بسم الله الرحمن الرحيم

كليـــــة نبتـــــه
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Medical Laboratory Sciences Program

Department of Microbiology

Student Practical log book

Semester 4

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Instructions

- **Wear gloves when in contact with body fluids, such as serum, plasma, urine or whole blood**
- **Wash your hands when gloves are removed or changed**
- **Perform procedures carefully to minimize aerosol formation**
- **Wear protective clothing such as laboratory coats or aprons when working with specimens**
- **Keep your hands away from your face**
- **Cover all superficial cuts before starting any work**
- **Dispose of specimens and other contaminated materials according to you laboratory's biohazard control procedure**
- **Keep your work area disinfected, disinfect tools and other items that have been in any contaminated area.**
- **Do not eat or drink or apply cosmetics while in the laboratory**

Practical No (1)

Microtome and sectioning

Microtome:-

A microtome is a mechanical instrument used to cut biological specimens into very thin segments for microscopic examination. Most microtome uses a steel blade and is used to prepare sections of animal or plant tissues for histology.

Types of microtome's:-

- Rocking microtome
- Rotary microtome
- Sliding and base sledge microtome.
- Freezing microtome.
- Ultra microtome.
- Vibration microtome.

General parts of microtome:-

- Knife holder.
- Block holder.
- Microtome wheel.
- Thickness adjustment.
- Reverse mechanism.
- Microtome base.
- Microtome cover.

Sectioning:-

Requirement of sectioning:

- Pencil.
- Ice .
- 50% - 70% alcohol.
- Forceps .
- Brush .
- Old knife.
- Water bath
- Oven .
- Thymol.
- Slide.
- Adhesive media.

    Ex: Egg albumin-gelatin – starch.

Sectioning cutting depends on:-

- Properly prepared tissue.
- A suitable microtome in good condition.
- The sharp knife.
- The skill of microtome.

Steps of sectioning:-

1. **trimming:**
   - Adjustment the thickness in 10 – 20 micron.
   - Use old knife.
   - Use ice.
   - Fix the object in the clamp, tighten securely.
   - Check the paraffin block will be correctly orientated in relation to the knife edge.
   - Removal of surplus wax above the tissue and exposure of the complete surface area of the specimen.
2. **cutting of sections:**
   - Replace the old knife.
   - Adjustment the thickness in 3 – 5 micron.
   - Sharp knife.
   - Use forceps
   - Ribbon section (serial sectioning consists of the preparation of consecutive sections, about ten sections).
   - Use 70% alcohol for spread and floatation.
   - Use pencil.
   - Slide.
   - Water bath for floatation
   - Use thymol as anti fungal
   - Use adhesive media .

3. **Drying of section :**

- Dry at room temperature
- Dry in oven

4. **Method of drying :**

- Drying for 16 – 24 hours in incubator at 37C .
- In oven or on hot plate at 45-50 C for two hours or more
- In oven or on hot pate at temperature high enough to melting the wax (55 – 65C) for 30 – 60 minutes.

Results:-

The section is ready for stain.

Assignment

1-Mention the parts of the microtome and functions:-

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2-Mentionthe equipment’s need of the sectioning and functions:-

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Practical No (2)

Haematoxylin and eosin stain

Introduction:

Haematoxylin :-

1. It is one of most widely used dyes in histology for demonstration of cell nuclei ,myelin , elastic fibers , neuroglia and muscle striations .

2. Extracted from the wood of small tree, haematoxylin campechianum (logwood).

3. Originated in Mexico and cultivated in Jamaica.

4. It has little or no staining capacity and requires oxidation to haematin .

5. Added mordant to haematin to became strong stain Oxidationofhaematoxylin.

1. Natural oxidation:-

By contact with air or sunlight.

E.x

PTAH , Ehrlich’s haematoxylin.

2. **chemical oxidation:-**

Ex:

Sodium iodate in Mayer's haematoxylin .

Mercuric oxide in Harris haematoxylin .

3. **Mordant:-**

Ex :

Aluminum ammonium sulphate(ammonium alum).

Aluminum potassium sulphate(potassium alum).

Ferric chloride or ferric ammonium sulphate (iron alum).

