WORK SHOP

Technique of histological study of animal tissue using light microscopy

تقنية دراسة الأنسجة الحيوانية باستخدام المجهر الضوئي

الاثنين و الثلاثاء 1/1439 /26-27 من 9 الى 2

فريق العمل

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INTRODUCTION

- Histology is the scientific study of the fine detail of biological cells and tissues using microscopes to look at specimens of tissues that have been carefully prepared using special processes, the technique for routine production of histological slides is the micro technique , this procedure involve many step.
  - The step are :-

Step 1: Tissue fixation

Step 2: Processing tissue

Step 3: Embedding

Step 4: Sectioning

Step 5: Staining

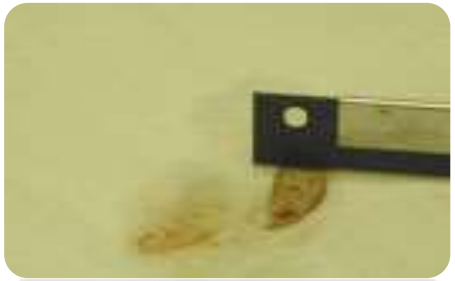
Step 6: Examination under a light microscope.

Tissue fixation

Step 1 : Tissue Fixation

- **The concept of fixation o biological tissue in order to understand biological function and structure has led to the development of many type of fixatives over the last century.**
- **The major objective of fixation has been to maintain clear and consistent morphological features.**
- **Your biological tissue samples should be transferred into fixative immediately after collection.**
- **Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles.**

Fixation cont.



- Biological tissue samples should be transferred into fixative immediately after collection.
- Slide preparation begins with fixation of your tissue specimen. For best results, your biological tissue samples should be transferred into fixative immediately after collection. Although there are many types of fixative, most specimens are fixed in 10% neutral buffered formalin. This will allow most tissues to become adequately fixed within 24-48 hours.
- Formalin containers should be capped and leak-proof, and labelled correctly.

- **Routine fixation** often uses a solution of formaldelhyde (formalin) to react with proteins and other organic molecules to stabilize cell structures. This solution is buffered and osmotically balanced to minimize shrinkage, swelling, and other collateral damage.

Processing tissue

Specimen transfer to cassettes

- After fixation, specimens are trimmed using a scalpel to enable them to fit into an appropriately labelled tissue cassette. Specimens should not be so big that they fill the cassette – they are trimmed so as not to touch the edges. Additionally, they must not be too thick (ideally they should be less than 4mm), otherwise they risk being "waffled" when the cassette lid is closed.

Tissue cassette

Processing tissue:

- This processor uses vacuum function to accelerate the speed to tissue processing for animal and human tissue automatically.
- The aim of this step is to remove water from tissue and replace with medium that solidifies to allow thin sections to be cut.

- **Three major steps:**

- **Dehydration:** is the first step, whish involves immersing the specimen in increasing concentrations of alcohol to remove the water and formalin from the tissue.
- **Clearing:** is the second step, which an organic solvent is used to remove the alcohol and allow infiltration with embedding agent.
- **Embedding:** is the final step, where specimens are infiltrated with embedding agent. Paraffin creates stability in the tissue so that it may be sectioned during microtomy
- Paraffin assists in preparing tissue for embedding.

- **The techniques of this machine:**

Standard tissue basket with capacity of up to 80 cassettes

- Specimen transfer to cassettes.
- 60% aqueous ethanol for four 30 min.
- 70% aqueous ethanol for four 30 min.
- 80% aqueous ethanol for four 30 min.
- 95% aqueous ethanol for 30 min.
- 95% aqueous ethanol a mixture with Zyline (1:1) for 30 min.
- Zyline 1 (1:1) for 30 min.
- Zyline 2 (1:1) for 30 min.
- In Oven (56- 60)˚
- zyline with wax (1:1) for 30 min
- Wax 1 for 30min.
- Wax 2 for 30min

Process	Solution	Time
Dehydration	70% alcohol	60 mins
Dehydration	90% alcohol	45 mins
Dehydration	Absolute alcohol	45 mins
Dehydration	Absolute alcohol	45 mins
Dehydration	Absolute alcohol	60 mins
Clearing	Xylene	60 mins
Clearing	Xylene	60 mins
Clearing	Xylene	60 mins
Infiltration	Paraffin Wax	30 mins
Infiltration	Paraffin Wax	60 mins
Infiltration	Paraffin Wax	90 mins
Blocking Out	Paraffin Wax	n/a

Embedding

Paraffin wax :

Paraffin continues to be the most popular infiltration and embedding in the histology laboratory.

The tissue is impregnated with wax which forms a matrix preventing tissue structure distortion during microtomy .

Embedding tissue in paraffin :

1) Open the tissue cassette, check against worksheet entry to ensure the correct number of
tissue pieces are present.

2) Select the mold, there should be sufficient room for the tissue with allowance for at least a 2 mm surrounding margin of wax.

