

Preparing educational tissue slides for students of the Department of Medical Laboratory Technology in southern technical collage

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Abstract

The goal of this study is to learn how to create a microscopic slide of plant or animal tissue and get it ready for compound microscope inspection. Without a doubt, those involved are students at all levels of the medical field as well as those interested in topics related to histology, embryology, anatomy, cell science, and animal and plant sciences. This study was conducted in the pathological analysis department of The Medical Technical Institute in Basra.

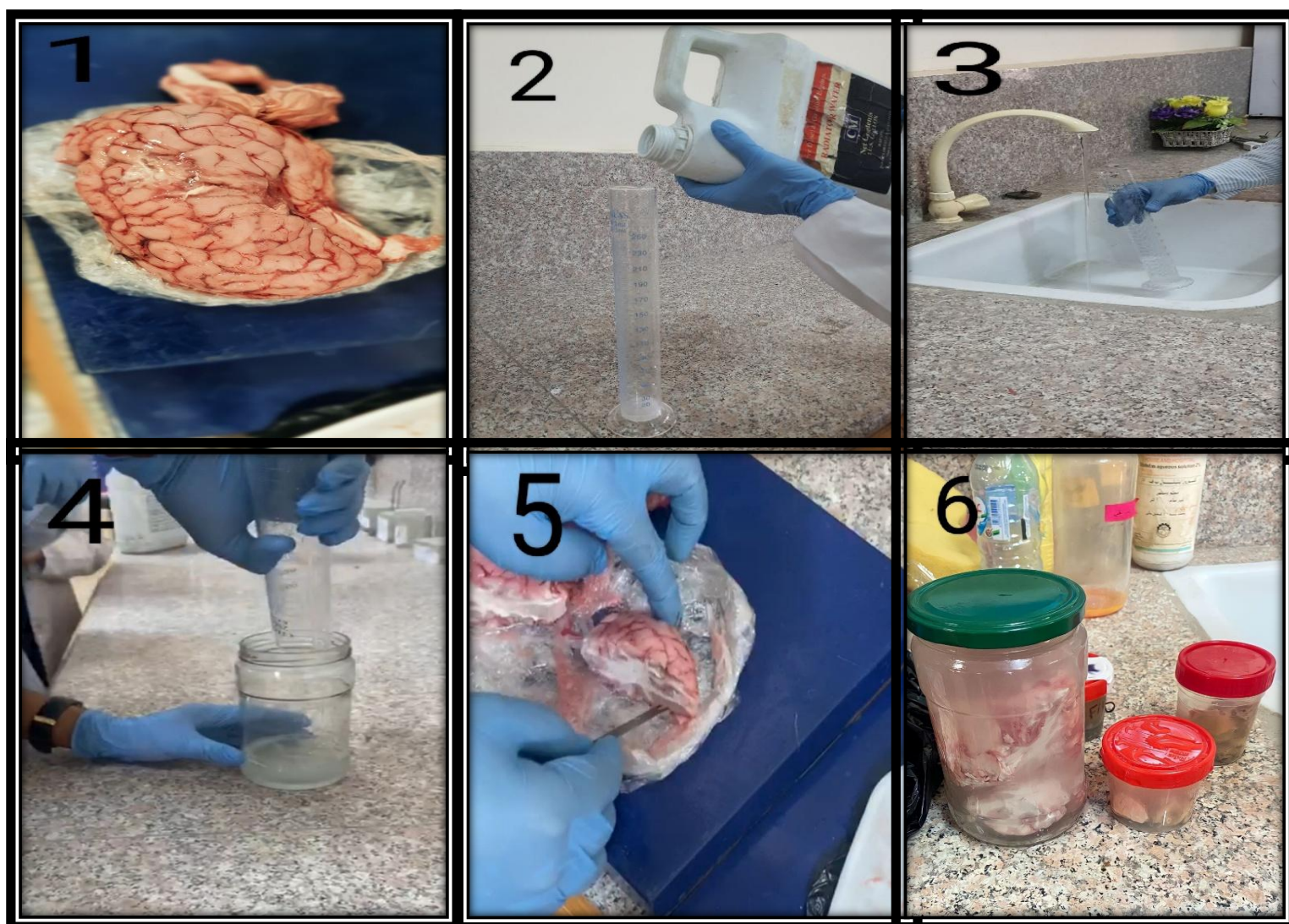
Key words: *Histology, slides, precreation, paraffin wax, cutting, dehydration .*

