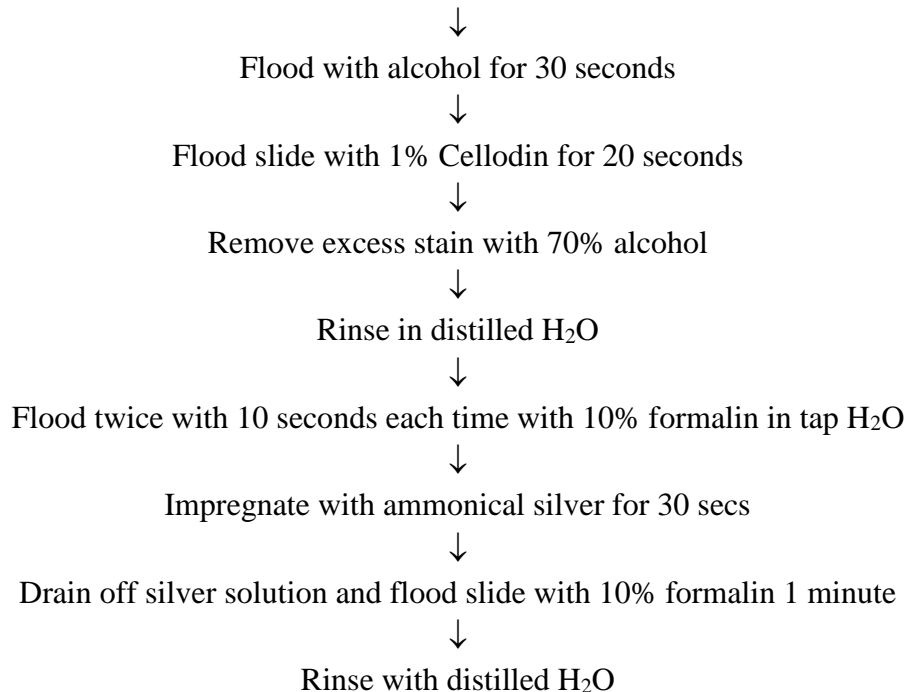
**Result:** Nuclear - Bluish red  
RBC - Pink  
Granules - Blue pink

### Silver Staining

Glees and Marsland's modification of Bleschowsky's method for paraffin sections.

**Preparation:** 20% silver nitrate + 20 ml add drop by drop until the precipitate is formed.

**Method:** Treated section with xylol for 1 minute to remove wax



**Result** Nerve cells, Axons, Dendrites

**Crystal** Linderstrom – long and Mognensen designed the first crystal in 1938 Coons and his colleagues redesigned it in 1951.