Classification of haematoxylin according to mordant:-

1. **Alum haematoxylin :**

Ex :

Mayer's , Harris , Ehrlich's haematoxylin

2. **Iron haematoxylin:**

Ex :

Weigert's , heidenhain's , iron haematioxylin.

3. **Phosphotungstic acid haematoxylin (PTAH).**
4. **Molebediniumhaematoxylin.**
5. **Lead haematoxylin.**
6. **Haematoxylin without mordant.**

*Haematoxylin stain cell nuclei red color but this color converted to blue by bluing methods.

Eosin:-

Stains cytoplasm – red or pink color (Counterstain) or called back ground stain.

Types of eosin:-

- Eosin yellowish.
- Eosin bluish.
- Eosin greenish.

Notes:-

-Eosin y (yellowish) is most commonly used and is readily soluble in water, less soluble in alcohol.

Assignments

1-Write of the followings:-

A- **Natural oxidized agents:-**

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B- **Chemical oxidized agents:-**

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C- **Classification of the haematoxylin according to mordant:-**

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Practical No (3)

Mayer's haematoxylin

Aim:-

To demonstrate cell nuclei and cytoplasm

Principle of haematoxylin:-

Haematoxylin it's self is not a stain, haematin is the major oxidation product, haematin is an acid dye have a poor affinity for tissue, so mordant substance is added to form mordant dye. Mordantdye reacts with the tissue to form tissue mordant dye complex (acid base reaction).

Solutions:-

1- Mayer's haematoxylin :

- Haematoxylin ……1g.
- Distilled Water ……..1000ml.
- Sodiumiodate ……....0.2g.
- Potassium alum ……..…50g.
- Citric acid …….....1g.
- Chloral hydrate ……..…50g.

2- Eosin y:-

- Eosin y……..1 g.
- D.water …….……..100ml.
- Glacial acetic acid.……..…..0.05ml.
- Small amount of crystal thymol or few drops of formalin.

Method:-

- Take section to water (removing artifact pigment).
- Stain with Mayer's haematoxylin for 10-20min.
- Blue in running tap. water for 8- 10 minutes.
- Counter stain in Eosin Y for 1-3 min.
- Dehydrate ,in 70%-90% absolute alcohol1-2.
- Clearxylene and mount in D.P.X.

Result:-

Nuclei:……

Cytoplasm:…….

Others:……

Comment:

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Assignment

1-Write about the followings:-

A-Principle of haematoxylin stain:-

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B -Preparation of the Mayer's haematoxylin stain:-

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C-Functions of these regents:-

1-Sodium iodate …….....

2-Potassium alum……

3-Citric acid…….

4-Chloral hydrate…….

5-Crystal thymol……..

6-Glacial acetic acid……

7-Eosin y……

D-Name of this stain……..

Practical No (4)

Harris's haematoxylin and Eosin

Aim:-

To demonstrate cell nuclei and cytoplasm.

Principle of hematoxlin:-

Haematoxylin itself is not a stain, haematin is the major oxidation product,haematin is acid dyes have a poor affinity for tissue, so mordant substance is added to form mordantdye. Mordant dye reacts with the tissue to form tissue mordant dye complex (acid base reaction).

Solutions :-

1. **Harris'shematoxylin.**

- Haematoxylin …….1g.
- Absolute ethanol……..…10ml.
- Distilled water ……..….200ml.
- Mercuric oxide ……0.5g.
- Ammonium or potassium Alum ….....20g.
- Glacial acetic acid…… 8 ml.

2. Eosin y:-

- Eosin y……..…1g.
- Distilled water ……100ml.
- Glacial acetic acid……0.5ml.
- Small amount of crystal thymol or a few drops of formalin

Method:-

- Take section to water.
- Stain in Harris' haematoxylin for 5-15 min.
- Wash in running tap water 2-3 min.
- Differentiate in 1% aqueous hydrochloric acid for few seconds.
- Blue in running tap water 5 min (stop acid reaction: microscope).
- Stain in 1% aqueous eosin 1-3 min.
- Wash in water (to remove excess stain)
- Dehydrate in alcohol and clear in xylene and D.P.X.

<u>Results:-</u>

Nuclei:-……..