I. Introduction

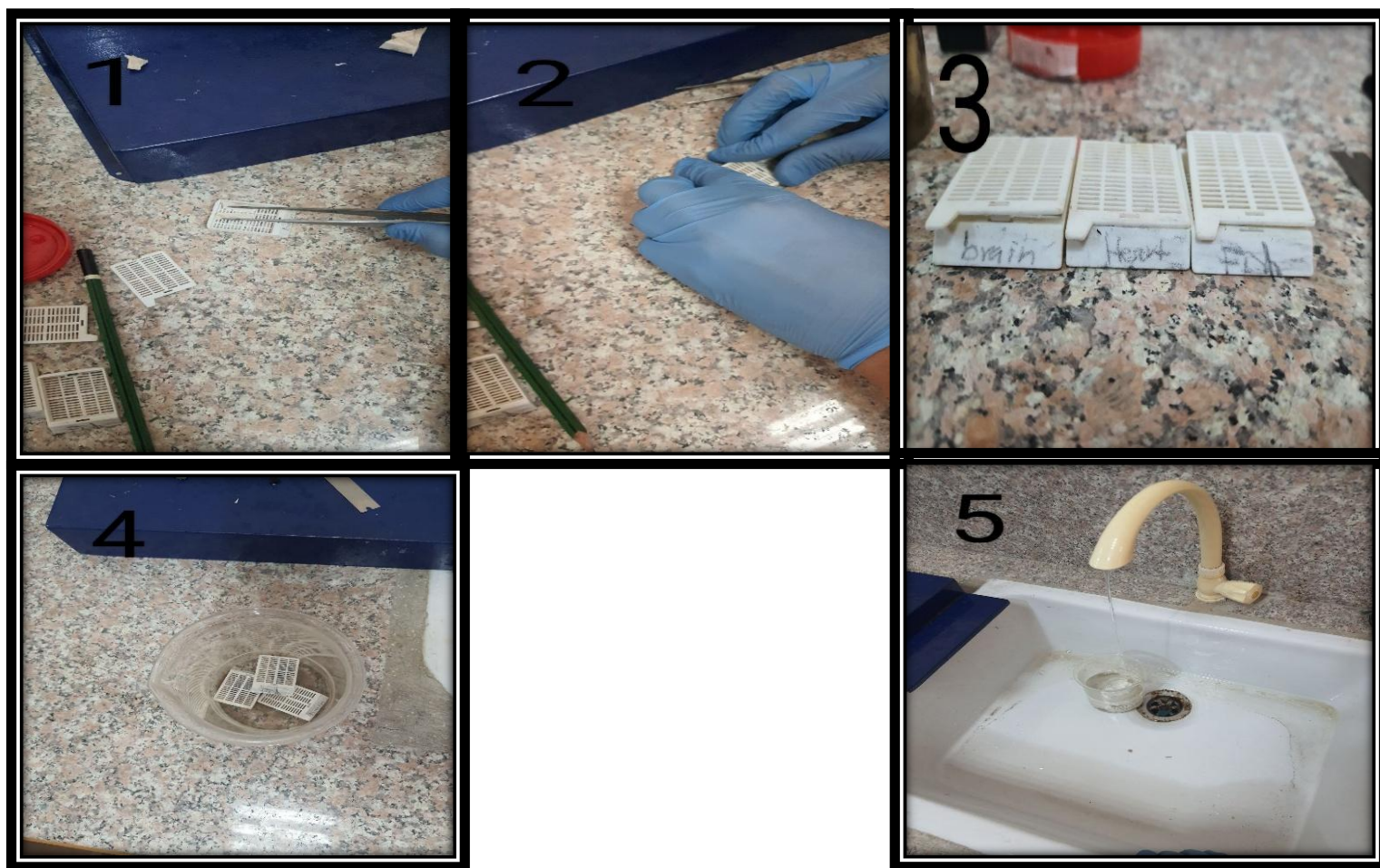
Often referred to as microscope glass slides, histology slides are devices used to demonstrate or examine the microscopic structure of cells, tissues, and organs as well as the relationship between structure and function. Often, these objects are biological specimens that are held or placed on thin, flat glass surfaces, usually coloured to highlight different structural details, and then examined under a microscope, a photomicrograph, often known as a micrograph and frequently produced from a glass slide ,a photo taken using a microscope to display a magnified or computerized image of a specimen or object is created. Consequently, it is a visual representation of an object's image created by a microscope. (Connett, 2017)In the study and use of histology, having access to a collection of histological glass slides and photomicrographs is crucial . using microscopes in fields like molecular biology, medicine, and others. The ability to produce tissue samples and apply the information to assess cells and tissues under a microscope is more important than merely copying the content from histology textbooks. Unfortunately, histology is still taught without laboratories most of the time, and a histology atlas is often used in its place because of the technical expertise required, the difficulty in preparing or obtaining high-quality slides, and the high cost of buying pre-made slide collections for teaching and demonstration. 2005's Sorenson and Brelje, The focus of this historical approach to histology is microscopy, which allows the observer to see minute or very small thin structures up close at a scale that facilitates straightforward inquiry and interpretation. (Ford and Shannon, 2021), Major changes to many schools' medical teaching curricula throughout the years have imposed serious limitations. depending on the amount of time allotted for teaching standard laboratory courses in the anatomical disciplines(Fitzharris, 1998; Hightower et al., 1999; Cotter, 2001). In order to see the true microstructure of cells, tissues, and organs, this research project was developed to promote efficiency or mastery in the creation of slides and photomicrographs. These microscopic preparations were crucial to the medical and research aspects up until the current day since they also seek to revive scientific or practical interest in conventional histopathology techniques and investigate the initial basis for illness diagnosis, also Teaching students of the Department of Laboratory Techniques in all faculties and institutes how to prepare tissue slides.

