SAWA UNIVERSITY

HISTOPATHOLOGY - FIRST COURSE

LAB -4- 5- Tissue processing

Sawa University — جامعـــــة ســـــــاوة الاهلية

College of health and medical techniques — كليـــــة التقنيـــات الصحيـة والطبيـة

Department of Medical Laboratories — قسم تقنيات المختبرات الطبية

Third Stage — المرحلـــــة الثالثـة

محاضرة رقم 5&6 — الجانب العملي

Lecture No. 1 — Practical

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THE AIM OF THE LECTURE

The aim of tissue processing is to infiltrate a tissue sample with a supportive medium, like paraffin wax, which provides enough rigidity to enable very thin sections to be cut for microscopic examination. This is achieved by removing water and replacing it with a solid support, allowing the tissue to be successfully sectioned with a microtome and then stained for viewing under a microscope.

INTRODUCTION:

Tissue Processing:

It is done in 4 stages. ***Dehydration, Clearing, Impregnating and Embedding***. It is important that all specimens are properly labeled before processing by graphite pencil written, type-written or India ink.

Tissue processing is a long procedure and required 24 hours. it can be done manually or mechanically.

Manual Tissue Processing:

In this process the tissue is changed from one container of reagent to another by hand.

Mechanical Tissue Processing: Automatic tissue processors:

1. there are different jars containing reagents. These are arranged in a sequence.

2. The tissue is moved from one jar to another by a mechanical device.

3. Timings are controlled by a timer which can be adjusted in respect to hours and minutes.

4. Temperature is maintained around 60oC.

5. The processing, whether manually or mechanically, involves the same steps.


SLIDE STAINING MACHINES

Tissue processing steps

1-Fixation.

2-Sectioning

3- Dehydration.

4- Clearing (or de-alcohlisation).

5- Impregnation in wax (Wax infiltration).

6-Embedding in paraffin block.

