AL-Mustaqbal University College

Pharmacy Department

Third stage

Practical Pathophysiology

(General introduction & Slide preparation)

Lab 1

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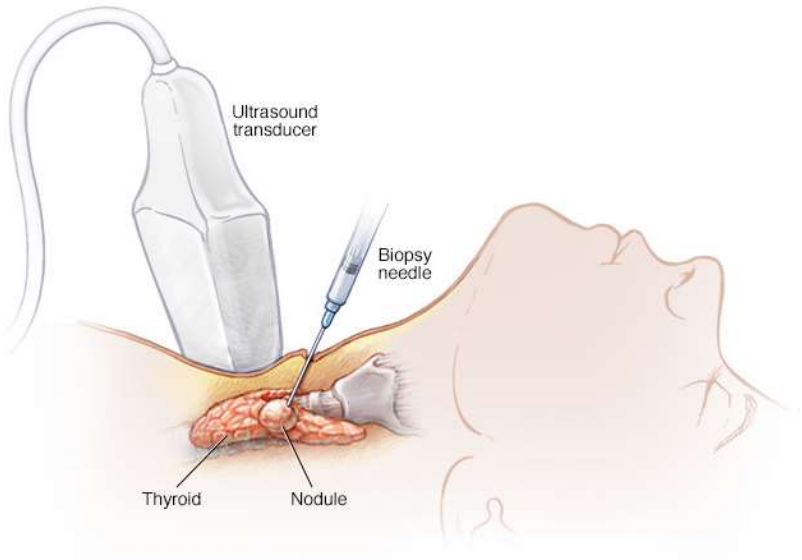
General introduction & Slide preparation

Pathology: The science of the causes and effects of diseases, it deals with the laboratory examination of samples (specimens) of body tissue for diagnostic or forensic purposes.

Biopsy: An examination of tissue removed from a living body to discover the presence, cause, or extent of a disease.



Autopsy: A post-mortem examination to discover the cause of death or the extent of disease.

Tissue preparation

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 as following.

1. Obtaining a fresh specimen

2. Fixation

3. Dehydration

4. Clearing

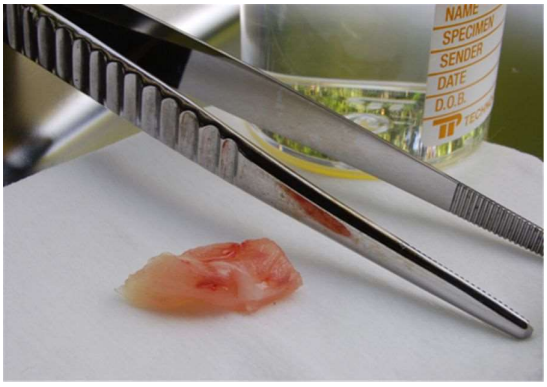
5. Wax infiltration

6. Embedding or blocking out

1. Obtaining a fresh specimen

• Fresh tissue specimens 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. Fixation

- To maintain the natural state of specimen.
- Fixation facilitates proper staining of tissue.
- Fixative should be 20-25 times of volume of specimen.

Characters of a good fixative:

*Penetrate quickly * Prevent tissue from shrinkage

Good fixatives: • Formaldehyde • Alcohol • Picric Acid • Potassium Dichromate etc.

Washing

- For removal of fixative.
- 6-8 hours.
- Under tap water.

3. Dehydration: Removal of water.

- Alcohols are most commonly used for dehydration.
- Water replacement with alcohol.
- Done by transferring the samples into ascending concentration of ethanol as follow:

- 70% ethanol for 2 hours.
- 80% ethanol for 2 hours.
- 90% ethanol for 2 hours.
- 100% ethanol for 2 hours.

4. Clearing

- Removal of dehydrating agent.
- Clearing agent, miscible with both embedding medium and dehydrating agent.
- Xylene (clearing agent)
- Two times for 1 hours each.
- **Other reagents:** Toluene, Chloroform, Benzene, Cedar wood oil etc.