II. MATERIALS AND METHODES

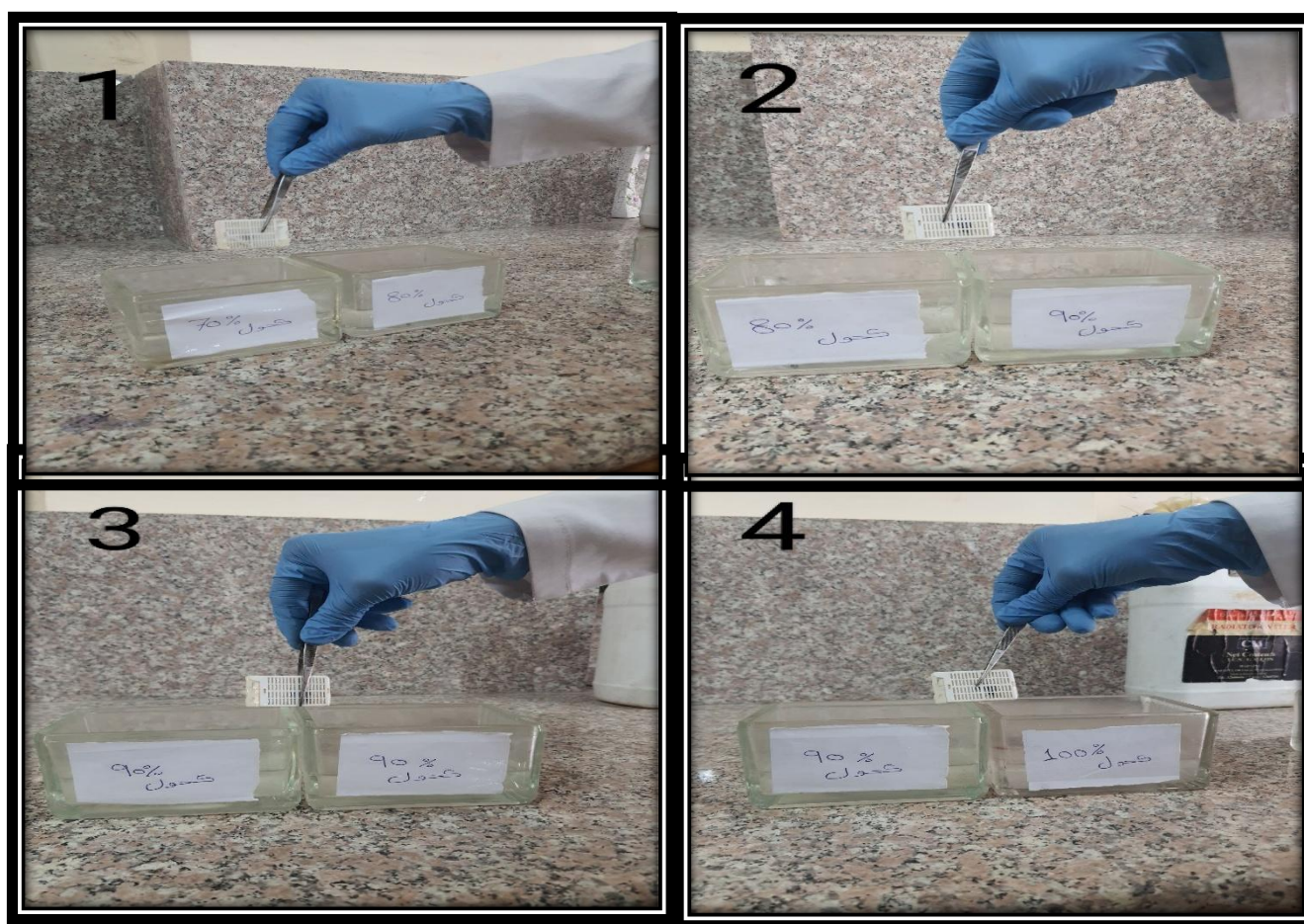
Fixation Procedure : We start with an empty, clean container, mark it with a pen, Wax pencil, or name it, and then add 10% formalin concentration and 90% tap water to it using a graduated cylinder. Finally, we chop the tissue into pieces that are around 4-5 micrometers in size ,then put the sample in the diluted formalin using forceps and close it and put It in a dark container .