7- sectioning and slide preparation

8- staining

9- mounting

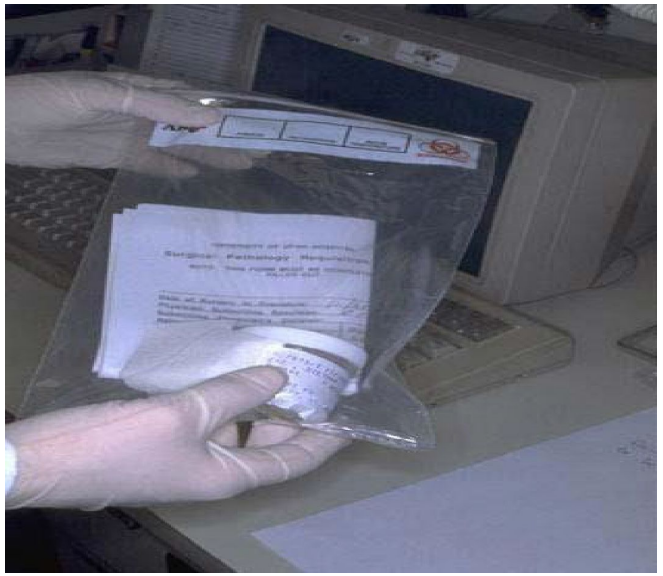
Fixation

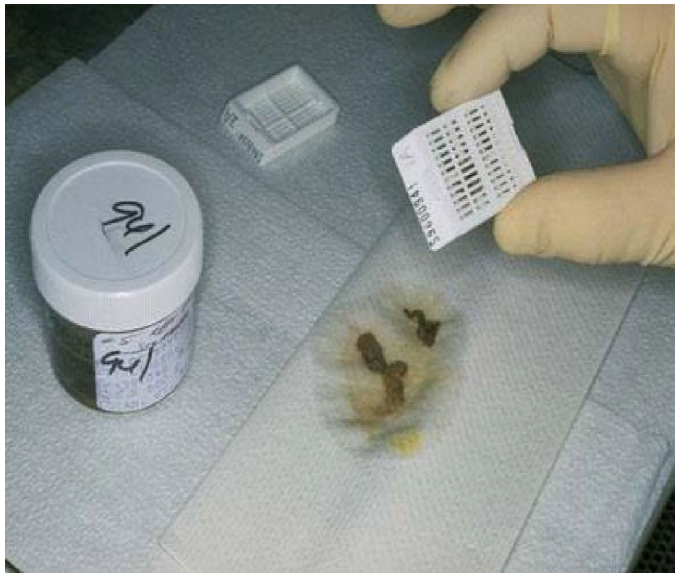
- Aiming to preserve a sample of biological materials (tissues or cells) as close to its natural state as possible in the process of preparing tissue for examination.

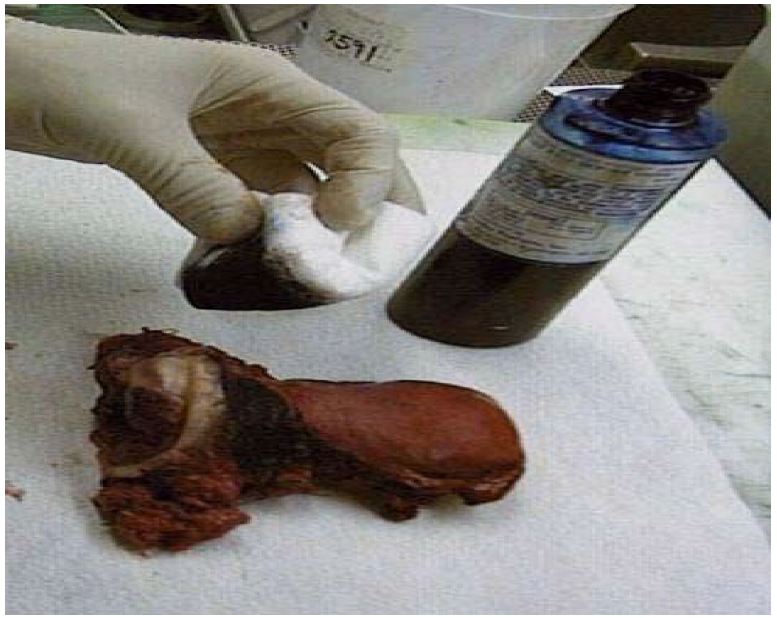
Factors affecting fixation: PH , temperature, volume ,Time interval

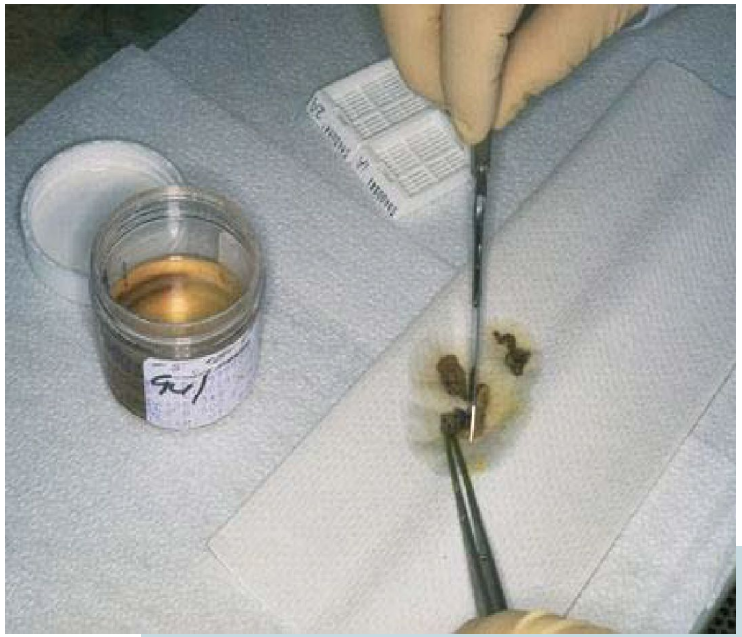
Sectioning

Each organ or tissue has a special procedure in sectioning.