Cytoplasm:-……

Others:-……..

Comment:-

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Assignment

1-Write about the followings:-

A - Preparation of Harris's haematoxylin stain:-

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B - Preparation of Eosin stain:-

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C- Preparation of 1%acid alcohol (name of the steps):-

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C - Functions of these regents:-

1-Mercuric oxide ……..

2-Ammonium or potassium Alum…….

D-Name of this stain……..

Practical No (5)

Ehrlich's haematoxylin and Eosin:

Aim:-

To demonstrate cell nuclei and cytoplasm.

Principle of hematoxylin:-

Haematoxylin itself is not a stain,haematin is the major oxidation product,haematin is acid dye have a poor affinity for tissue, so mordant substance is added to form mordantdye. Mordant dye reacts with the tissue to form tissue mordant dye complex (acid base reaction).

Solution:-

1- Ehrlich's haematoxylin:-

- Haematoxylin……2 g.
- Abs alcohol ……100ml.
- Glycerol ……...100ml.
- Glacial acetic acid….10ml.
- Potassium alum sulphate ……10--14g.

2- 1% acid alcohol (differentiation fluid):

- abs of alcohol……70ml(70% alcohol).
- ofConc. HCl …… 1ml.

(Remove 1ml to add 1ml HCL)

3-Eosin y:-

- Eosin y ……..1g.
- D.W ……..100ml.
- Glacial acetic acid……..…0.05ml.
- Small amount of crystal thymol or a few drops of formalin.

Method:-

- Take section to water.
- Stain with Ehrlich haematoxylin for 20 – 45 minute.
- Differentiate in 1% acid alcohol for few seconds.
- Bluing and running tap water 8 – 10 minute .
- Counter stain with eosin for 1 – 3 minute's.
- Dehydrate in 70% - 90%- abs 1,2 (just rinse).
- Clear in xylene mount in D.P.X.

Results:-

Nucleaus:-……

Cytoplasm:-……

Comment:-…….

Assignment

1-Write about the followings:-

A - Preparation of the Ehrlich's haematoxylin stain:-

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B - Types of bluing:-

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C - Functions of these regents:-

1-Glyerol ……...

2- Potassium alum sulphate ……..

3- Bluing and running tap water used in method…….....

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D-Name of this stain……..

Practical No (6)

Weigert'sHaematoxylin and Eosin

Aim:-

To demonstrate cell nuclei and cytoplasm.

Principle of haematoxylin:-

Haematoxylinit self is not a stain, haematin is the major oxidation product, haematin is an acid dye have a poor affinity for tissue, so mordant substance is added to form mordant dye. Mordant dye reacts with the tissue to form tissue mordant dye complex (acid base reaction).

Solutions:-

3- Weigert'shaematoxylin (nuclear stain):

Solutions A :(stain):

- Haematoxylin…….……1g.
- Alcohol…….....100ml.

Solutions B (mordent):

- 30% aqueous ferric chloride ……....4ml.
- Conc.HCl……1ml.
- Distilled water …….….95ml.

Working solution: take equal volume from A&B.

4- 1% acid alcohol (differentiation fluid):

- Absolute alcohol……70ml(70% alcohol).
- Conc.HCl ……..1ml.

  (Remove 1ml to add 1ml HCL)

*Small amount of crystal thymol or few drops of formalin.

Method:-

- Take section to water.
- Stain with iron haematoxylin mixture for 5-15mintues.
- Rinse in water.
- Differentiate in 0.5 – 1 % HCL for few second.
- Blouing in running tap water 8 – 10 min.
- Counter stain with eosin for 1-3 min.
- Dehydrate in 70% -90% - abs alcohol 1, 2 (just rinse).
- Clear in xylene & mount D.P.X.

Results:-

Nuclei:……

Others:……

Comment:……

Assignment

1-Write the following questions:-

A - Preparation of the Weigert'shaematoxylin stains:-

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B- Functions of these regents:-

1-30% aqueous ferric chloride

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D-Name of this stain……

Notes:-

- D.P.X:Mixture of distrene (apolystrene) – aplasteicizter (tricresylphosphate)&xylene.
- BPS: (butyl, phthalate styrene).