Washing : A clamped capsule Is used in Which the tissue Is placed then it is closed and placed inside the cap under tap water stream for (24h) and label the capsule by using pencil.



Dehydration: Firstly we prepare different concentration of alcohol gradually in ascending order as: 70%.. 80%..90%..100.% ... Then pass the specimen through this series alcohol into each container for 15 min_1 h depending on the type of the tissue, the specimen passed twice in the 90% concentration and twice in the 100% concentration for improvement of dehydration process .



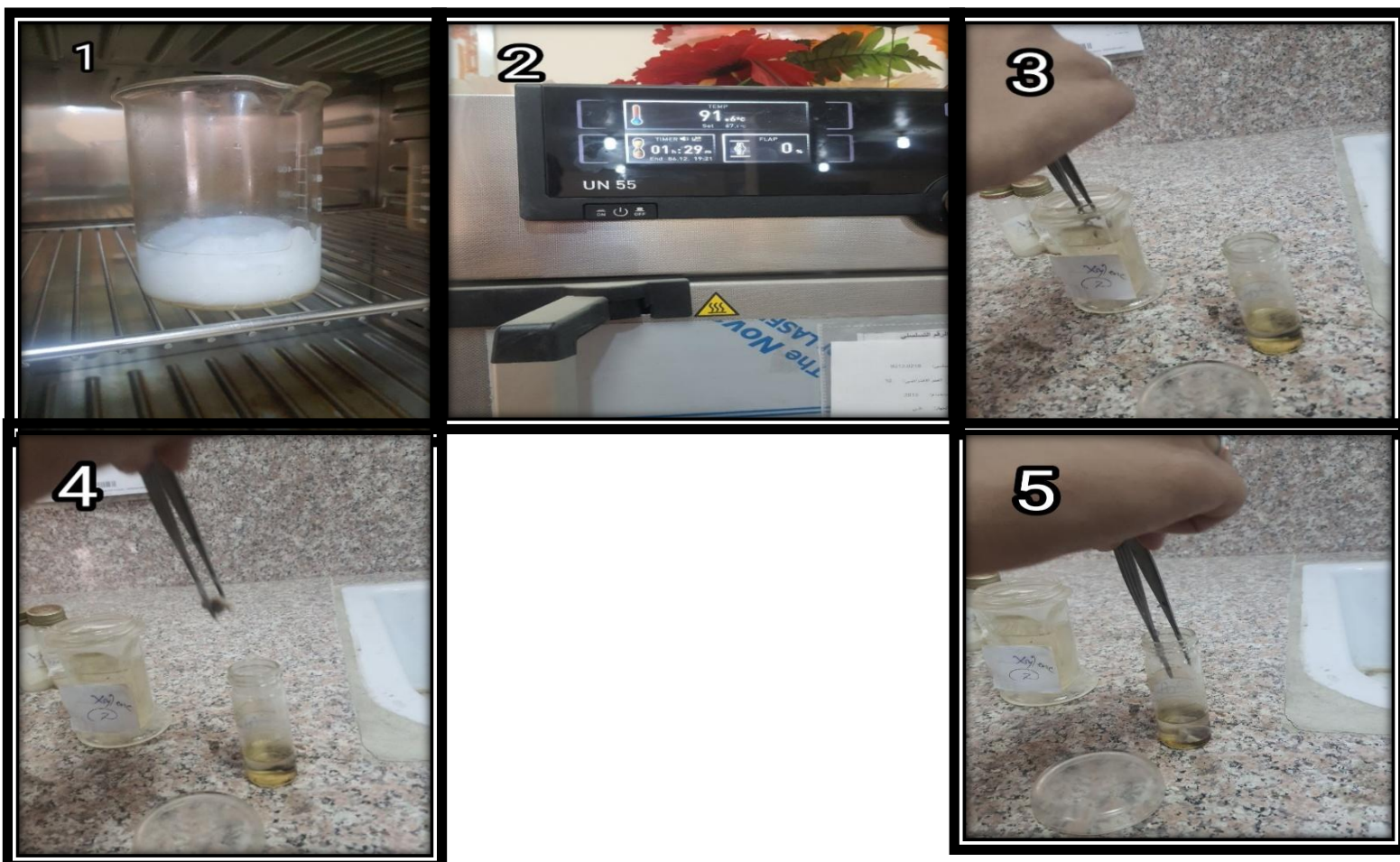
Clearing :Before starting this process, we prepare two beakers and put the clearing agent (Xylene) in them . The volume of the solution in the beaker is not much, We use glass beaker to be able to monitor the transparency of the specimen, The specimen is placed freely in the beaker, This process is fast so it is not important to write the specimen information on the beaker, We put the beaker under the light to observe the specimen , and if the tissue acquired degree of transparency we transfer the specimen using forceps to the other beaker , when the specimen reaches the full transparency, we take it out using forceps and start the next process. the time in both beakers depends on the type of tissue.