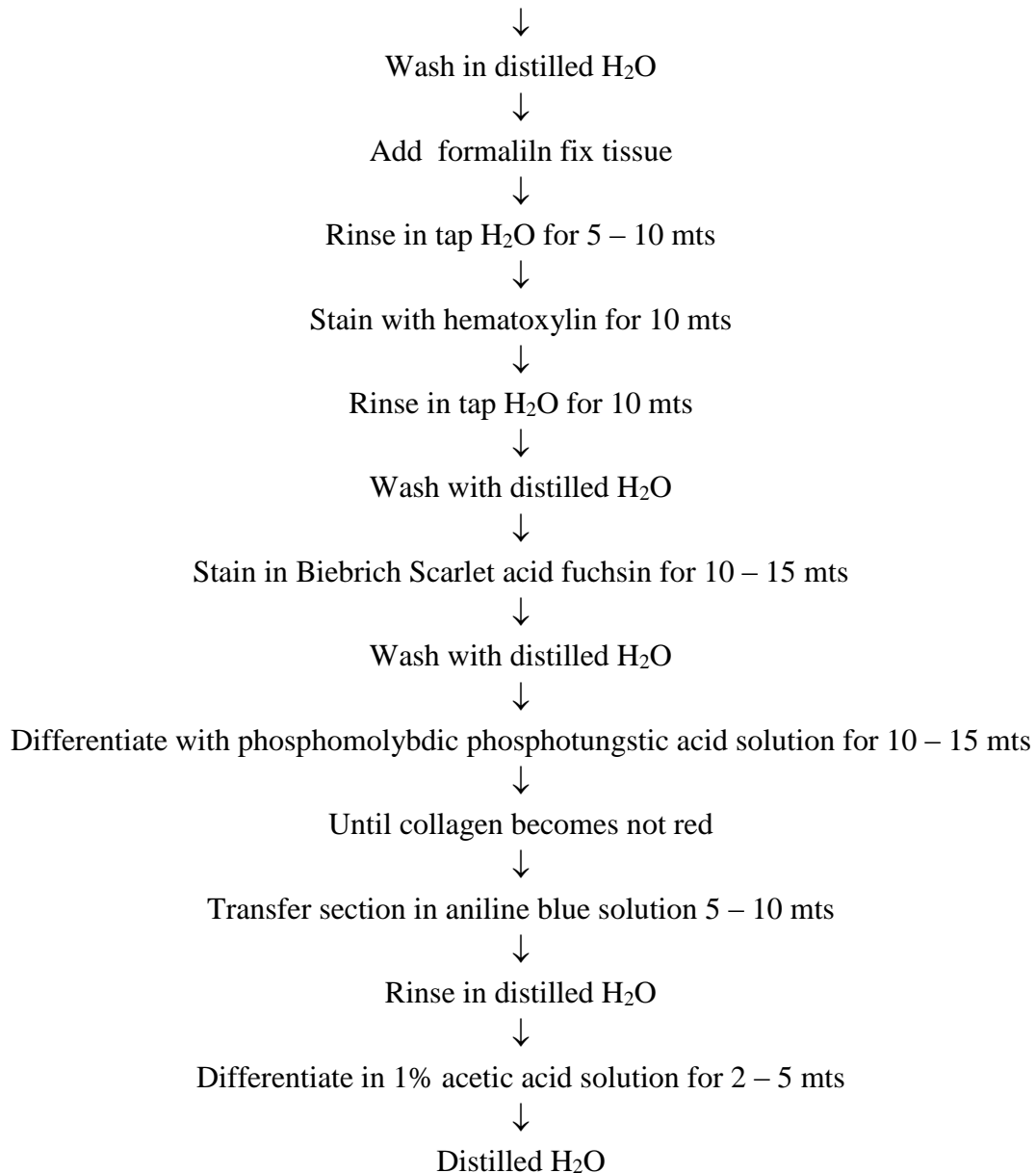
- The tissue may be frozen (embedded in ice)



This method is used for the detection of collagen fibers in tissues such as skin, heart etc.

**Method:**

Deparaffinize and rehydrate through 100% alcohol, 95% alcohol, 70% alcohol

**MOLECULAR PATHOLOGY – FISH****In situ – Hybridization**

- Is a powerful technique for localizing specific nucleic acid targets within fixed tissues and cells.
- ISH is similar to that of blot hybridizations the nucleic acid probe is synthesized, labeled purified and annealed with the specific target.

- Fluorescent in situ hybridization (FISH)
- Fish modern cytogenic technique that can be used to detect small deletions and duplications.
- It can be used to detect how many chromosomes present in each cell and to confirm rearrangements.
- Fish specifically detects one specific area of a chromosome only.
- It uses a very small chemical that glows brightly when it detects the specific region on a chromosome.

Eg. Deletion → one bright spot can be seen instead of two

Duplication → three bright spot can be seen instead of two.

### Principle

The hybridization reaction identifies (or) labels target genomic sequences so their location and size can be studied.

DNA (or) RNA sequence from appropriate chromosome – specific probes are first labeled with molecules which are later identified through fluorescence microscopy.

Labelled DNA (or) RNA probe is then hybridized to the metaphase chromosome.

After washing and signal amplification the specimen.

This technique is rapid, simple to implement offers great probe stability.

It is used to detect → entire chromosome, chromosome specific regions.

Single – copy unique sequence.

### Methodology