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 melted paraffin.

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

6. Embedding or blocking out

- Allow the melted wax to be solidify.
- It gets rigid.

A. Orient tissue

1. cross section

2. longitudinal section

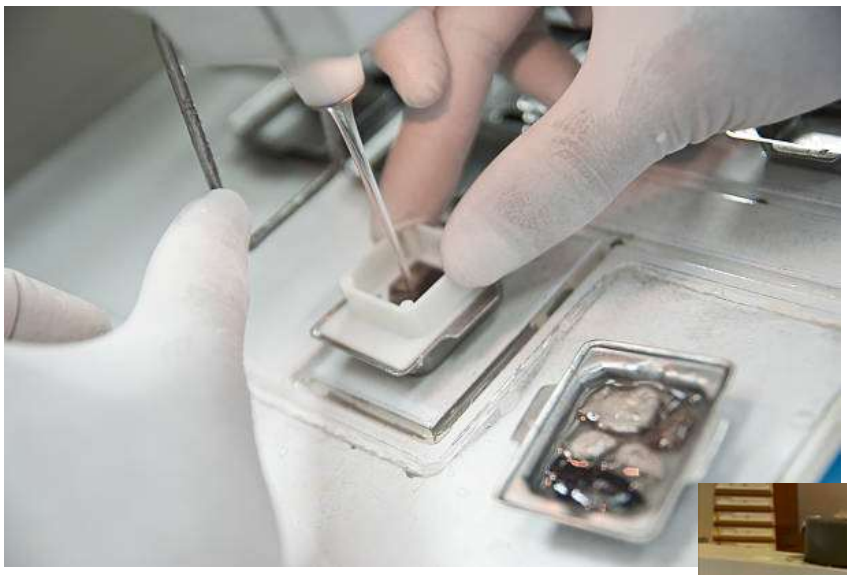
B. Dissection orientation

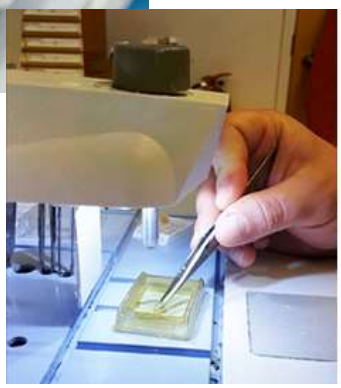
C. Avoid bubbles

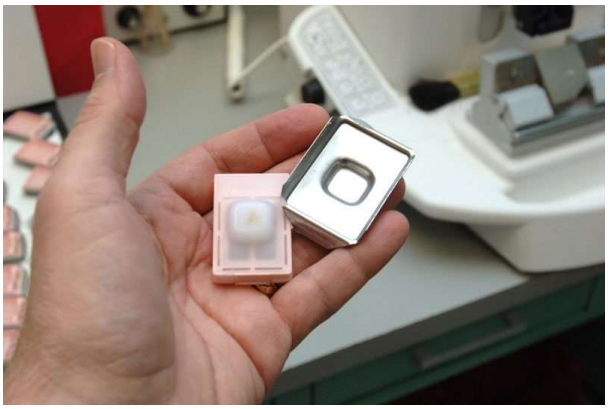
Embedding

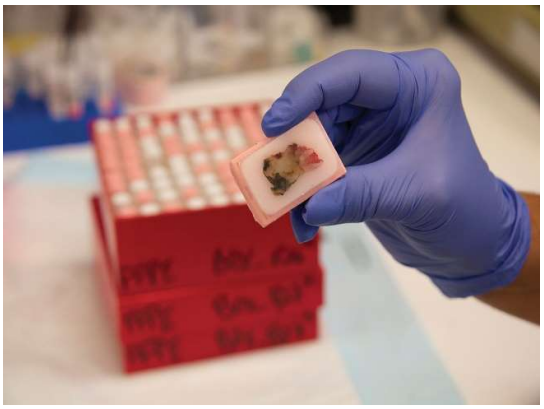
- **Procedure**

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