Infeltration we have prepared the tools and materials , and we set the specimen with the wax in the liquid, we use a closed vessel (that contains a lid) then transfer the tissue from the beaker to the first container ,and then transfer it to



the second container in order to ensure the improvement of the infeltration process by using forceps ,the time for this process is 2hr for each container .



Blocking : Warm the forceps and transfer the infiltrated tissue tissue from container to the mold(at the middle) and set up the liquid wax into the mould and then put the capsule above the liquid wax immediately and press the capsule until the wax is allowed to come out and then left the mould for same time until transfer to sold state by put it in the refrigerator or left it at room temperature.

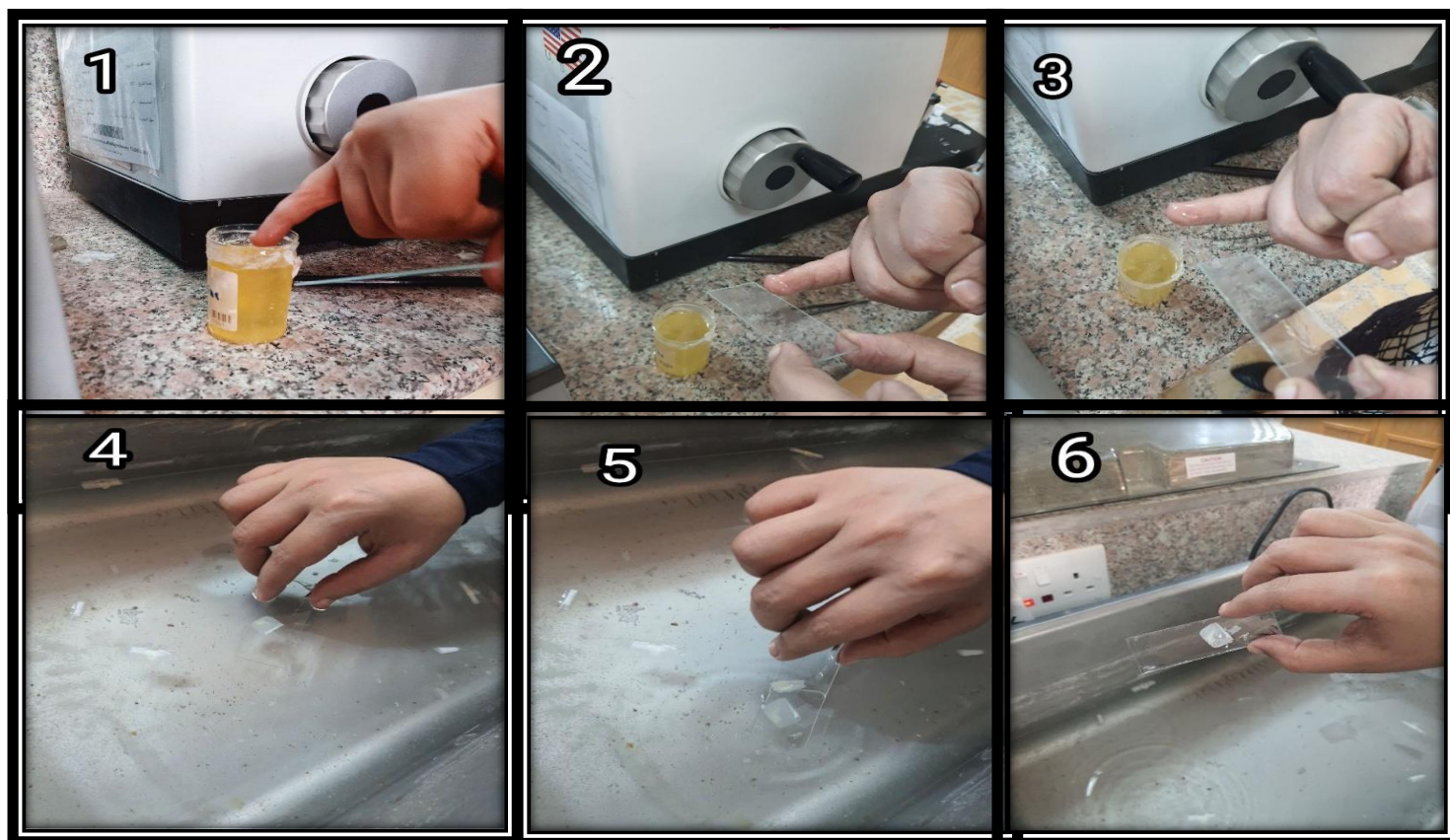
Cutting : The microtome's chuck holds the block in place. The block's cutting surface ought to go parallel to the knife's. A good section requires an angle of clearance of just 2 to 5 degrees. The tissue in the block is sliced with a moderate, soft stroke. The tissue parts resemble ribbons. The terminal portion of the ribbon is brushed away from the knife edge while the tip is kept in place with forceps. In the event that there is any trouble The cutting surface should be slowly warmed by warm water to get the flat section.