Dehydration

- This process must be carried out so that the embedding medium can infiltrate properly.
- Alcohol in various dilutions is usually used.
- water from the tissues should be removed because water is not miscible with wax
- prevent tissue shrinking.

• Clearing

- As paraffin wax is insoluble in alcohol, it must be replaced by a fluid that is miscible with or is a solvent of paraffin wax, to get rid of alcohol & make the tissue more translucent.
- **Xylol:** cheap and rapid in action, though tends to harden tissue on prolonged application

Infiltrating and Embedding of the tissues

Infiltration: process by the tissue with supporting medium to facilitate its cutting by microtome knife This is allowed to occur at melting point temperature of paraffin wax, which is 54-60oC.Volume of wax should be about 25-30 times the volume of tissues.

Total duration of (2- 4)hours is sufficient for routine impregnation. The duration of infiltration 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. Xylene is easiest way to remove.

- Embedding (Blocking).

 Embedding media are all materials used by histological technique to infiltrate such **as paraffin** , support and enclose specimens which are to be cut into thin sections and orient the tissue for proper embedding.

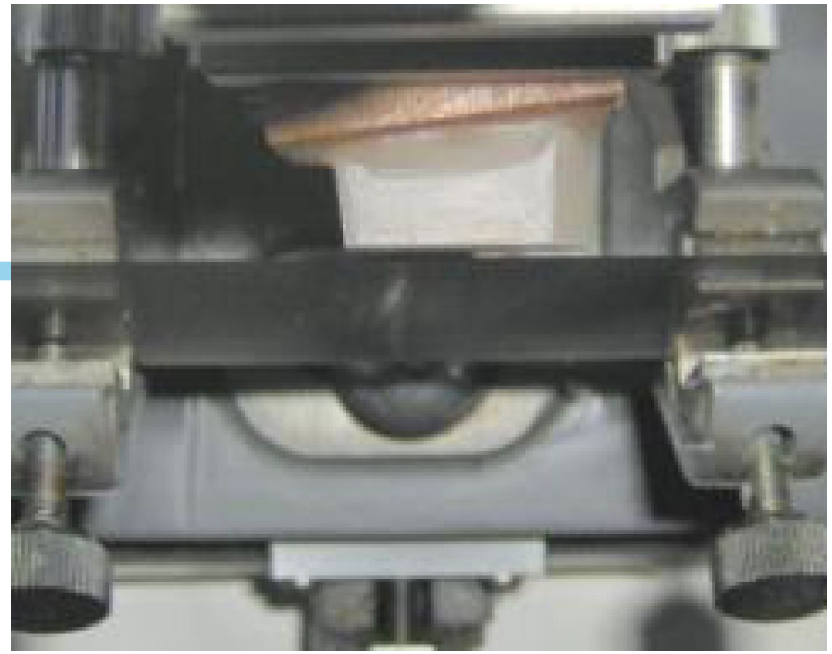

TISSUE EMBEDDING CENTER

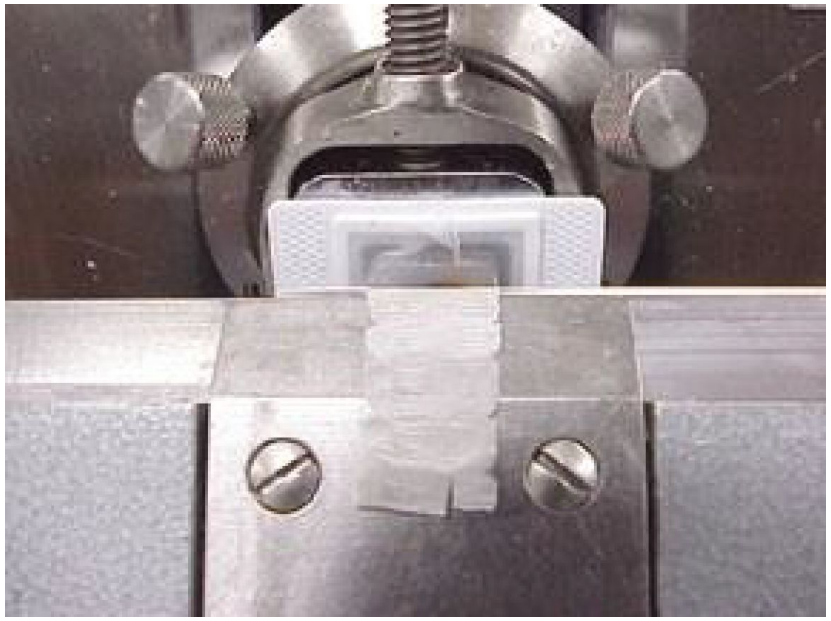

ARAPLAST

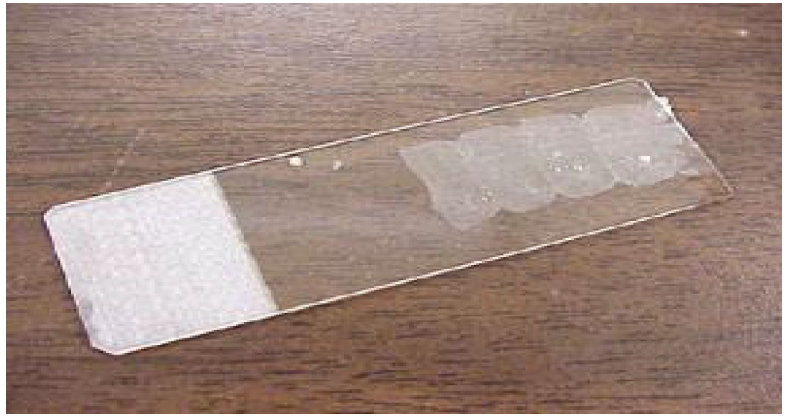
Sectioning

- Embedded tissues, to be cut into thin sections of 3- 5 µ with a microtome.
- Cut sections are, floated on a warm water bath
- Helps to spread the specimen and remove wrinkles.
- Floated sections are picked up on an adhesive coated glass slide.
