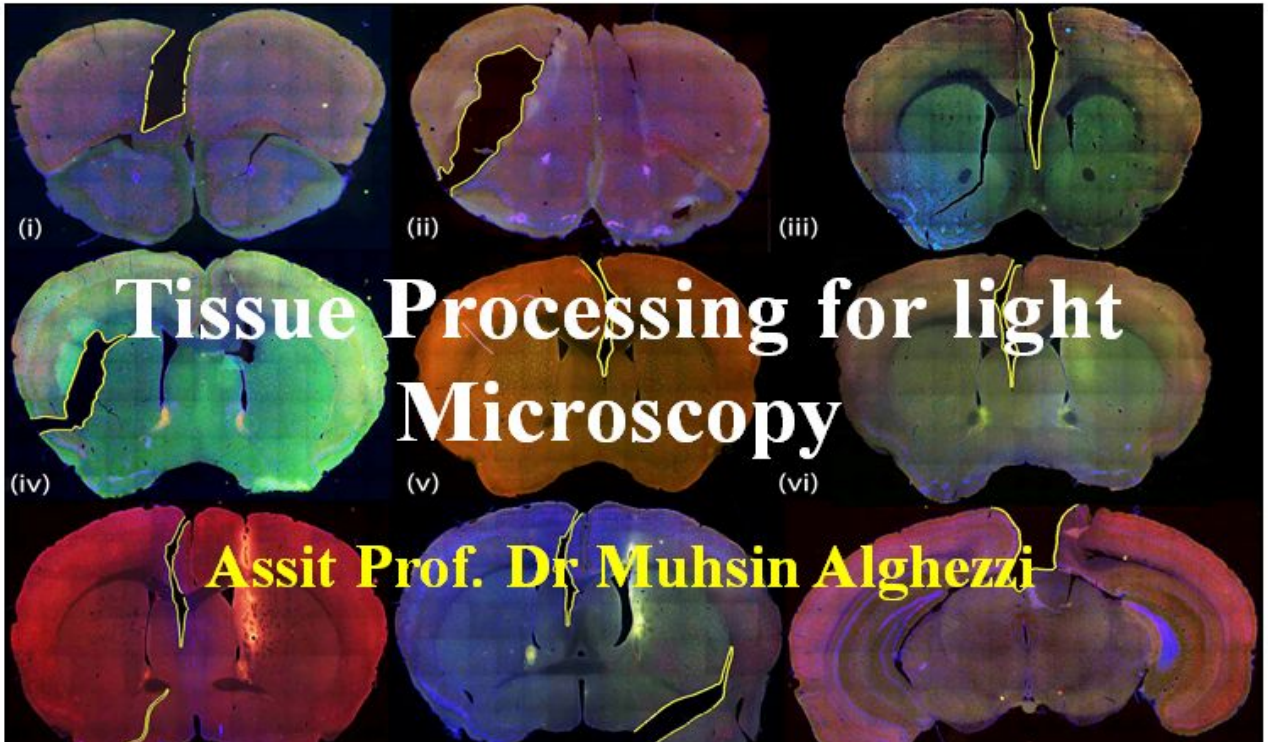
Cihan University-Erbil
College of Science
Department of G-Biology

Presentation



Tissue Processing for light Microscopy

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Tissue Preparation

- **Introduction**
- "Tissue processing" describes the steps required to take animal or human tissue from fixation to the state where it is completely infiltrated with a suitable histological wax and can be embedded ready for section cutting on the microtome.

- **Importance**

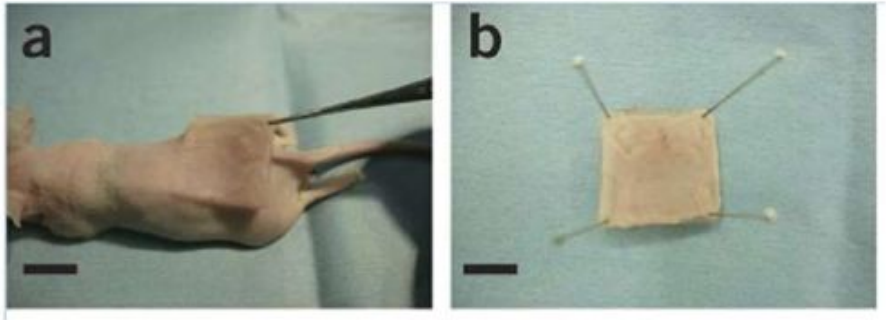
- Microscopic analysis of cells and tissues requires the preparation of very thin, high quality sections (slices) mounted on glass slides and
- appropriately stained to demonstrate normal and abnormal structures.

