وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الجنوبية
المعهد التقني العمارة
قسم تقنيات المختبرات الطبية

الحقيبة التدريسية لمادة

شرائح نسيجية

Techniques Histological

الصف الاول

تدريسي المادة

أ.م .د. نضال عبدالله هاشم

الفصل الدراسي الثاني

جدول مفردات مادة شرائح نسيجية

المفردات	الاسبوع
Mounting , Adhesives	**1**
Staining , classification of stains	**2-3**
Staining section	**4-5**
Methods of staining	**6**
Types of stains , preparation of stain and oxidation of some stains	**7-8**
Stains solvents ,factors affecting staining , storage of stains , how to choose . stain	**9**
Decalcification , bone tissue	**10**
Examination for second term	**11-12**
Tissue slide , Freezing microtome	**13-14**
. Final examination	**15**

Purpose :- prepare slides for histopathology and cytology

- A) In general

Students can prepare permanent slides for different body organs

- B) Specifically student can do

1-.Permanent stained tissue slides and body fluid smears

2-.Fix and preserve tissue specimen

Target group :

Students of second stage /techniques of medical laboratory

Educational techniques used :

1-blackborad and pens

2-interactive whiteboard

3-Data show

4-laptop

First week

Mounting , Adhesives

Educational objective:

The student should be able to :

1- Know important mounting step deal with histological sections .

2- Know important Adhesives that deal with histological sections.

3-Know types of mounting and adhesive agents.

Lecture duration:

Theory (2) hour+ practical (3) hour /weekly.

Activities Used:

1- Interactive classroom activities

2- Brainstorming questions

3- Group activities (if required)

4- Homework

5- Online homework (classroom)

6- Quick written exam .

Mounting& Adhesives

Once sections are cut, they are floated on a warm water bath that helps remove wrinkles. Then they are picked up on a glass microscopic slide, which is covered by a thin film of adhesive that provides good adhering of the sections.

Types of adhesives:-

1. Albumin:

a. Mostly used in histopathological labs.

b. Used for more adhering of sections.

c. Source of albumin is egg, bovine, or human albumin

d. Preservative should be used to prevent putrefaction.

e. Glycerol is added to albumin to increase viscosity & prevent complete drying.

2. **Gelatin:**

a. Gelatin adhesives provide affirmer attachment of sections than albumin.

b. 0.5% in distilled water with preservative is satisfactory before use it should be heated gently to melt the gelatin and then used.

3. Starch:

a. Starch adhesives provide greater adhesion than gelatin.

b. lts disadvantage is of interfering with many dyes.

c. Since it is carbohydrate, its use isn't recommend when detecting for these substances

4. **Cellulose:**

a. 1% methyl cellulose solution found to be a good adhesive.

b. It does not interfere with commonly used dyes.

5. Sodium silicate:

a. Used by diluting the commercial syrup up to 10 (1:100)

b. Good adhesive.

c. Tend to stain with most dyes.

6. Resins:

a. A greater adhesion is made by using epoxy resins(araldite).

b. This adhesive, diluted 1in 10 with acetone, it should be painted onto clean slide immediately before use.

❑ Mountants :

- DPX (Distrene Dibutyl phthalate Xylene).
- Canada Balsam
- Colophonium resin
- Terpene resin

Mounting media:

There are two types of mounting media :

1- Aqueous media – used for material which is unstained , stained for fat or metachromatically stained

2- Resinous media : for routine staining.

Aqueous media :

Generally it is used for temporary mounting media, hours, deys, or weeks .This include unstained preparations ,some florescent techniques and most enzymes techniques.

Resinous media :

This used for permanent preparations .They are used for routing working except when the substance to stain or the dyes are soluble in the dehydrating, clearing or mounting media.

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture.

Q1/ Mention advantage of the following :

1- cellulose

2-gelatin

3-sodium silicate

4-albumin

Q2/Mention the diluting fluid for the following :

1- sodium silicate

2- resins

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

Second-third week

Staining , classification of stains

Educational objective:
The student should be able to :
1- Know important staining step deal with histological sections .
2- Know classification of stains.
3-Know types of stains.

Lecture duration:
Theory (2) hour+ practical (3) hour /weekly.

Activities Used:
1- Interactive classroom activities
2- Brainstorming questions
3- Group activities (if required)
4- Homework
5- Online homework (classroom)
6- Quick written exam .

