

**Mustansiriyah University**

**College of science**

**Biology Dept.**

**Zoology**

**4<sup>th</sup> class**

**Laboratory Technique {*Histological Technique*}**

**(4)**

## (cutting)

- For **light microscopy**, a glass knife mounted in a microtome is used to cut 4-6  $\mu\text{m}$ -thick tissue sections which are mounted on a glass microscope slide.
- For transmission **electron microscopy**, a diamond knife mounted in an ultra microtome is used to cut 50-nm thick tissue sections which are mounted on a 3-mm diameter copper grid. Then the mounted sections are treated with the appropriate stain.
- **Frozen** tissue embedded in a freezing medium is cut on a microtome in a cooled machine called a **cryostat**.







# Staining

- ❑ Staining is a process by which we give color to a section.
- ❑ There are hundreds of stains available.

## Classification of Stains:

- ❑ Acid stains
- ❑ Basic stains
- ❑ Neutral stains



## H&E stain :

- Hematoxylin (water based dye).
- Eosin (counter stain).

They stains nucleus & cytoplasm

## Procedure of staining:

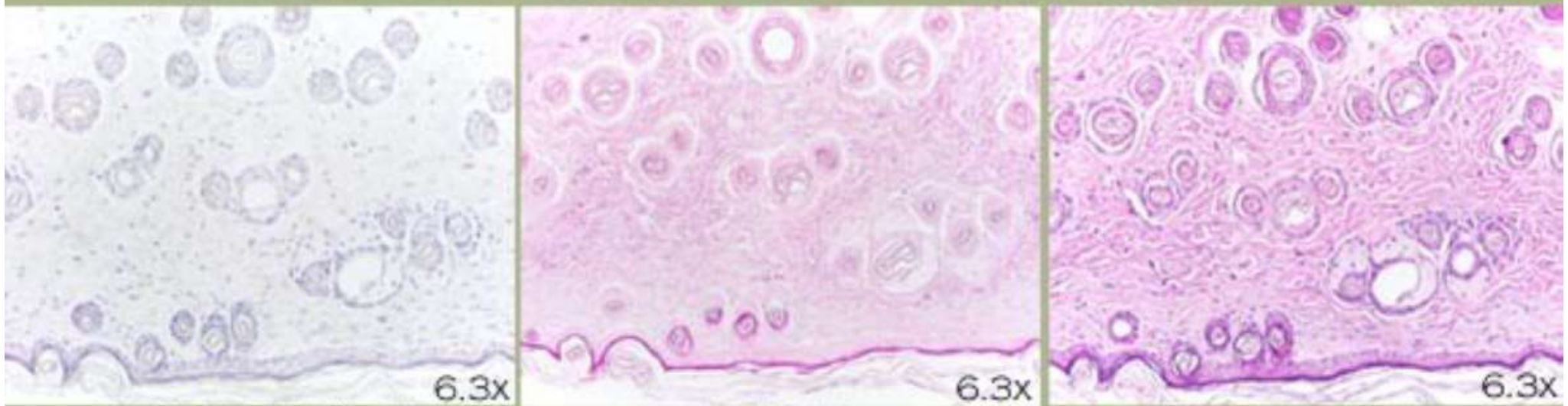
There are two types of staining,

- Manual Staining
- Automatic staining

## Hematoxylin and Eosin (H & E)

- ❑ H & E is a charge-based, general purpose stain.
- ❑ Hematoxylin stains acidic molecules shades of blue.
- ❑ Eosin stains basic materials shades of red, pink and orange.
- ❑ H & E stains are universally used for routine histological examination of tissue sections.





HEMATOXYLIN

EOSIN

H & E STAIN

## Staining Procedure

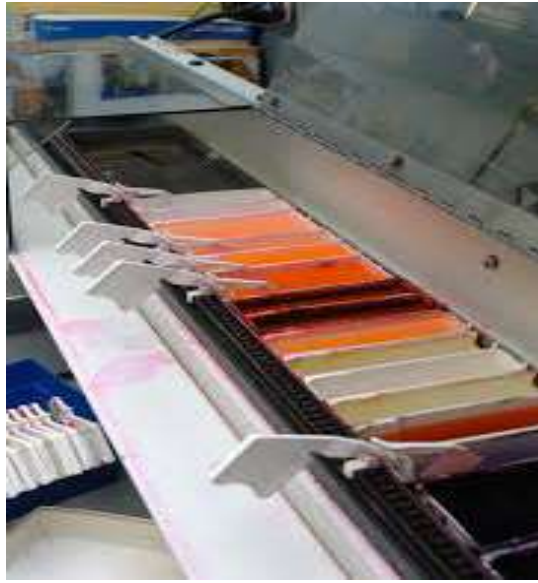
- ❑ Deparaffinize and hydrate to water
- ❑ Mayer's hematoxylin for 15 minutes
- ❑ Counterstain with eosin from 15 seconds to 2 minutes depending on the age of the eosin, and the depth of the counterstain desired
- ❑ Dehydrate in 95% and absolute alcohols, two changes of 2 minutes each or until excess eosin is removed
- ❑ Clear in xylene, two changes of 2 minutes each

## Manual Staining

- ❑ In a small laboratory when a few slides are stained daily, this is the method of choice.
- ❑ Although it is time consuming .
- ❑ Different reagent containers are placed in a special sequence and the slides are removed from one container to another manually.

<https://www.youtube.com/watch?v=ncj8JVsnZGU>

<https://www.youtube.com/watch?v=P0cZKCfyUwE>



# Automatic staining

In this procedure an automatic stainer is required.

- It has a timer, which controls the time.
- It has a mechanical device which shifts the slides from one container to next after the specified time.

Advantages of automated stainer are:

- 1) It reduces the man power
- 2) It controls the timing of staining accurately
- 3) Large number of slides can be stained simultaneously
- 4) Less reagents are used

## Note:

Slides stained either manually or by automatic stainer, pass through same sequences.