- **• It involves several steps that are following.**

- **1. Obtaining a fresh specimen**
- **2. Fixation**
- **3. Dehydration**
- **4. Clearing**
- **5. Wax infiltration**
- **6. Embedding or blocking out**
- **7-Sectioning**
- **8- Staining**
- **9- Covering**
- **10- Examining**

1- Obtaining a fresh specimen

- Fresh tissue specimens will come from various sources.
- A sharp blade or knife must be used for tissue collection.
- Obtain a thin layer of tissues about 1 cm thick.
- Careful handling
- Fix the specimen as soon possible.
- Wash specimen with normal saline for maximum penetration of fixative.



2- TISSUE FIXATION

- **Aim of Fixation:**
- 1- To prevent autolysis and bacterial attack.
- 2- To fix the tissues so they will not change their volume and shape during processing.
- 3- To prepare tissue and leave it in a condition which allow clear staining of sections.
- 4- To leave tissue as close as their living state as possible, and no small molecules should be lost.

2. Fixation

•Fixation is a reaction between the fixative and proteins in the specimen which form a gel, so keeping every thing as their in vivo relation to each other.

Fixative should be 20-25 times of volume of specimen.

Characters of a good fixative:

*** Penetrate quickly * Prevent tissue from shrinkage**

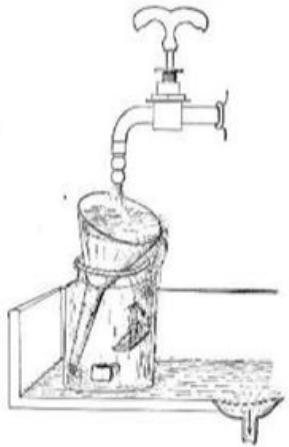
•

Types of Fixative- (Good fixatives)

- Potassium Dichromate.
- Acetic acid.
- Formaldehyde 10%.
- Ethanol.
- Glutaraldehyde.
- Methanol.
- Picric acid.
- Osmic acid (Osmium tetroxide).

Washing

- For removal of fixative.
- Under tap water.



- **3. Dehydration**
- ☙| Removal of water.
- ☙ Alcohols are most commonly used for dehydration.
- ☙ Water replacement with alcohol.
- ☙ Different % of alcohol are used for different time periods.
- ☙ 30%,50%,70% Alcohol for 2 hours.
- ☙ 80%,95% for 1,1 hours.
- ☙ 95% for 2 hours.
- ☙ 100% for ½ hours two times.

Types of dehydrating agents

- • Ethanol
- • Methanol
- • Acetone
- Tissues may be held and stored indefinitely
- in 70% ethanol without harm

4. Clearing

1- Removal of dehydrating agent.

2- Clearing agent > miscible with both embedding medium and dehydrating agent.

- **Zylene (clearing agent)**
- **Tow times for 1- 2 hours.**

Types of Clearing Agents

- ((Choice of a clearing agent depends upon many factors……))
- • **Xylene.**
- • **Toluene.**
- • **Chloroform.**
- • **Benzene.**
- • **Propylene oxide**

5- Wax infiltration:

- Replace xylene with paraffin.
- Immerse in melted paraffin.
- Remove all bubbles, xylene.

Procedure

Two baths of melted paraffin.

(a) Leave the tissue for 1 hour in malted paraffin.

(b) Put in fresh paraffin and melt it again.

6. Embedding or blocking out

Allow the melted wax to be solidify.

It get rigid.

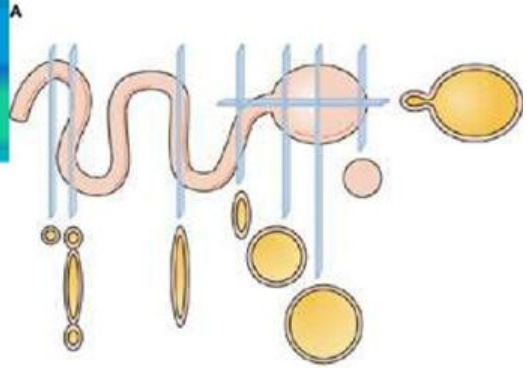
A. Orient tissue

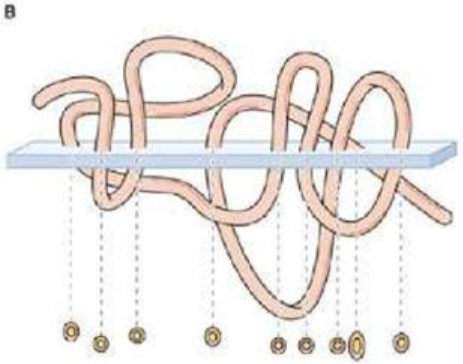
1. cross section
2. longitudinal section