Staining :

Staining is accomplished by soaking tissue in a solution of one dye or more. Staining is employed to give both contrast to the tissue as well as highlighting particular features of interest.

Staining Terminology

1- Stock Solution:-

This term has two meanings:-

a. The solutions that used in bulk every day. (formalin, alcohol, etc).

b. The solutions that prepared in high concentrations and then diluted on working (on the day of use).

2- Working solution:-

The solutions that were prepared from the stock solution on the day of using.

3- Decolourization:-

It is a partial or complete removal of stain from the stained sections.

4- Bleaching:-

Is a complete removal of stain or any color from tissue.

5- Mordant:-

Is an intermediate agent used to link the regressive dye strongly to the tissue (ex: alums, Fecl3).

6- Counter stain:-

Is one dye or more that added to the main stain to bring out the differences between tissues or cell components.

Ex: eosin → HX.

Van Gieson → Verhoeff's stain

7-Acidophilic stain:-

The dye that stains the acidic components of the cell and it is basic in nature (ex: HX stain).

8- Basophilic stain:-

The dye that stains the basic components of the cell, it is acidic in nature

9- Metachromatic stain:

A stain that gives a colour different from its original colour

Ex: Methylene blue (after staining) red colour

Toluidine blue (after staining) red colour

10-Polychromatic stain:-

It is a compound of dyes that gives different colours for tissue (ex: acid dyes, fuchsine, basic dyes, alcians, and Masson's trichrome).

11-Dewaxing:-

Is the complete removal of paraffin wax from tissue sections by using a clearing agent (xylol, toluene, benzene).

12-Differentiation-:

Is a partial removal of the dye from the tissue by using (ex: 1% acid alc. for HX stain; fecl3 for Verhoeff's stain).

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture.

Q1/ Write differences with the following :

1-Metachromatic ,polychromatic

2- physical staining ,chemical staining .

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

Fourth Week

Methods of staining, Types of staining

Educational objective:
The student should be able to :
1- Know staining methods .
2-Know types of stains.
3-Know Nature of stains

Lecture duration:
Theory (2) hour+ practical (3) hour /weekly.

Activities Used:
1- Interactive classroom activities
2- Brainstorming questions
3- Group activities (if required)
4- Homework
5- Online homework (classroom)
6- Quick written exam .

Staining Reactions, methods & types:

A) Staining reactions:

1-Direct staining (absorption)

Stain diffuses into tissue without causing any change, it is simple diffusion (ex: Eosin).

Stain →Tissue (Eosin stain)

2- Indirect staining:

Stain needs mordant to colour the tissue, by making a linkage between and the dye and the tissue (ex: Haematoxylin , Bests carmine).

Stain →Mordant →Tissue

Indirect Stain (Haematoxylin Stain)

3-Physical staining-:

It is simple solubility of the dye into cell components as in the staining of fatty tissue (ex: Sudan 3), fatty substance will stain orange.

4- Chemical staining-:

A new substance is formed as a result of dye- tissue interaction which is usually irreversible (ex: P.A.S reaction).

5. **Adsorption phenomenon:**

It is an ionic attraction in which the staining is influenced by affinity of acid to base or vice versa. These ions accumulate on the surface of one of cell components.

B) Methods of staining:-

1. **Vital staining:-**

It is the injecting of the dye into the living body. This is done for research.

2. Routine staining:-

Routine dyes that stain all tissue components with minute differences except nucleus &cytoplasm (ex: Eosin stains cytoplasm with pink colour, Haematoxylin stains nucleus with blue -violet colour).

3. Special staining:-

In which dyes stain some tissues, bacteria, fungi, some cell secretion, and components inside and outside the cell.

C) Types of staining:-

1. Regressive staining:

In this type of staining all the tissue will be stained and then the extra stain removed by using certain solution called: differentiator as 1% acid alcohol used with haematoxylin.

2. Progressive staining:-

The stain couldn't be removed from the tissue after staining as in using eosin.

Nature of stains:

Dyes are divided according to its nature into:

a) Natural stains : These are of natural origin such as Haematoxylin (plant origin), Carmine (animal origin), and Orcine (animal origin).

b) Synthetic stains : These are manufactured in factories, such as: Eosin, Thionine, Light-green, Neutral red, Orange green.

