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

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Abstract

The purpose of this research is to learn how to make a microscopic slide of animal or plant tissue and prepare it for examination by means of a compound microscope. There is no doubt that those concerned with this are students of medical sectors at all stages and those concerned with matters of histology, embryology, anatomy, cell science, animal and plant sciences, and this research was done in the pathological analysis department in The Medical Technical Institute in Basra specifically.

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

I. INTRODUCTION

.Histology slides, often known as microscope glass slides, are items typically made to show or investigate the microscopic structure of cells, tissues, and organs as well as how structure and function are related. These items are frequently biological specimens that are fixed or supported on thin, flat pieces of glass, usually coloured to highlight different structural details, and then examined under a microscop, 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 . medicine, molecular biology, and other areas utilizing microscopes. Instead of simply copying the material from histology textbooks, scholars must be able to create tissue samples and use the knowledge to evaluate cells and tissues under a microscope. However, histology is frequently taught today without laboratories, and a histology atlas is frequently used as a replacement due to the technical know-how needed and/or the difficulty often faced in preparing or obtaining good quality slides as well as the high cost of purchasing ready-made slide collections for teaching and demonstration. Sorenson and Brelje (2005), This historical approach to histology, which is centered on microscopy, enables the observer to have an extremely close look at minute or very small slender structures at a size that makes investigation and analysis easy(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). Therefore, this research project has been created to encourage efficiency or mastery in the production of slides and photomicrographs for the purpose of visualizing the actual microstructure of cells, tissues, and organs. It also aims to rekindle scientific or practical interest in traditional methods of histopathology, also study the first basis for diagnosing the disease, so these microscopic preparations were of great importance in the medical and research aspect until the present time, also Teaching students of the Department of Laboratory Techniques in all faculties and institutes how to prepare tissue slides.

II. MATERIALS AND METHODS

Sampling of tissue:

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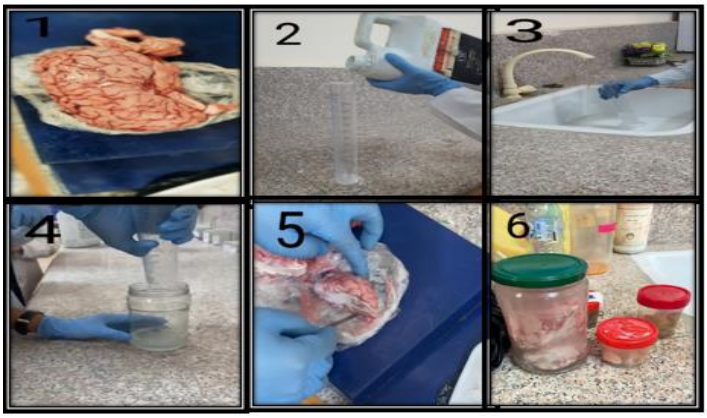
This procedure entails the collection of tissue through postmortem dissection, surgical excision, or biopsy. access to people. Ethics and laws regarding both data and tissue samples make it difficult and sometimes impractical to work with tissue.

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.

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Infelteration we have prepared the tools and materials , and we set the specimen with the wax in we use a closed vessel (that contains a lid) then transfer the tissue from the beaker to the the liquid, first container ,and then transfer it to the second container in order to ensure the improvement of the infiltration 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 block is fixed in the chuck of the microtome. The cutting surface of the Block should be parallel to the knife. The Angle of clearance should be only 2–5° to have Good section ,The tissue in the block Is cut by gentle, smooth and slow stroke. The Ribbon-like tissue sections are produced. The Tip of the ribbon is held by forceps, and the end part of the ribbon is removed from the Knife edge by brush. In case of any difficulty To get the flat section, the cutting surface Should be gently warmed by warm water

8- floating ,mounting and drying:

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With the forceps, the individual sections are separated from each other. As mentioned before, the temperature of the Water bath should be constantly maintained Below the melting point of the paraffin wax. The slide is placed vertically within the water bath in front of the tissue, and when the tissue is touched, the slide is Withdrawn vertically from the water.