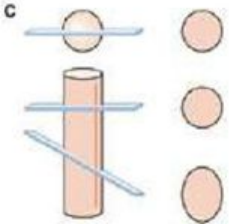
B. Dissection orientation

Avoid bubbles

The process by which tissues are surrounded by a medium such as agar, gelatin, or wax which when solidified will provide sufficient external support during sectioning.





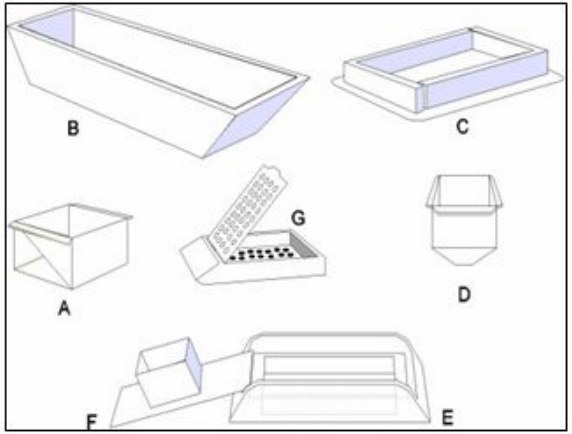


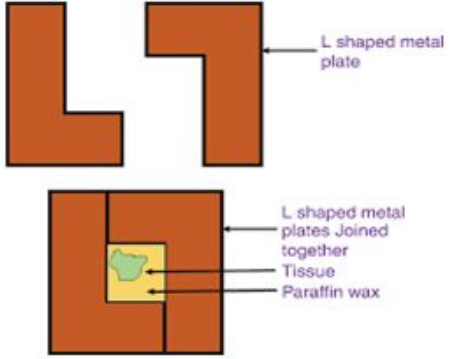
Embedding

ꕥProcedure

1. Place tissue cassette in melted paraffin
2. Fill mould with paraffin
3. Place tissue in mould
4. Allow to cool.

Tissue processing
Embedding moulds:





(A) paper boat;
(B) metal bot mould;
(C) Dimmock embedding mould;
(D) Peel-a-way disposable mould;
(E) base mould used with embedding ring (F) or cassette bases (G)

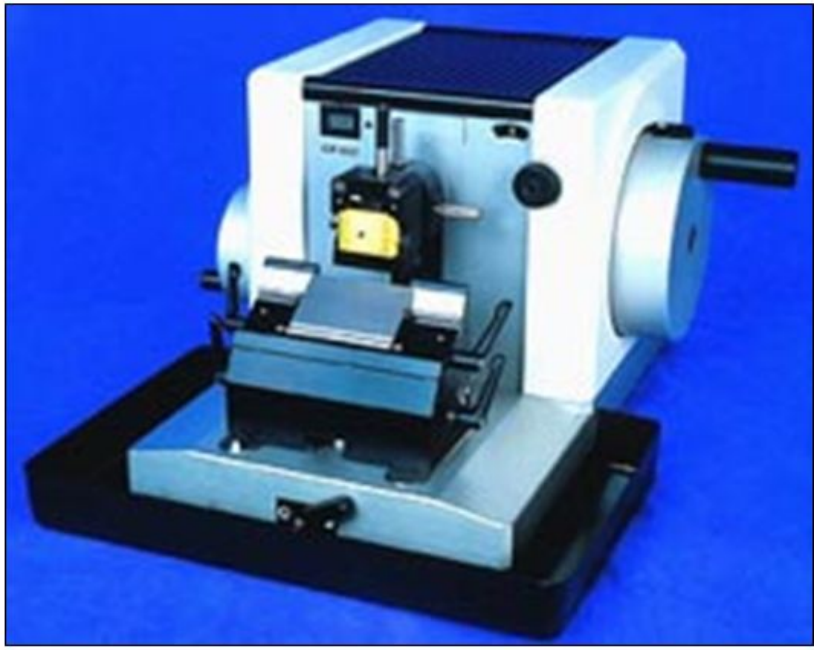
Sectioning – Trimming the Block

Untrimmed tissue block

Trimmed block with excess paraffin removed and block face in a trapezoid shape

7- CUTTING

- using the microtome



A microtome is a mechanical instrument used to cut biological specimens into very thin sections for microscopic examination.