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture

Q1/ Mention differences between Regressive staining, Progressive staining.

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

Five week

Types of staining

Educational objective:
The student should be able to :
1-Know types of stains.
3-Know Nature of Hematoxylin

Lecture duration:
Theory (2) hour+ practical (3) hour /weekly.

Activities Used:
1- Interactive classroom activities
2- Brainstorming questions
3- Group activities (if required)
4- Homework
5- Online homework (classroom)
6- Quick written exam .

Hematoxylin :

its dark red color in the natural state, and to its method of manufacture from wood. Hematoxylin is a natural dye extracted by boiling the wood of the South American and West Indian logwood tree The active dye is not hematoxylin itself, but its oxidized product, hematin. The later can be bought, but is expensive, and the same results can be had simply by oxidizing the crude hematoxylin.

Oxidation is of two types:

1) Natural oxidation: In the early days oxidation was carried out by making up the hematoxylin solution, plugging the container with cotton, and leaving it exposed to light and air for 6weeks to several months.

2) Chemical oxidation: It was soon found that oxidation (also called ripening) could be achieved much faster by adding a wide variety of oxidizing agents, such as, alum, iodate, and mercuric oxide.

*** The addition of glycerin to several formulas is said to guard against over-oxidation and perhaps to retard fungal growth.**

*** Its lifetime is shorter than the naturally oxidized hematoxylin.**

Hematoxylin lakes:

Used alone, hematoxylin is a poor stain, but in combination with various metallic salts (mordents) which link it to the tissue, it is one of the best nuclear stains known. It has also been used to detect metals in tissue, to stain mitotic figures, fibrin, muscle cross striations and other tissue elements. The combination of hematoxylin plus mordant is called a hematoxylin lake. Most mordents are incorporated into the hematoxylin staining solution;
but in Heidenhain's iron hematoxylin the tissue sections soaked in the mordant before staining.

*** Hematoxylines could be classified according to which mordant is used as follows :**

1- Alum hematoxylin

2- Iron hematoxylin

3- Tungsten hematoxylin

4- Molybdenum hematoxylin

5- Lead hematoxylin

6- Hematoxylin without mordant

1. Alum hematoxylin :

This group is mostly used in the hematoxylin eosin staining and produce good nuclear stain, the mordant is aluminum in the form of :

A) Potash alum (aluminum potassium sulfate). .

B) Ammonium alum (aluminum ammonium sulfate

All alum hematoxylins, whatever their formula may be used as either progressive or regressive stains.

Differentiation: is done in dilute acid (usually acid alcohol because of hematoxylin's greater solubility in alcohol). Differentiation is stopped immediately by simply washing the slides in water .

Blueing: because most alum hematoxylin formulae are fairly acid, the nuclei at first be stained the purplish color of the acid dye. Changing their color to blue gives a much better contrast with the usual red counter stains.

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture

Q21/ 1-Mention disadvantageous of alum Hematoxylin

2-using alcohol for diluting acid of hematoxylin (differentiation).

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

Six week

alum hematoxylins

Educational objective:

The student should be able to :

1-Know types of alum hematoxylins stains.

3-Know features of alum Hematoxylin

Lecture duration:

Theory (2) hour+ practical (3) hour /weekly.

Activities Used:

1- Interactive classroom activities

2- Brainstorming questions

3- Group activities (if required)

4- Homework

5- Online homework (classroom)

6- Quick written exam

The mostly used alum hematoxylins are:

A. Ehrlich's HX.

B. Mayer'sHX.

C. Harris'sHX.

D. Carazzi's HX is used for urgent frozen sections.

A) Ehrlich's HX (1886):

a) An excellent nuclear stain.

b) It stains mucins including polysaccharide of cartilage.

c) It is recommended for the staining of bone and cartilage.

d) It is good for staining tissues which have been stored for a long time in formalin.

e) It is useful to stain tissues which were exposed to acid decalcification.

f) It is not ideal for frozen sections.

g) It ripens naturally for about 2 months, and this period of time could be shortened by placing the stain in un-stoppered bottle, in warm sunny place (window-ledge).

h) It lasts for years (in bulk).

i) It retains its staining ability in a Coplin jar for some months.