8- floating ,mounting and drying:

Individual portions are separated from one another using forceps. As previously stated, it is important to continuously keep the water bath's temperature below the paraffin wax's melting point. The slide is positioned vertically in front of the tissue in the water bath, and it is removed from the water vertically when the tissue is contacted.



Mounting:The tape is affixed to the slide using Meyers albumin after placing one drop of albumin with a light smear by the finger on the slide, then the slide is inserted in the water bath obliquely so that the wax tape in the water is picked up, after that the slide is lifted from the basin and shakes slightly to remove excess water From slide and wax tape

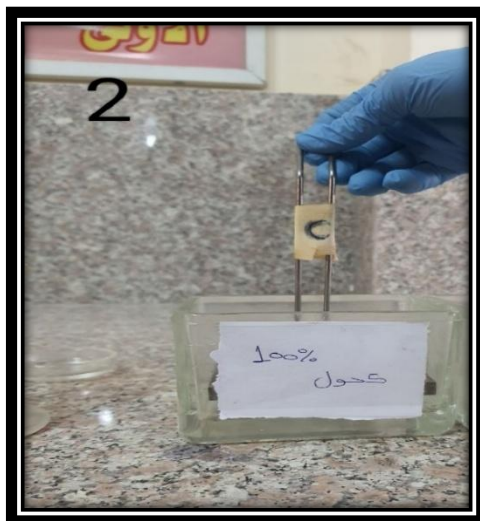


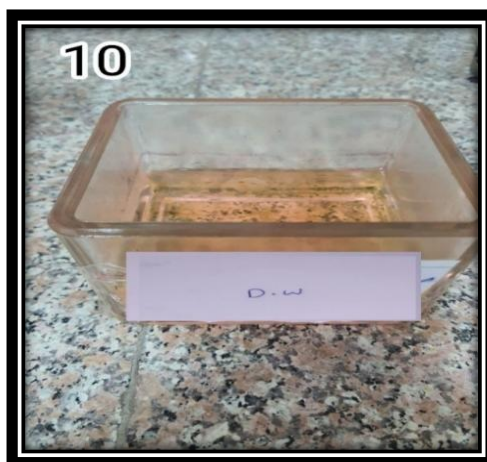
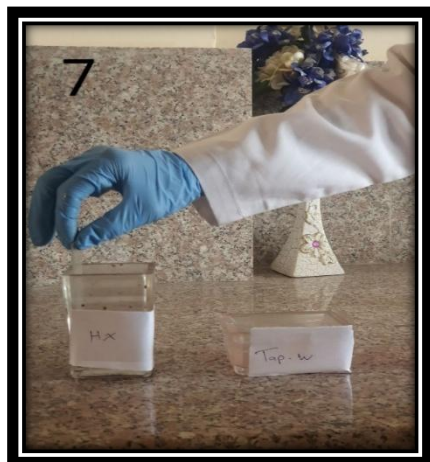
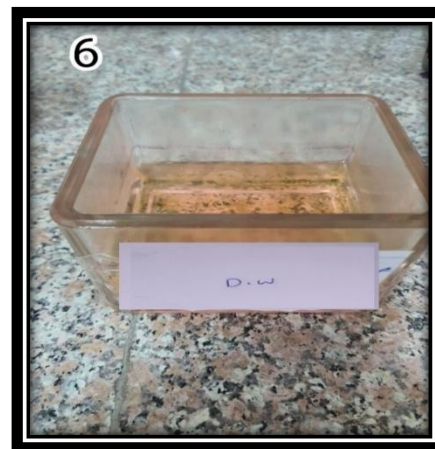
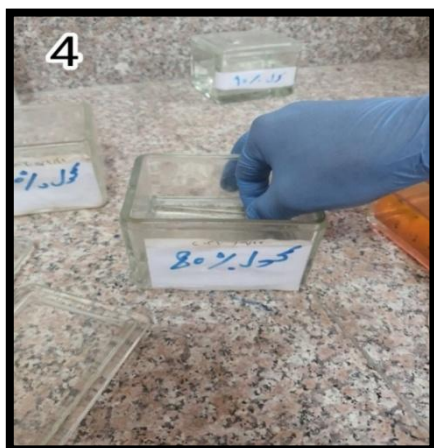
Drying The slide containing the section is kept in slide rack. The Slides are now kept in Room temperature to get dry.