• Most microtomes use a steel blade and are used to prepare sections of animal or plant tissues for histology.

Microtome knives:

- Steel knives
- Disposable metal blades
- Diamond knives
- Glass knives
- Non-corrosive knives for cryostats

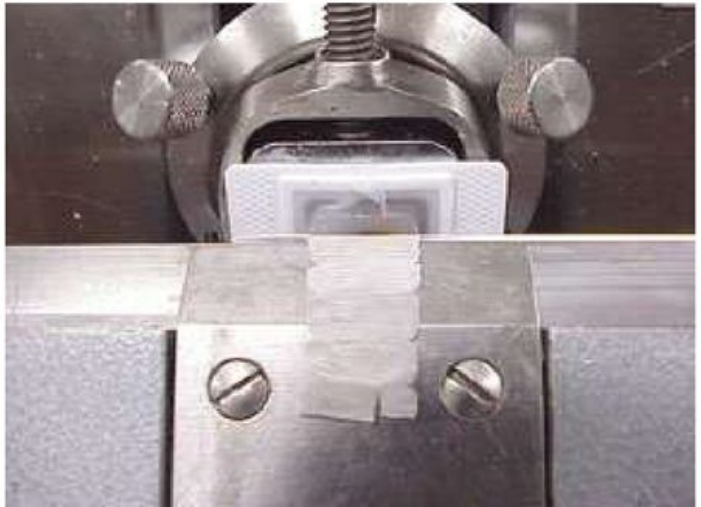
Sectioning

Procedure

1.Place tissue block in microtome with wide edge of trapezoid lowest, and parallel to knife

2.Advance blade toward block

3.Begin sectioning



Mounting sections

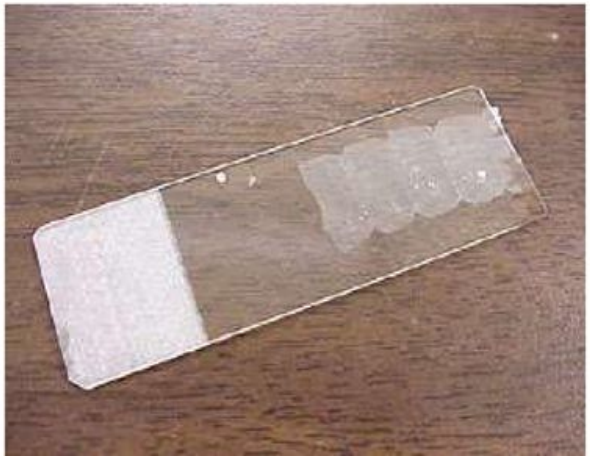
A. 40o C water bath

1. Flattens paraffin section
2. Permits mounting on slide

B. Gelatine & albumin

C. Glass slides

D. Oven / air dry



8- Staining

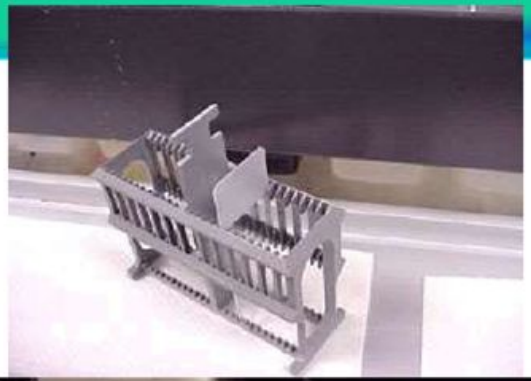
Procedure;

1. Slide rack
2. Solutions;

a. Rehydration

b. stain

c. dehydration



• Staining Procedure

• Deparaffinize and hydrate to water

If sections are Zenker-fixed, remove the mercuric chloride crystals with iodine and clear with sodium thiosulphate

• Stain with haematoxylin for 15 minutes

• Stain with eosin from 15 seconds to 2 minutes.