B) Mayer's HX (1903):

a) This stain chemically ripened with sodium iodate.

b) It is useful as a progressive stain when cytoplasmic components are stained with special stain that it may be destroyed or de-colored by differentiation.

c) It is used as a counter stain in the demonstration of glycogen and in various enzyme histochemical techniques.

d) The time for staining is (5-10 min) until the nuclei are stained, then blued without differentiation.

C) Harris's HX:

a) It is chemically ripened with mercuric oxide.

b) Since mercuric oxide is highly toxic, it is replaced by sodium or potassium iodate.

F) Carazzi's HX :

a) It is chemically ripened using potassium iodate.

b) It remains stable for about (6 months).

c) It is very good for frozen sections when used as a double strength solution with a very short staining time.

Staining time with alum HX depends on:

1. Type of hematoxylin (Ehrlich's HX, 20-45min, Mayer's HX, 10-20min).

2. Age of stain. As stain ages, it needs more time for staining.

3. Intensity of use of stain. A heavily use of the stain will weaken the power of the stain.

4. Using stain regressively or progressively.

5. Length of time of fixation, acid decalcifying solution or frozen sections (pre-treatment of the tissue).

6. Post-treatment of the sections (subsequent acid stain).

7. Personal choice.

Disadvantages of alum hematoxylins:

These stains are sensitive to any subsequently applied acid staining solutions (Van-Gieson stain). In this case we can use iron mordant HX, which resists the effect of acidity.

2- Iron hematoxylins:

In these hematoxylin solutions, iron salts are used both as the oxidizing and as mordant, these salts are:

a) Ferric chloride

b) Ferric ammonium sulfate

These iron salts are strong oxidizing agents so it can be used as a differentiating fluid after hematoxylin staining.

The most commonly used iron HX are:

1. Weigert's HX.

2. Heidenhain's HX

3. Loyez's hematoxylin for myelin

4. Verhoeff's hematoxylin for elastic fibers

Iron hematoxylins are used in two ways:

a) Prepared separately and then mixed with the mordant and the oxidant immediately before use (as in Weigert's HX).

OR

b) Preparing the iron salt solution separately and use before staining with HX (Heidenhain's & Loyez's hematoxylins) .

The iron hematoxylins are capable of demonstrating a much wider range of tissue structures than the alum hematoxylins.

It takes longer staining time than the alum ones.

Weigert's HX:

a) Naturally ripened for 4weeks.

b) Just before use, ferric chloride is added to the stain. Iron solution acts as an oxidant and mordant.

c) Time for staining is 15-30min.

Heidenhain's HX:

a) Naturally ripened for 4weeks.

b) Ferric ammonium sulfate is used as an oxidant/mordant.

c) The above solution is used as the differentiating fluid.

d) Sections first treated with the iron solution, then stained with HX until it over-stained.

e) Differentiation is done by using the iron solution and controlled under microscope.

f) It can be used to demonstrate many structures according to the degree of differentiation .

g) Time for staining is 1 hour .

Eosin:

This stain can be used to stain cytoplasm, collagen, and muscle fibers which are called eosinophilic. Eosin is used to differentiate between cytoplasm and different types of connective tissue fibers and matrices by staining the differing shades of red and pink. Is a fluorescent red dye resulting from the action of bromine on fluorescin, It can be used to stain cytoplasm, collagen, and muscle fibers which are called eosinophilic. Eosin is most often used as a counter stain to HX in H&E staining. This dye could be obtained in these types:

1- Eosin Y (eosin yellowish, water or alcohol soluble) .

2- Ethyl eosin (eosin S, alcohol soluble) .

3- Eosin B (eosin bluish, erythrosine B) .

For staining eosin Y is typically used in concentration of 1-5% w/v, dissolved in water or ethanol. A small concentration (0.5% of acetic acid) usually gives a deeper red color to the tissue. For preventing mold growth in aqueous solution, thymol is sometimes added.

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture

Q1/Enumerate types of iron HX

Q2/Mention features of eosin ,and types .

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

Seven-eight week

Stain solvents, factors affecting staining , storage of stains

Educational objective:

The student should be able to :

1-Know types of alum hematoxylins stains.

3-Know features of alum Hematoxylin

Lecture duration:
Theory (2) hour+ practical (3) hour /weekly.