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

DryingThe 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

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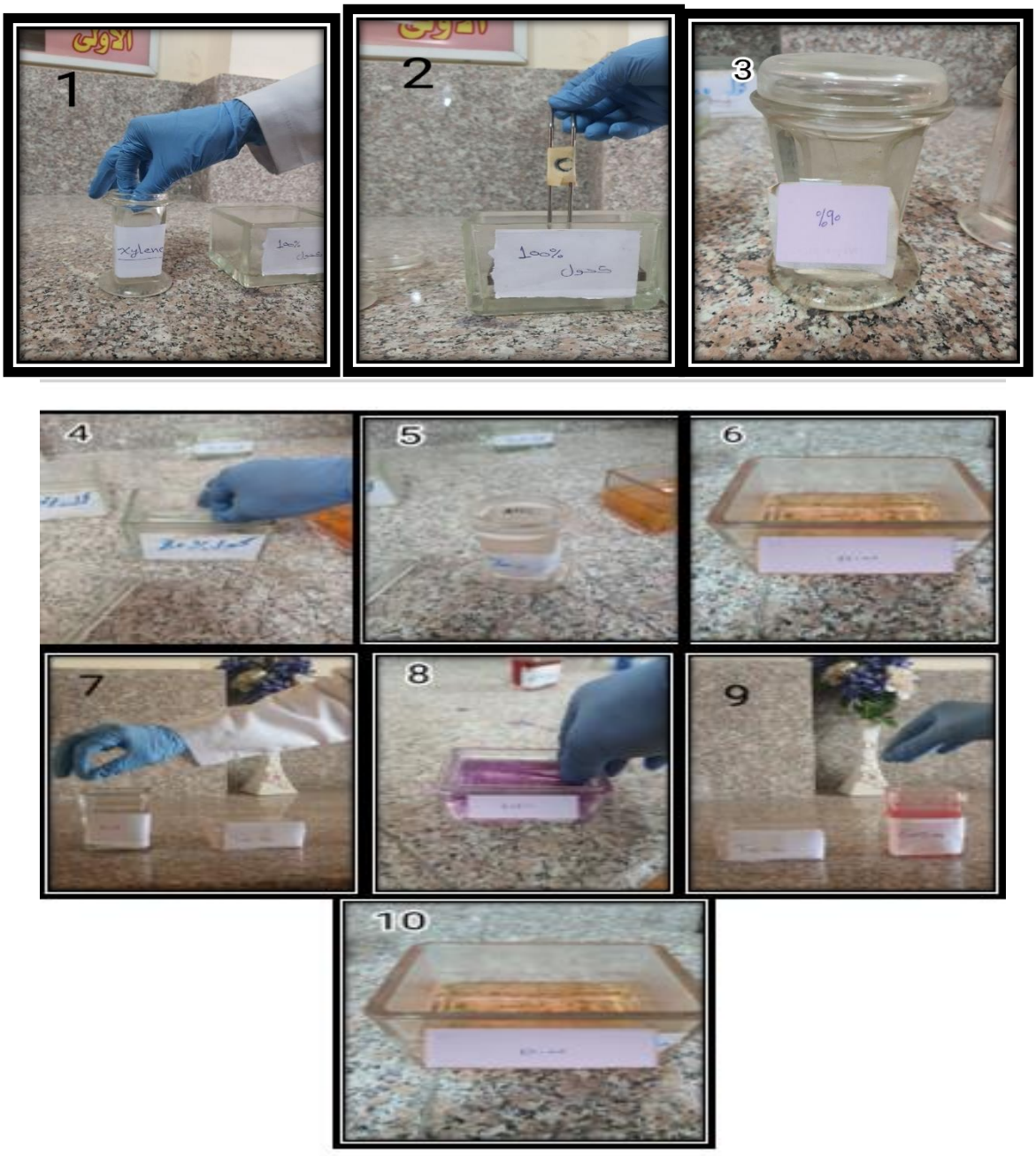
. stain with 1% eosin for 1/2mi.....(staining the cytoplasm)