depending on the age of the eosin, and the depth of the counterstain desired

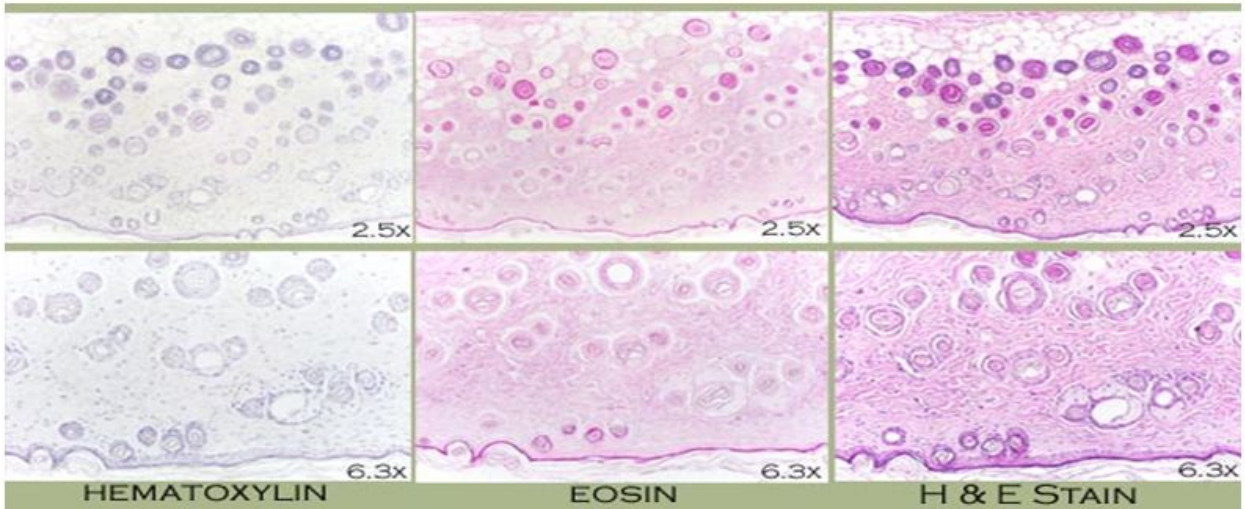
• Dehydrate in 70% .. 95% and absolute alcohols, two change of 2 minutes each or until excess eosin is removed

• Clear in xylene, two changes of 2 minutes each.

Hematoxylin and Eosin (H & E)

H & E is a charge-based, general purpose stain.

- Haematoxylin stains base molecules shades of blue.
- Eosin stains acidic materials shades of red, pink and orange.
- H & E stains are universally used for routine histological examination of tissue sections.



- **Basic dyes stain:**
- **Heterochromatin**
- **Nucleic acids**
- **Ribosomes**
- **Cartilage**

- **• Acidic dyes stain:**
- **Filaments**
- **Mitochondria**
- **Collagen , Muscle fibers**

Additional Dyes

Many tissue components can not be stained with (Hematoxylene and Eosin).

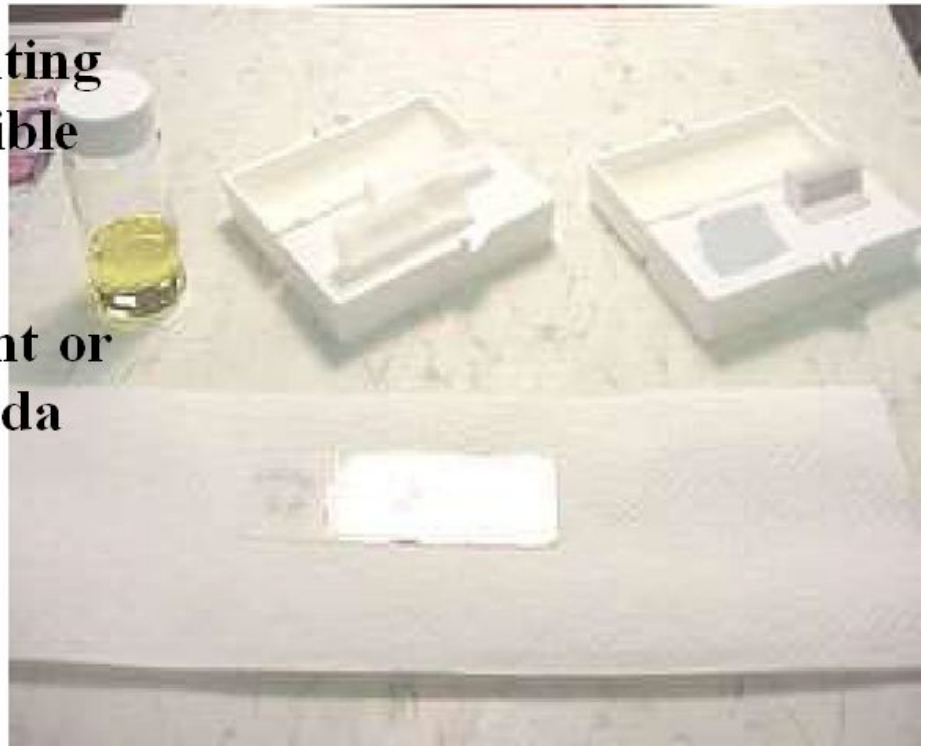
Other dyes are used to specifically stain certain tissue components:

- **Resorcin-Fuchsin** **for elastic fibers**
- **Silver stain** **for reticular fibers and basement membrane**
- **Periodic-Acid Schiff (PAS) Reaction for CHO**
- Trichrome (Masson or Mallory)
- Toluidine blue- CT- breast biopsy
- Giemsa -blood cells

9- Coverslipping:

A. Coverslip & mounting medium (not miscible with water).

B. Mount in Permount or Histoclad or Canada Balsam.



Fixation and wax embedding schedule for FAA

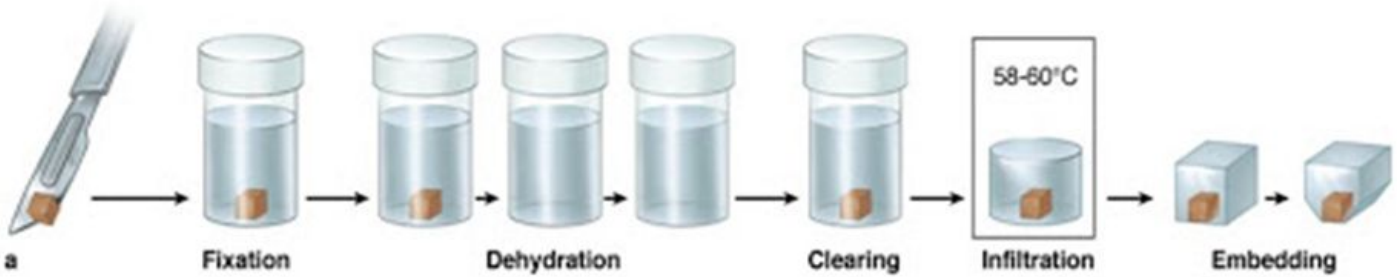
1.	Fixation		
2.	70% ethanol	2 h	overnight
3.	90% ethanol	30 min	1h
4.	100% ethanol	30 min	1h
5.	100% ethanol	30 min	1h
6.	Cedarwood oil	4 h	overnight
7.	Toluene **	15 min	1/2 h
8.	Toluene **	15 min	1/2 h
9.	Wax/Toluene **	15 min	1/2 h
10.	Wax 1	15 min	1/2 h
11.	Wax 2	15 min	1/2 h
12.	Embedding		