Activities Used:

1- Interactive classroom activities

2- Brainstorming questions

3- Group activities (if required)

4- Homework

5- Online homework (classroom)

6- Quick written exam

Stain solvents, factors affecting staining , storage of stains

Stain is a combination of dyes and pigments suspended in a solvent. Soluble dyes dissolve in compatible solvents and provide greater grain

clarity, meaning the grain shows through the stain. Insoluble pigments are finely ground coloring materials that disperse but do not dissolve in the solvent.

DESCRIPTION. Solvent-Based Stain System is a solvent-based, fast dry stain that is formulated for spray and wipe application. Solvent-Based Stain System can also be hand applied with a rag or stain pad and then wiped off with a clean rag.

Factors That Affect Dye Binding .

Stain uptake is dependent upon the affinity the tissue element has for the dye component of the stain solution. Affinity refers to the tendency of a dye to transfer from a staining solution onto the tissue section. Factors that affect affinity and ultimately staining include the following:

1- Concentration of the dye - The greater the concentration of the dye, the more the dye is bound to tissue components.

2-Temperature - An increase in temperature increases the rate at which the dye diffuses throughout the tissue sample. It can also alter tissue components so that they are more receptive to dye penetration.

3-pH of the staining solution - Cells and other tissue elements often have an affinity for stains/dyes with specific pH ranges. Therefore, the pH of the staining solution can have a direct impact on the ability of a dye to bind with its intended tissue element .

4-Tissue fixation - Fixation alters and reorganizes certain molecular structures within the tissue sample such that there is increased permeability for staining. Unfixed tissue elements have limited binding sites for dyes .

5- Mordants - Mordants are chemicals that may be needed to bind dyes to certain tissue elements. They can also be used to intensify the staining results.

6- chemical or reagent which oxidation or reducing.

Difficulties in staining :

1-the staining solution have deteriorated and need to be replaced .

2- if staining solution employs haemotoxylin ,the staining solution may not be ripe.

3-the fixative and or the decalcifying solution may not have been washed out.

4- the tissue may be not fixed well.

5- tissue that have been stored for long period in 70% alcohol or 10% formalin ,do not accept the dye solution readily ,often required special handling

Special stain for staining some tissue structures :

1- Fat : used sudden III stain.

2- Amyloid : used Congo red stain

3- Mucin : used Mayer mucicarmine red

- Mucin appear deep pink to red
- Nuclease appear black
- General view for section yellow

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture

Q1/Enumerate factors effect on staining

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

Nine week

Decalcification , bone tissue

Educational objective:

The student should be able to :

1-Know types of important of Decalcification

3-Know methods of Decalcification

Lecture duration:
Theory (2) hour+ practical (3) hour /weekly.

Activities Used:

1- Interactive classroom activities

2- Brainstorming questions

3- Group activities (if required)

4- Homework

5- Online homework (classroom)

6- Quick written exam

Decalcification, bone tissue :

Bone tissue

Some tissues contain calcium deposits which are extremely firm, and will not sectioned properly with paraffin embedding. The bulk of mineral of bone is a crystalline substance" hydroxyapatite", formed mainly of Calcium phosphate and hydroxyl ions .

Bone specimen processing:

1. Biopsy: is taken by using a good fine saw (jewellery saw). Before sawing bone into slabs, it is advisable to remove connective tissue and tendon because they drag in the blade. Specimen size is 3-5 mm for hard bones & 4-6mm for soft bones.

2. Fixation: must be done immediately for (24-48 hours) and during this time specimen should be cleaned. The common & suitable fixative used for most biopsies is 10% neutral or buffered formalin, because it penetrates well and renders the soft tissue resistant to the acids present in the decalcification fluid.

3. Washing: is done thoroughly by running tap water to remove the excess fixative.

4. Decalcification: In order to obtain satisfactory paraffin or celloidin sections of bone it is necessary to remove the minerals & thus soften the tissue. This is carried out by treatment with reagents which react with calcium. These reagents are:

a. Acids to form calcium salts.

b. Chelating agents to pick up calcium ions.

Decalcifying agents:

1. Acid decalcifiers: there are two groups:

a. Strong acids: nitric and hydrochloric acid in (5-10%) aqueous solutions. They decalcify rapidly so fixation time should be not more than (24-48 hours) because of their serious deterioration of stain ability. These acids used only for urgent biopsy specimen .