- Glass slide kept on a slide warmer at 580 temp for 20 min to ensure adhesion
- Egg albumin with additives

- commonly used adhesive







Staining

- Biochemical technique of adding a class-specific dye to a substrate (DNA, proteins, lipids, carbohydrates) to qualify or quantify the presence of a specific compound
- Commonly used Staining procedures

- Gram staining
- Haematoxylin and eosin (H & E) staining
- Papanicolaou staining (PAP)
- PAS staining
- Masson's trichrome staining

H & E stain/ Haematoxylin & Eosin stain

- Most popular staining method in histology.
- Most widely used stain in medical diagnosis.
- The staining method involves application of the basic dye haematoxylin, which colors basophilic structures with blue-purple hue, and alcohol-based acidic eosin Y, which colors eosinophilic structures bright pink.

Mounting

- The stained section on the slide must be covered with a thin glass coverslip to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the tissue section for years to come
- Mounting medium is used to adhere
- the coverslip to the slide DPX & Canada balsam commonly used.

Summary of Paraffin Wax Embedding:

1. Fixation 24-48 hrs
2. Dehydration
   - 50% alcohol 1 hour
   - 70% alcohol I 1 hour
   - 90% alcohol II 1 hours
3. Washing
4. Clearing :Xylene I 15 minute --Xylene II 15 minute
5. Wax Impregnation :Paraffin wax 1 1 hour

Paraffin wax II 1 hour---Paraffin wax III 1 hour

6. Embedding

Questions

1-what is tissue processing in histopathology?

2-What is the process of processing a sample in histopathology?

3-What are common problems in tissue processing?

4-How many steps are there in tissue processing?

Any questons?