zz. Wash with D. W



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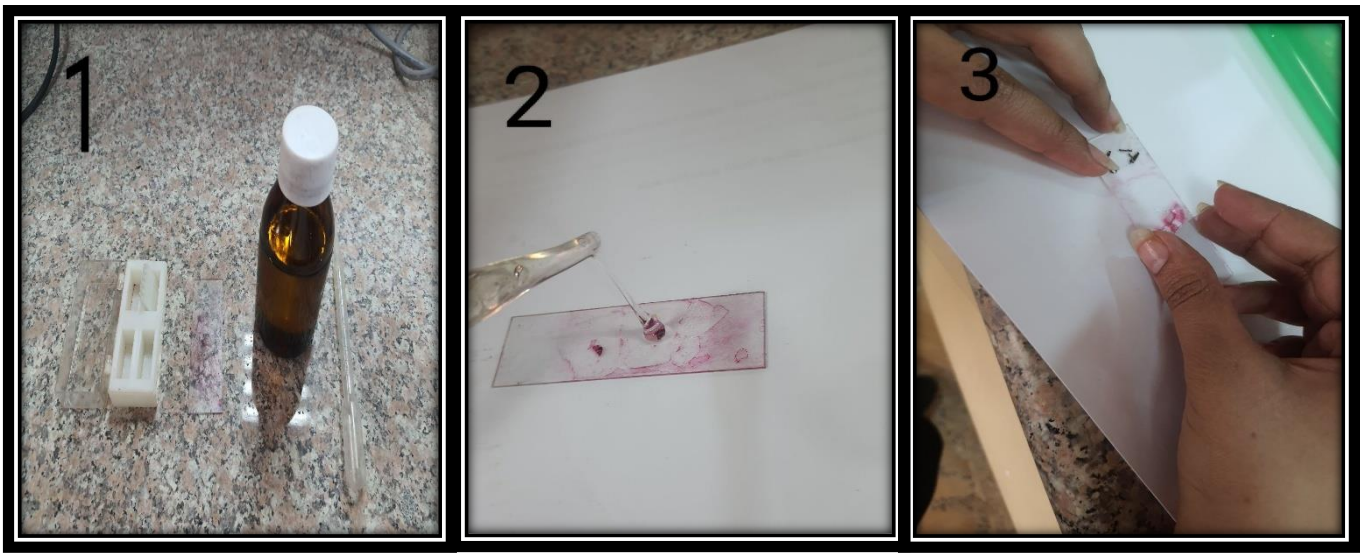
Mounting : We put a drop of Canada balm on a glass slide Then we put **a cover slide slowly on the slide.**



III. RESULTS AND DISCUSSION

As in figure A the fixation process shows the colour of the tissue and stiffness are changed homogenously ,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

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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 nike aye. The infiltration agent's job is to completely infiltrate the tissue with paraffin wax after removing the clearing agent from it. In doing so, the tissue will be able to solidify and form a wax block from which tiny histological slices can be cut.

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).

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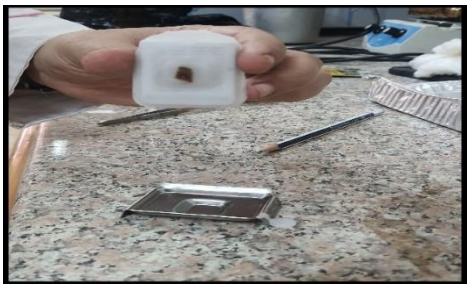
Figure Show the result of embedding process



The embedding method could provide single-cell resolution and be compatible with partial histochemical staining, and the embedded tissues had suitable hardness and stability for continuous sectioning within weeks at room temperature. Additionally, the chemistry and physics of paraffin samples were very stable, and they could be stored at room temperature for months.(Zhao *et al*, 2020).

For successful microscopic examination, Floating: the ribbon is wrinkle free it is necessary to have thin sections of the tissue by cutting.Water Bath (Floatation Chamber) Water bath is used to float the tissue after cutting, It is necessary to prevent formation of any air bubbles within the water bath. (Lynch, 1969). Water Bath (Floatation Chamber) Water bath is used to float the tissue after cutting, 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

Hematoxyline reacts like a basic dye with a purplish blue colour. It stains acidic, or basophilic, structure including the cell nucleus (which contains DNA and nucleoprotein) and organelles that contain RNA such as ribosomes and the rough endoplasmic reticulum. Eosin is an **acidic** dye that is typically reddish or pink. It stains basic, or acidophilic, structures which includes the cytoplasm, cell walls, and extracellular fibres. (Sanderson, 1994)

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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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