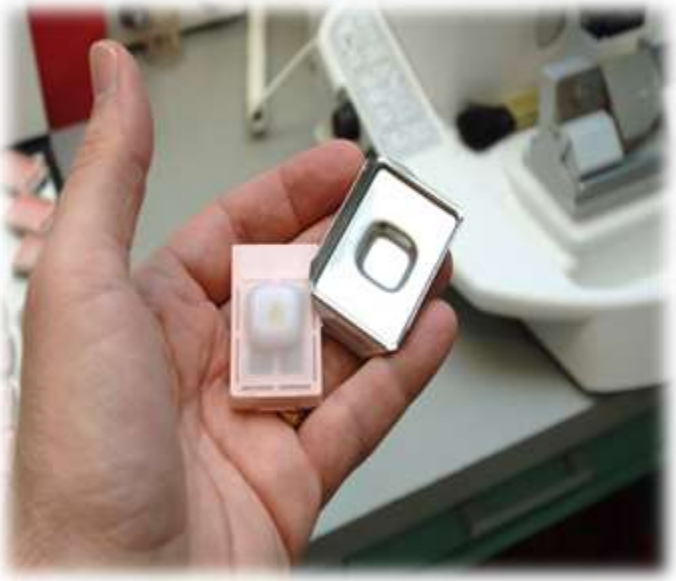
3) Fill the mold with paraffin wax.

4) Using warm forceps select the tissue, taking care that it does not cool in the air; at the same time.

5) Place the tissue in the mold according to the side to be sectioned.This side should be facing down against the mold. A small amount of pressure may be used in order to have more even embedding.

6) Chill the mold on the cold plate, orienting the tissue and firming it into the wax with warmed forceps. This ensures that the correct orientation is maintained and the tissue surface to be sectioned is kept flat.

7) Insert the identifying label or place the labeled embedding ring or cassette base onto the mold.

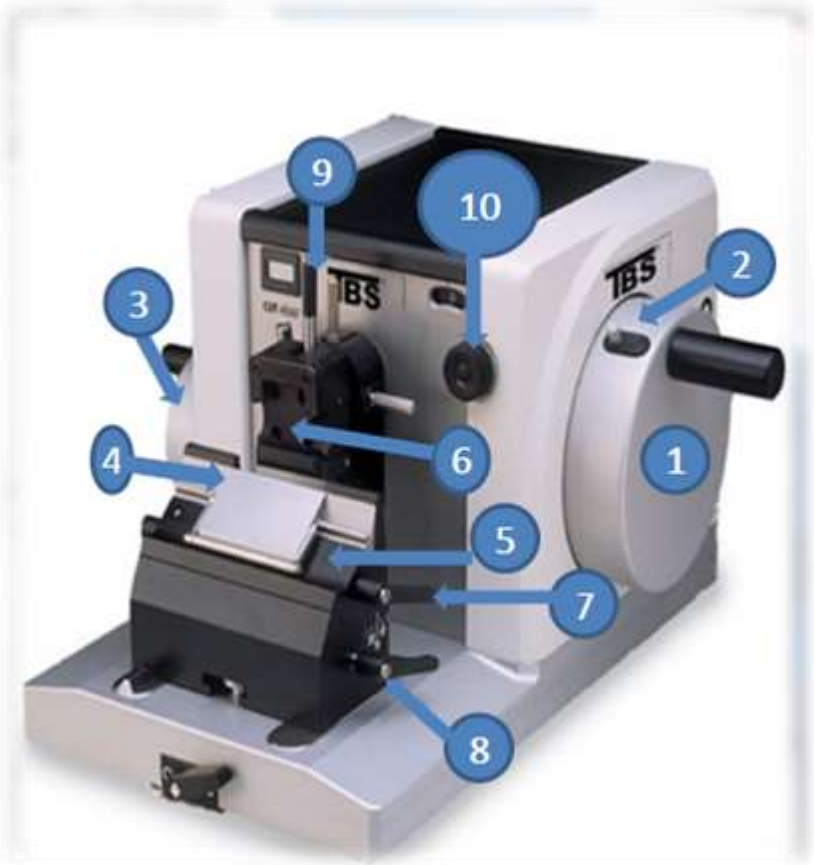


Sectioning with Microtome

Cutting The Specimen

- using the Rotary microtome
- A microtome to sectioning biological specimens a tissue sample into very thin slices (sections)
- thicknesses varying from 2 - 25 micrometers thick.

Amicrotome has the essential machinery of a slicer:



1.crank hand
2.safety lock
3. crank knife
4.cutting edge knife (blade)
5.the knife holder
6.a specimen holder
7. Knife regulating rod for inclining angle
8.Knife fixing knob
9.Paraffin block fixing screw
10. Sectioning thickness regulating knob

1- Sectioning:

- Microtome > 5-10 microns
- Ribbon of sections
- Put The sections (very quickly) in Absolute alcohol (until the sections spread).
- Taken on hot water bath at 42 ˚C to flatten out

2- Picking up sections

- **Floating sections onto slides Carefully arrange the sections in the center of the slide (that helps remove wrinkles)**

3-Microscope slide preparation:

- Taking the section onto slide
- Flat, no air bubbles, no stretch or breaks.
- Taken on hot plate at 48 ˚C to dry .

Staining

HAEMATOXYLIN & EOSIN

- Standard and widely used staining procedure. Haematoxylin is used for nuclear staining. Eosin is used for cytoplasmic staining.

METHOD OUTLINE

- 1. Haematoxylin is firstly used to stain the nucleus with dark blue, violate or black.
- 2. Secondly, Eosine is used to stain the cytoplasm (collagen, keratin) red.

Jar Number	Chemical solution	Time by (Sec)
1	Xylene	60
2	Xylene	60
3	Xylene	60
4	Absolute Alcohol	60
5	Absolute Alcohol	60
6	96% Alcohol	60
7	Dis. Water	90
8	Haematoxylin	10 min
9	Tab water	90
10	96% Alcohol	30
11	Eosin	2 min
12	Absolute Alcohol	60
13	Absolute Alcohol	60
14	Xylene	60
15	Xylene	60
16	Xylene	60

Examination under a light microscope

Examine the slides

- **Finally :**

  Examine the slides by microscope.

Thank you …