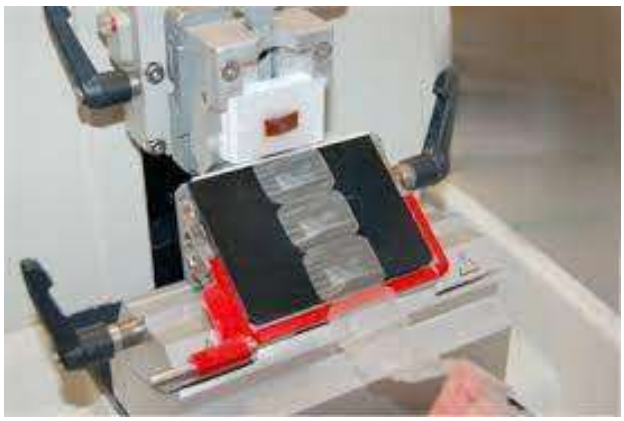


Sectioning – Trimming the Block

Untrimmed tissue blocks trimmed using a sharp scalpel and excess paraffin removed and block face in a trapezoid shape.

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. Gelatin & albumin

C. Glass slides

D. Oven / air dry

Staining

Procedure

1. Slide rack

2. Solutions

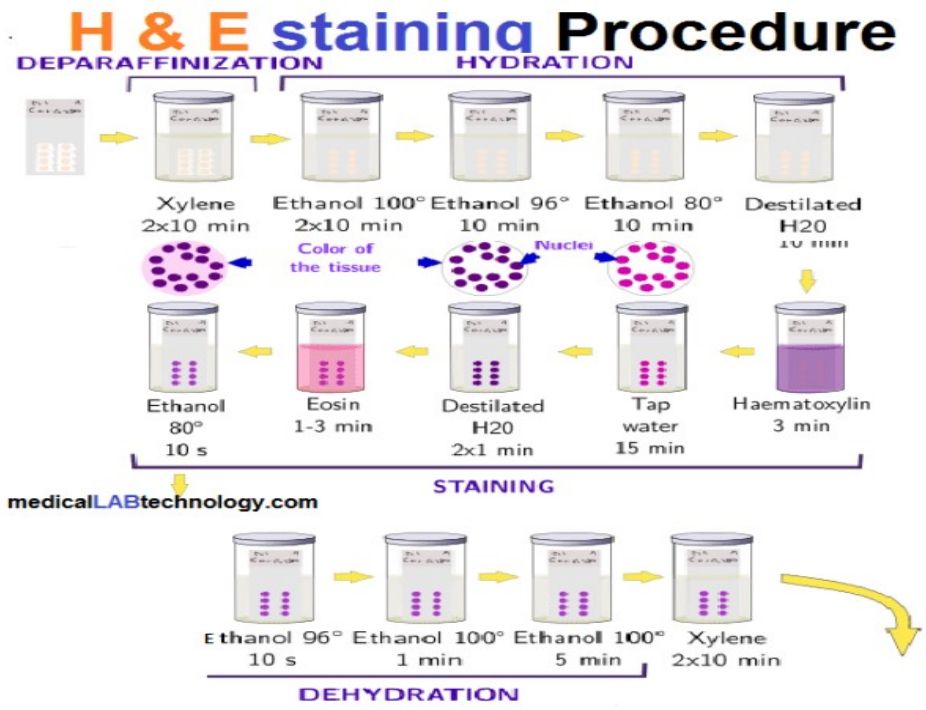
a. rehydration

b. stain

c. dehydration

Routine stains

Hematoxylin & Eosin



Cover slipping

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

B. Dehydrate

C. Clearing agent