Staining :Procedure:

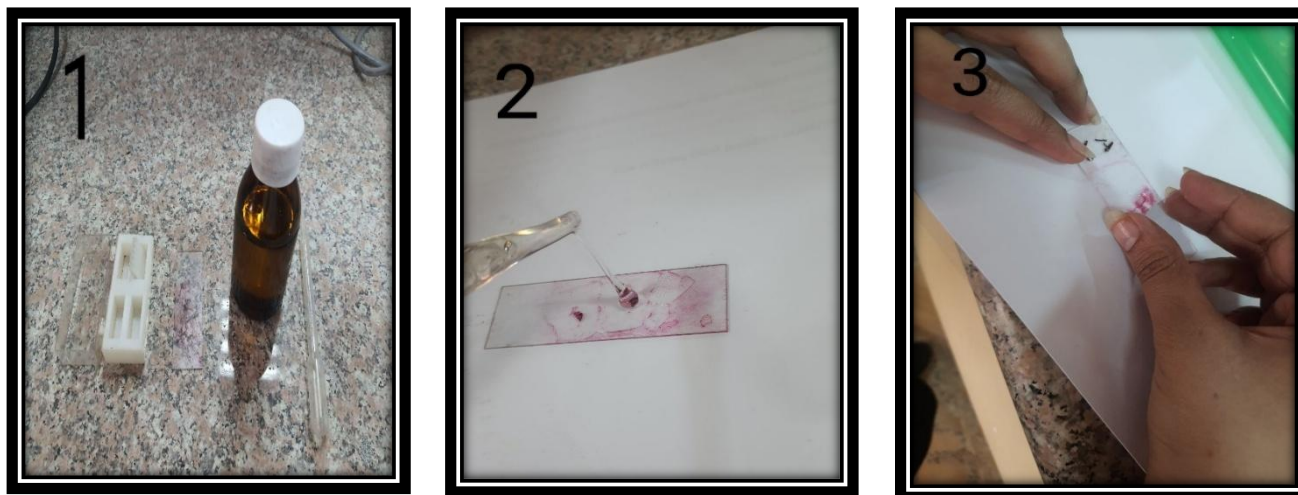
- . Take the slide to first xylen for 2min
- Take the slide to 100% alcohol for 2min
- Take the slide to 90% alcohol for 2min
- . Take the slide to 80% alcohol for 2min
- . Take the slide to 70% alcohol for 2min
- . wash with D.W for 1min
- . Take the slide to hematoxylin for 5min.....(staining the nucleus)
- . Wash with tap water for 2min
- . stain with 1% eosin for 1/2mi.....(staining the cytoplasm)
- zz. Wash with D. W



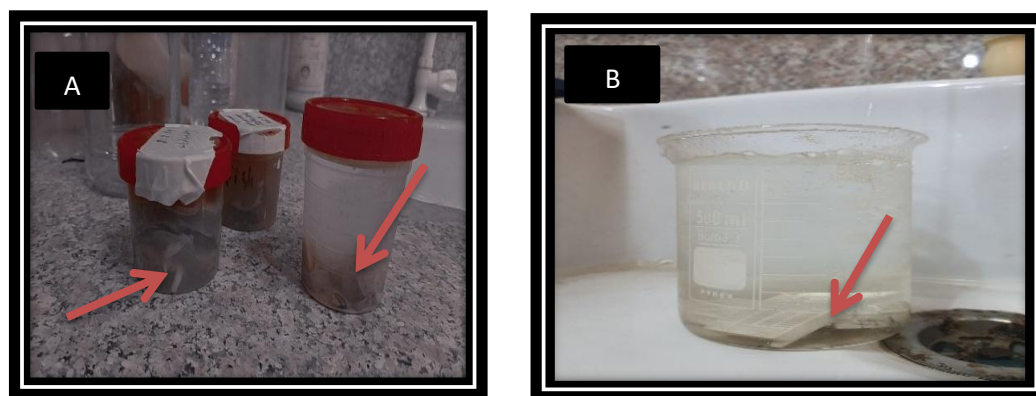


Mounting : We put a drop of Canada balm on a glass slide Then we put a **cover slide slowly on the slide.**

III. RESULTS AND DISCUSSION



As in figure A the fixation process shows the colour of the tissue and stiffness are changed homogeneously, this done by fixed materials, Fixation must be done as quickly as feasible after the tissues have been removed in order to prevent autolysis, putrefaction, as well as osmotic shock, deformation, and shrinkage of the tissue. Unfortunately, Fixatives could inadvertently generate artifacts that make it difficult to evaluate the cellular ultrastructure (Thavarajah R *et al*, 2012)



In the figure B the washing process shows Change the colour of the water to be clearance, As the tissues were being washed, the water's color changed to a clear one. Before moving on to the following stage, this is utilized to remove any extra fixative that was employed. This is due to the possibility that fixative will prevent correct staining or leave behind precipitates (artifacts) (Mathieson *et al*, 2016)

