# 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 $H_2O$
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$^oC$ for 30 – 45 mts.
↓
Rinse rapidly with $H_2O$

↓

Transfer to a Coplin jar containing haematoxylin solution 56°C for 30 - 43 mts

↓

Rinse rapidly in water

↓

Excess haematoxylin stain can be removed by 5% or 2% iron alum

↓ Wash it distilled $H_2O$

Then stain with cosin for 30 mts

↓

Wash with running tap $H_2O$ for 5 mts

↓

To remove excess stain

**Observation**

| | |
|---|---|
| Mitochondria | Chromosomes |
| Nuclear membrane | Centrioles |
| Muscle fibres | Yolk |
| | Chromatin |

Phosphotungstic acid haematoxylin (PTAH)

Composition – Haematein, Phosphotungstic acid, Distilled $H_2O$

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

Preparation: Dissolve the hematein and the phosphotungstic acid separately in distilled $H_2O$ using gentle heating and followed by cooling.

Staining: Bring tissue section to $H_2O$ and treat with

↓

Iodine and sodium thiosulphate

↓

Add 0.25% aqueous $KmnO_4$ for 5 mts

↓

Wash in $H_2O$ for 2 mts

↓

Rinse in distilled $H_2O$

↓

Place in 5% oxalic acid for 10 mts

↓

Wash in water (distilled $H_2O$)

↓

Stain in PTAH for 12 – 24 hrs

↓

Dehydrate with 95% absolute alcohol

↓

**Observation**

| | | |
|---|---|---|
| Nuclear | | to remove excess red straining |
| 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 $H_2O$, 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 37ºC

↓

Stain with may – Grunwald stain

↓

Diluted in 1:5 buffer

↓

pH 6.8 for 10 – 15 mts

↓

Rinse in buffer solution

↓

Stain in Giemsa strain

↓

Diluted 1:10 with pH 6.8

↓

Buffer for 12 hours
↓
Wash in buffer
↓
Add glycerin – ether
↓
Remove excess blue stain
↓
Rinse in buffer to dehydrate

**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
↓
Flood with alcohol for 30 seconds
↓
Flood slide with 1% Cellodin for 20 seconds
↓
Remove excess stain with 70% alcohol
↓
Rinse in distilled $H_2O$
↓
Flood twice with 10 seconds each time with 10% formalin in tap $H_2O$
↓
Impregnate with ammonical silver for 30 secs
↓
Drain off silver solution and flood slide with 10% formalin 1 minute
↓
Rinse with distilled $H_2O$

**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)

- Sections are cut using freezing microtome
- If the tissue is frozen and sectioned supported by the ice
- Section may be cut and stained
- 3 basic methods of fixing frozen sections to slides
- Albuminized slides
- Gelatinized slides
- Celloidinization
- Cryostat consists of a microtome of any type preferably rust proof
- Which is enclosed and operated with in a deep freeze cabinet.
- The cabinet is fitted with a double glass window and a door through which material may be passed in and out.
- The cabinet is equipped with a fluorescent light and fan to ensure the circulation of cold air.
- The cabinet temperature may be regulated between -10$^{o}$C to – 40$^{o}$C
- Crystat can be used as an alternative to a freezing microtome for rapid sectioning.
- Microtome may be adjusted to cut sections from 2 to 5 micron.

It is most important that a very sharp knife is used.

A small piece of filter paper is placed on the surface of the block holder.

A drop of water is placed on it, followed by tissue. The water freezes almost instantly holding the tissue firmly.

**Cutting**: Knife is placed in the cabinet alteast 15 mts before cutting

↓

The brush is used to prevent the section from curling at the beginning of the cutting stroke

↓

Tissue section is attached to warm slide directly (touching the slide)

↓

Sections is air dired

↓

Staining carried out

Eg. Fluorescent antibody technique

Trichome staining (staining collagen).

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

↓

Wash in distilled $H_2O$

↓

Add formaliln fix tissue

↓

Rinse in tap $H_2O$ for 5 – 10 mts

↓

Stain with hematoxylin for 10 mts

↓

Rinse in tap $H_2O$ for 10 mts

↓

Wash with distilled $H_2O$

↓

Stain in Biebrich Scarlet acid fuchsin for 10 – 15 mts

↓

Wash with distilled $H_2O$

↓

Differentiate with phosphomolybdic phosphotungstic acid solution for 10 – 15 mts

↓

Until collagen becomes not red

↓

Transfer section in aniline blue solution 5 – 10 mts

↓

Rinse in distilled $H_2O$

↓

Differentiate in 1% acetic acid solution for 2 – 5 mts

↓

Distilled $H_2O$

**MOLECULAR PATHOLOGY – FISH**

Insitu – 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**

Make a probe complementary to the known sequence

↓

Probe is labeled with fluorescent marker. Eg. Rhodamine, Acryflavine

↓

Put chromosome on a microscopic slide

↓

Denature them

↓

Denatured the probe

↓

Add it to micoroscopic slide

↓

Probe hybridize to its complementary site

↓

Wash of the excess probe

↓

View chromosome in a fluorescent microscope

↓

Probe will show one (or) more fluorescent signal in the microscope

↓

Depending on how many sites it can hybridize