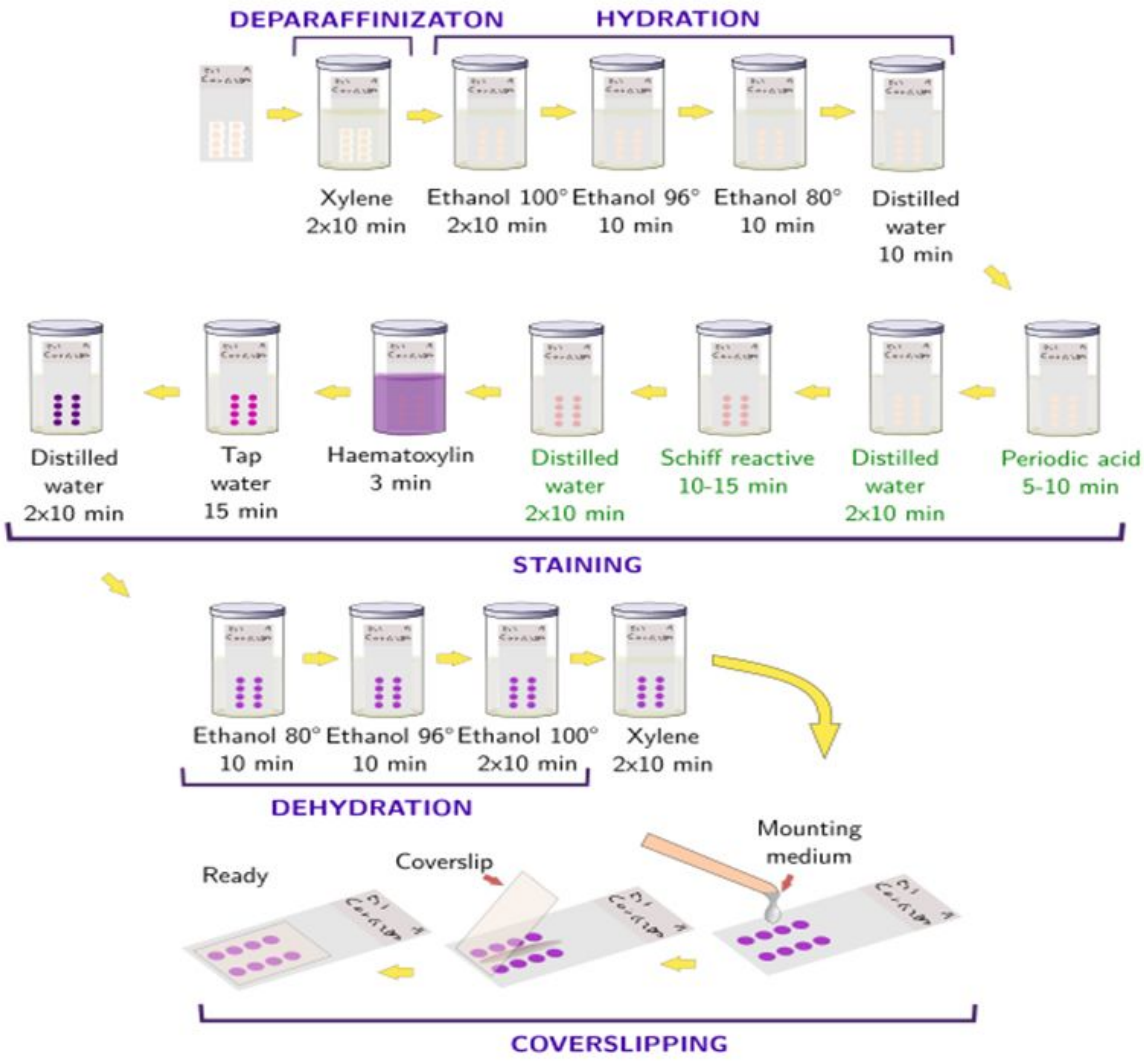
** or Trichloroethylene

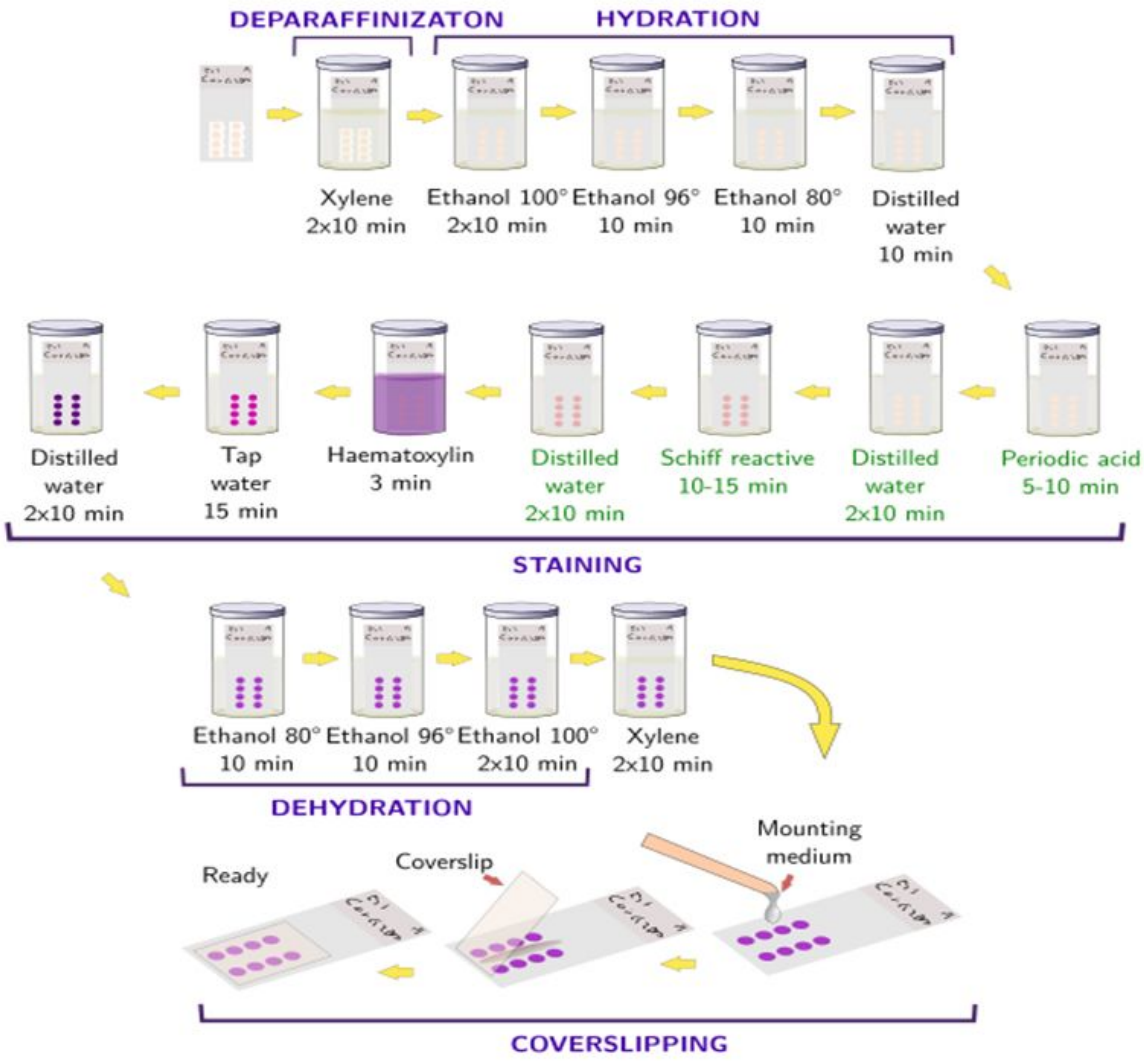
Wax staining schedule (Ehrlich's haematoxylin)

1.	Xylene 1	5 min	dewax
2.	Xylene 2	2 min	intermediate
3.	Abs. alc.1	2 min	
4.	Abs. alc.2	2 min	
5.	90% alc.	2 min	
6.	70% alc.	2 min	
7.	Haematoxylin	20 min	nuclear stain
8.	Rinse water	1 min	
9.	Acid alc.2 (70% alc +1/2 HCl)	brief dips	
10.	Running water	5 min	blueing
11.	70% alc.	2 min	
12.	Eosin (1% Eosin + 90% alc)	1/2 min	counterstain
13.	90% alc.	1 min	
14.	Abs alc.2	2 min	
15.	Abs alc.1	2 min	
16.	Xylene 2	2 min	intermediate
17.	Histoclear		awaiting mounting

Tissue processing




DEPARAFFINIZATON
HYDRATION
Xylene
2x10 min
Ethanol 100°
2x10 min
Ethanol 96°
10 min
Ethanol 80°
10 min
Distilled
water
10 min
Distilled
water
2x10 min
Tap
water
15 min
Haematoxylin
3 min
Distilled
water
2x10 min
Schiff reactive
10-15 min
Distilled
water
2x10 min
Periodic acid
5-10 min
STAINING
Ethanol 80°
10 min
Ethanol 96°
10 min
Ethanol 100°
2x10 min
Xylene
2x10 min
DEHYDRATION
Ready
Coverslip
Mounting
medium
COVERSLIPPING


DEPARAFFINIZATON
HYDRATION
Xylene
2x10 min
Ethanol 100°
2x10 min
Ethanol 96°
10 min
Ethanol 80°
10 min
Distilled
water
10 min
Distilled
water
2x10 min
Tap
water
15 min
Haematoxylin
3 min
Distilled
water
2x10 min
Schiff reactive
10-15 min
Distilled
water
2x10 min
Periodic acid
5-10 min
STAINING
Ethanol 80°
10 min
Ethanol 96°
10 min
Ethanol 100°
2x10 min
Xylene
2x10 min
DEHYDRATION
Ready
Coverslip
Mounting
medium
COVERSLIPPING

- 10- Examining

Thank You