To guarantee that all water has been eliminated, it is crucial to incorporate two stages using 100% absolute alcohol. Since water is inimical to the majority of embedding media (such as paraffin wax), the dehydration process is crucial. It is necessary to switch the tissue between polar (like water) and non-polar (like chemical reagents like xylene) agents. It is impossible to "clear" tissue that is just partially dehydrated. The tissue stays opaque and appears milky when it is subjected to a subsequent clearing chemical (like xylene). The tissue will need to be re-dehydrated as a result. (Klatt, 2018), tissue stays opaque and appears milky when it is subjected to a subsequent clearing chemical (like xylene). The tissue will need to be re-dehydrated as a result (Klatt, 2018),

Clearing The tissue become transparent. The dehydrating agent must be taken out of the tissue at this point and swapped out for a wax-based solvent. When the dehydrating agent (for example, ethanol) and the impregnating medium/embedding agent (for example, paraffin wax) are not miscible, a clearing agent should be applied. It is a wax solvent that has to mix with the embedding and dehydrating agents .(Willey, 1979)

Figure Show the result of clearing process



In infiltration process the result shows in the cutting process ,because when the paraffin material inter to the pores of the tissue this not be visible to the nuke aye. The purpose of the infiltration agent is to remove the clearing agent from the



tissue and then use paraffin wax to fully penetrate it. This will allow the tissue to harden and create a wax block that can be sliced into microscopic histological slices.

Show the result of infiltration process

After the infiltration procedure is finished, a solid block containing the tissue must be obtained. The most used embedding technique is paraffin, which works well for decalcified hard tissues and thin sections of 3-6 m soft tissues .(Mescher, 2016).

Figure Show the result of embedding process



In addition to being compatible with partial histochemical labelling and offering single-cell resolution, the embedding technique produced tissues that were sufficiently stable and hard for continuous sectioning in a matter of weeks at room temperature. Furthermore, paraffin samples' chemistry and physics were incredibly stable, allowing for months of room temperature storage.(Zhao *et al*, 2020).

For successful microscopic examination, Floating: It is vital to trim tiny portions of tissue since the ribbon is wrinkle-free. The water bath, also known as the flotation chamber, is used to float the tissue after it has been sliced. It is important to keep any air bubbles from forming inside the water bath. Lynch, 1969. After cutting, the tissue is floatable using a water bath (floatation chamber), In this process, the wax strip is unfolded to avoid the kinking that occurs in the wax strip, as well as to show the histological structures more clearly after staining (Bancroft & Gamble, 2002.).

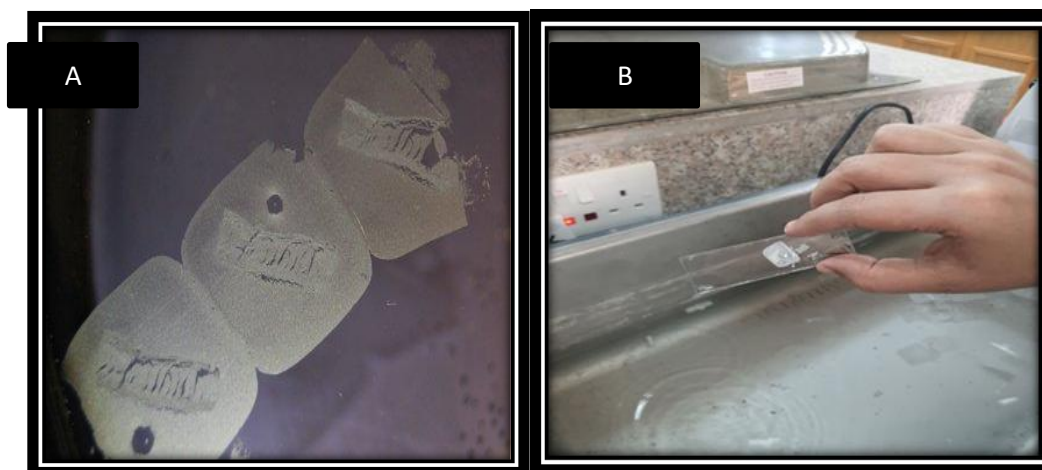


Figure A show the result of floating process and figure B show the result of mounting process

Hematoxylin takes on the hue of purplish blue, much like a basic dye. Acidic or basophilic structures are stained by it, such as the cell nucleus (which includes DNA and nucleoprotein) and RNA-containing organelles such as the rough endoplasmic reticulum and ribosomes. Reddish or pink in colour, eosin is an acidic dye. Basic or acidophilic structures, such as the cytoplasm, cell walls, and extracellular fibres, are stained by it. (Sanderson, 1994)



Show the result of staining and cover slid process



IV. Conclusion

1-The fixation, processing, embedding and staining process make the tissue more suitable to be observed microscopically.

2-Fixation keeps the cell in their original form while halted the effect of autolysis and putrefaction

3-The process of making prepared slides must be in sequential steps and with extreme precision

4-The specific times and times for each operation must be noted to avoid failure in preparation

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