|  |  |  |  |
| --- | --- | --- | --- |
| **Salahaddin University-Erbil** |  |  | **Subject: Microtechniques** |
| **College of Science** |  | **Exam. Duration: 1 hour** |
| **Department of Environment**  |  | **Date: 28/ 4/ 2024** |
| **3rd Stage** |  | **Midterm exam**  |
|  |

****

***Q1/* Define the following (20 Marks)**

 1-Fixative 2- Vital stain 3-Dry mounts 4-impregnation 5- Water bath

***Q2/* Answer the following (30 Marks)**

1- Sliding Microtome

 2- Prepare Carnoy’s fixatives.

1. Mention the Properties of the fixative
2. What is the different between mountants and mordants.
3. Metachromatic staining
4. Write about the Maceration Method.

***Q3/* Write True or False, with correct false one (40 Marks)**

1. Farrant's medium is very useful medium for mounting sections for fluorescent microscopy.

2- In Sliding microtome the knife is moved horizontally against a fixed block which

 progresses against it in an inclined plane.

3- necropsy is the examination of the body of a dead person.

 4-It is rapidly clear the tissue from 95% alcohol, hardens tissue the least of all the transition

 solvent, but it is difficult to eliminate from tissue during wax infiltration; as benzen.

 5- DPX it dissolved in xylene as a 20% solution. It is most commonly used

 6- Hydration is essential to remove the wax completely, otherwise subsequent

 stages will not be possible.

 7- The fixation consists of two steps preservation and killing

 8- Methylin and Toluidine blue are commonly used for quick staining of frozen selection

 using their to stain nucleus and cytoplasm differently.

 9- After mounting the cover slip can be ringed by clear nail polish for storage, this process

 called clearing.

 10- Sliding microtome could be slice thicknesses of sample about >1 µm.

1-X Apathy's medium 2-√ 3-√ 4-X cedar wood oil 5-√ 6-X Deparaffinisation

7-√ 8- X Thionin and Toluidine blue 9-X Permanent seal 10-X laser Microtome

**Fixative**: it is a chemical substance which will preserve the shape, structure, relationship and chemical constituents of tissue and cells after death.

**Vital staining:** staining of structures in living cells, either in the body or in a laboratory preparation ex. 1-Trypan blue. 2-Janus green is taken up by living cells and stains the mitochondria.

Whole mounts (dry mounts): is the most basic technique, is used for small size specimens that do not need cutting or sectioning e.g. observing hair, pollen, dust, spore and dead matter as insects, part of flower, filamentous algae.

**Infiltration:**

It is the complete removal of clearing reagents by substitution of paraffin or any such similar media (paraplast, paraplast plus, gelatin, agar embedding and celloidin). Impregnation with paraffin wax takes place in an oven heated to 56-62ᴼC depended up on the melting point of the wax in use.

**Water bath (for stretching the section)**

The paraffin section floated on a water bath (temperature of water bath less than melting point of paraffin wax, around 5 degree).

**Carnoy’s fixatives** -

Absolute ethanol - 60 ml

Chloroform - 30 ml

Glacial acetic acid - 10 ml

**Mountants:**

Histological sections which need to be examined for any length of time or to be stored, must be mounted under a cover-slip. There are two types of mounting media:

1. Aqueous media: used for material which is unstained, stained for fat, or mechanically stained.

2. Resinous media: For routine staining.

**Mordants:** substances that cause certain staining reactions to take place by forming a link between the tissue and the stain. The link is referred as lake. Without it the dye is not capable of binding to and staining the tissue ex. Ammonium and potassium aluminum sulfate.

**Properties of the fixative**

1. Coagulation and precipitation of proteins in tissues.

2. Penetration rate differs with different fixatives depending on the molecular weight of the fixative, best fixative which penetrate fast.

3. pH of fixatives – Satisfactory fixation occurs between pH 6 and 8. Outside this range, alteration in structure of cell may take place.

4. Solubility of fixative- all should be soluble in a suitable solvent, prefer in water.

5. The fixative should be cheap, non-toxic and non-inflammable. The tissues may be kept in the fixative for a long time.

7. Concentration- it is important that the concentration of fixative is isotonic.

**Sliding Microtome-** the knife is moved horizontally against a fixed block which progresses against it in an inclined plane. The sliding microtome can be used for paraffin-wax embedded sections although it was designed for cutting celloidin embedded sections.

**The clearing agents**

**1-Amyl acetate, methyl benzoate and methyl salicylate**

These are used as nitrocellulose solvent in double embedding technique, low toxicity, but they have strong odours necessitate good laboratory ventilation.

**2-Benzene**

It is gentle and rapid then xylene and toluene, it is probably the best transition solvent, but toxicity and carcinogenicity has preclude exclude, it’s used in histology.

**3-Butyl acetate**

This is used as xylene substitute and nitrocellulose solvent.

**4-Carbon tetrachloride**

Because of its high toxicity, now rarely used in histology and similar to chloroform properties.

**5-Cedar wood oil**

It is rapidly clear the tissue from 95% alcohol, hardens tissue the least of all the transition solvent, but it is difficult to eliminate from tissue during wax infiltration.

**6-Chloroform**

This is expensive, volatile, but slowly penetrate. It causes less brittleness than xylene and often used on dense tissues.

**7-Terpens**

Are isoprene polymers derived from plants, have low penetration rate and there are difficult to eliminate from paraffin wax.

**8-Trichloroethanol**

It is commonly used as xylene substitute.

**9-Xylene and toluene**

These are clear rapidly and tissues are rendered transparent.

Maceration Method: When studying individual or isolated cells, the transverse or longitudinal sections of the samples are not enough to clarify those cells, so used this method. Various solutions are used to separate a tissue into its individual cells. These solutions dissolve or weaken the middle lamella so that the cells are easily separate apart.

Metachromatic staining: there are certain basic dyes that will differentiate particular tissue components by giving them a different color to that of original dye, ex. Toluidine blue, methylene blue methyl violent, thionin crystal violent.