

Mustansiriyah University

College of science

Biology Dept.

Zoology

4th class

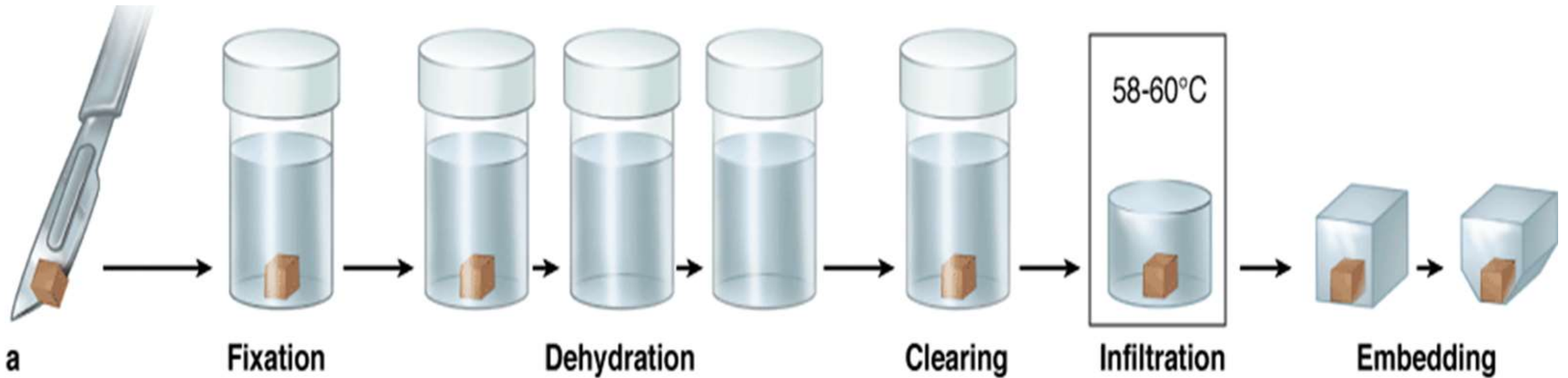
Laboratory Technique {*Histological Technique*}

(3)

Tissue Processing:

- 1) Dehydrations.
- 2) Clearing.
- 3) Impregnation.
- 4) Embedding

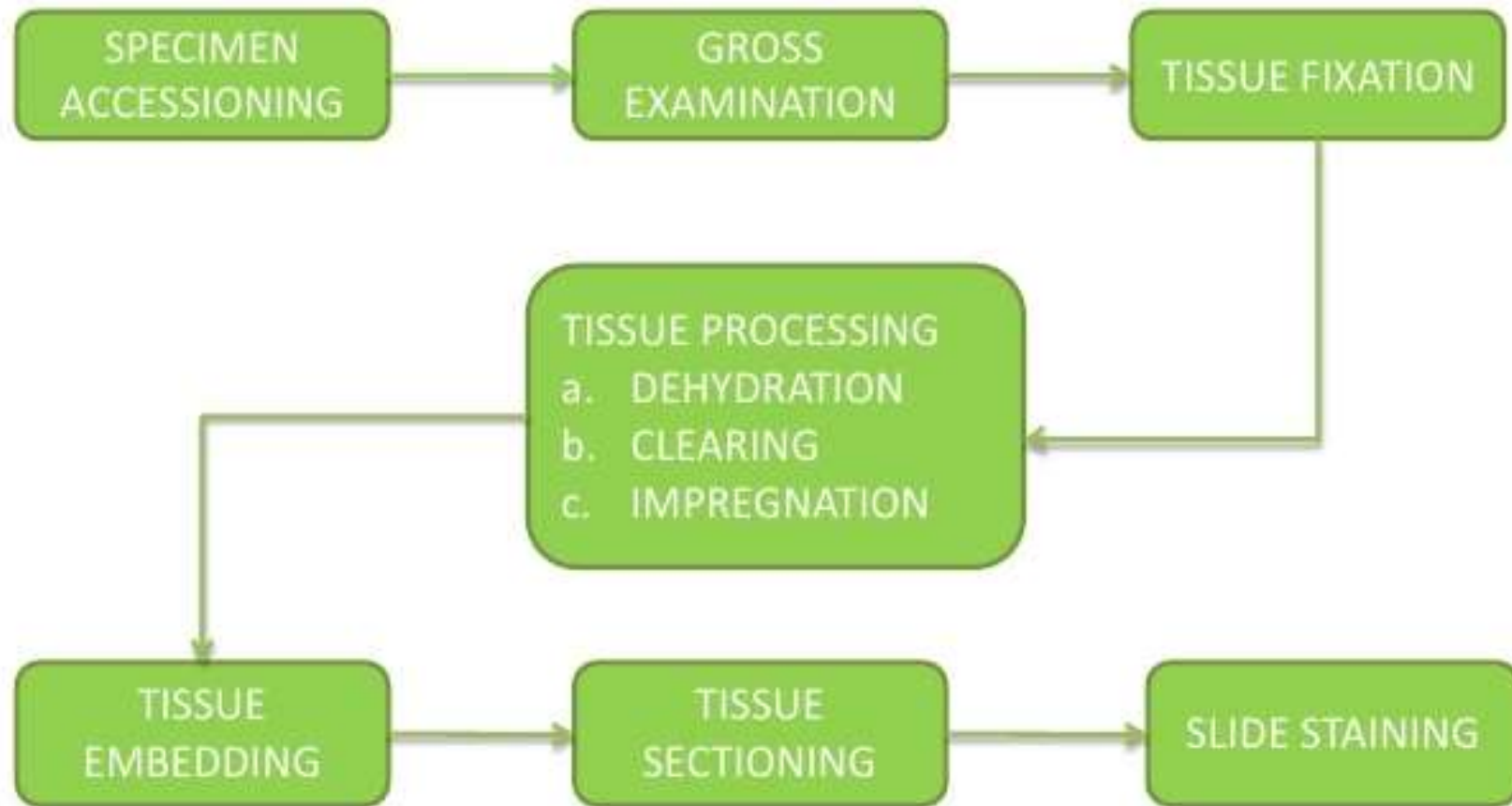
It requires 24 hours and done in many stages.



Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition*: <http://www.accessmedicine.com>

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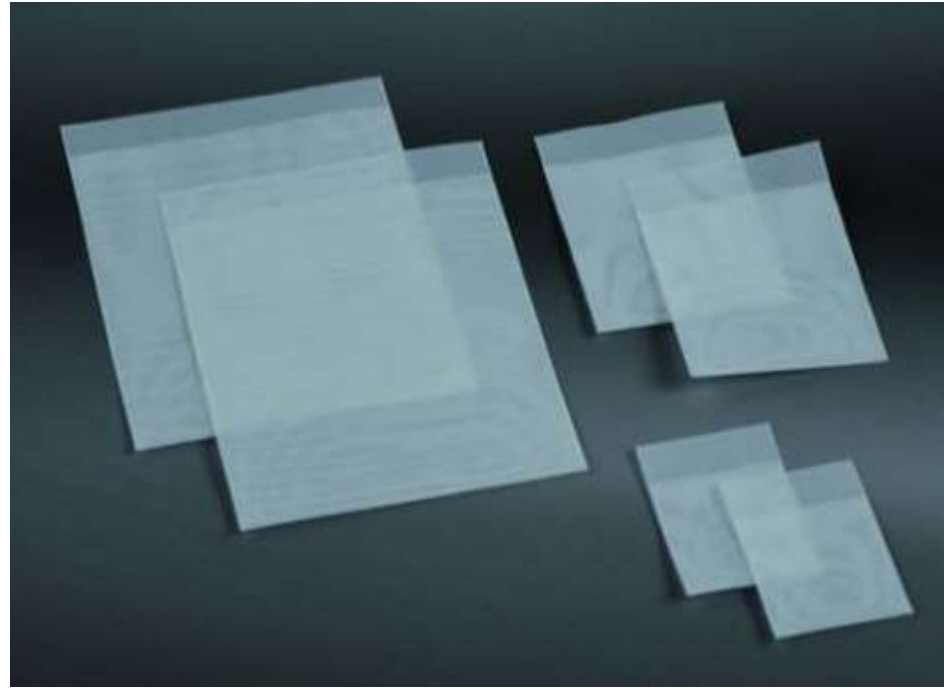
HISTOLOGY PROCEDURE





R.K. Tissue Embedding Cassettes





Tissue Processing

- In order to cut thin sections of the tissues, it should have suitable hardness and consistency when presented to the knife edge.
- It is important that all specimens are properly labeled before processing is started.

Types of tissue processing

1. Manual Tissue Processing:

2. Mechanical Tissue Processing:

➤ Mechanical Tissue Processing

- ❑ In this the tissue is moved from one jar to another by mechanical device.
- ❑ Timings are controlled by a timer which can be adjusted in respect to hours and minutes.
- ❑ Temperature is maintained around 60°C.





Clearing

- During dehydration water in tissue has been replaced by alcohol.
- The next step alcohol should be replaced by paraffin wax.
- As paraffin wax is not alcohol soluble, we replace alcohol with a substance in which wax is soluble. This step is call clearing.



□ Clearing of tissue is achieved by any of the following reagents:

1. Xylene
2. Chloroform
3. Benzene
4. Carbon tetrachloride
5. Toluene

Note:

Xylene is commonly used. Small piece of tissue are cleaned in 0.5 - 1 hour; whereas larger (5cm or more thick) are cleaned in 2-4 hours.

xylene is routinely used for clearing tissues.

Impregnation with Wax

- ❑ This is allowed to occur at melting point temperature of paraffin wax, which is 54-60°C. Volume of wax should be about 25-30 times the volume of tissues.
- ❑ The duration of impregnation depends on size and types of tissues and the clearing agents employed.
- ❑ Longer periods are required for larger pieces and also for harder tissue like bones and skin as compared to liver kidney, spleen, lung etc.



Embedding with Wax

- In order to distinguish the overlapping cells in a tissue and the extracellular matrix from one another, the histologist must embed the tissues in a proper medium and then slice them into thin sections,
- paraffin embedding replaces tissue water with paraffin wax, enabling the block to be cut readily, the xylene-permeated block



Embedding

- the tissue is placed in a suitable container of melted paraffin until it is completely infiltrated,
- once the tissues become completely saturated, melted wax occupies spaces formerly occupied by water,
- after the tissue is impregnated with paraffin, it is placed into a small receptacle, covered with melted paraffin, and allows to harden, forming a paraffin block containing tissue.



Embedding

- Impregnated tissues are placed in a mould with their labels and then fresh melted wax is poured in it and allowed to settle and solidify.
- Once the block has cooled sufficiently to form a surface skin it should be immersed in cold water to cool it rapidly.
- After the block has completely cooled it is cut into individual block and each is trimmed.
- Labels are made to adhere on the surface of the block by melting the wax with a metal strips sufficiently warmed.