1.Aqueous nitric acid:

Nitric acid 5 – 10 ml

Distilled water to 100 ml

2.Formalin – Nitric acid:

Formaldehyde (37-40%) …….10 ml

Nitric acid…… 10 ml

Distilled water ……..80ml

Decalcification with nitric acid takes short time (1-2days) with changing solution every day or twice a day, but tissue stain ability is less than formic acid which takes long time for decalcification.

b. Weak acids:

These are formic, picric and acetic acid is the only weak acid used for decalcification .It s concentration is (5-10%) aqueous solution or with additive, such as formalin or buffer.

1. Aqueous formic acid:

90% formic acid…….. 5-10 ml

Distilled water to …….100 ml

2. Formic acid formalin:

90% formic acid ……5-10ml

Formalin…… 5 ml

Distilled water to ……..100 ml

3.Buffered formic acid:

90% formic acid 35 ml

20% aqueous sodium citrate 65 ml

Washing after acid decalcification is necessary by using running tap water for 3-8hr to remove the last trace of the decalcification.

2- Chelating agents:

The mostly used for decalcification is ethylene diamine tetra acetic acid (EDTA), These processes are slower than acid decalcification and cause little or no effect on tissue elements; it does not affect tissue stain ability. Time required to complete decalcification of dense cortical bone may be as long as (6-8 week).

a) Hillman and Lee (1953)

EDTA disodium salt…….. 5.5 gm

Distilled water…….. 90 ml

Formalin ……..10 ml

b) Neutral EDTA

EDTA disodium salt…… 250 gm

Distilled water ……1750 ml

If solution is cloudy, adjust to pH 7 with about 25gm of sodium hydroxide. **For better decalcification**:

1. Volume of decalcifying solution should be (50-70 times) the specimen size.
2. Bone specimen should be near surface of the decalcifying fluid to ensure settling of salts.
3. Bone specimen should not be left longer than the specified time because it will swell & unattainable.

Testing the end of decalcification:

1. Physical test:

This test could be done either by inserting a needle into the bone piece. If the needle inters the bone easily, then the bone is ready to be processed; this will injure the tissue.

OR: If the bone bends easily & flexible, then it is decalcified.

2. Chemical test:

It is used to detect of calcium in the decalcifying fluid by using:

1. Concentrated ammonia

2. Saturated aqueous ammonium oxalate

Method:

a) To (5ml) of decalcifying fluid add a small piece of litmus paper.

b) Add concentrated ammonia drop by drop with shaking after each drop until the solution becomes neutral to litmus paper.

c) Add (5ml) of solution (2), shake well and allow to set for 30minutes.

Result:

1. If precipitate forms after the addition of ammonia, it means that considerable amount of Calcium is present; so it is unnecessary to proceed further with the test, and the decalcifying fluid should be changed.
2. If precipitation occurs after the addition of ammonium oxalate, it means that less calcium is present, and the decalcifying fluid should be changed.
3. If the solution remains clear after (30min), it means that decalcification is

complete.

Special stain for Bone Tissue

(Schmoral's-thionin Stain)

Fixative:

Any fixative other than those containing mercuric chloride is preferred.

Result:

Lacunae & canaliculi dark brown –black

Bone matrix red or brownish – yellow Cells

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

Ten week

Frozen sections

Educational objective:

The student should be able to :

1-Know method of frozen tissue processing

3-Know methods of frozen sections Staining

Lecture duration:

Theory (2) hour+ practical (3) hour /weekly.

Activities Used:

1- Interactive classroom activities

2- Brainstorming questions

3- Group activities (if required)

4- Homework

5- Online homework (classroom)

6- Quick written exam

Frozen sections:

Frozen section is a rapid way to fix and mount histology sections, which helps in diagnosis of a pathological process (during surgery). It is applied in routine histology especially that of central nervous system and histochemistry. Frozen sections; it can be cut from fixed or unfixed tissue. The principle of cutting frozen sections is by freezing tissue embedded in a freezing medium & cut on a microtome in a cooled machine called cryostat (refrigerated box containing a microtome). The temperature is around (-20 to -30 C).

Fixation:-

1) 10% formal calcium at 4c is suitable for most enzyme histochemistry & lipids.

2) Formal ammonium-bromide is used for neuropathology.

3) Fixative containing mercury, dichromate & alcohol should be avoided, as they tend to make tissue brittle & difficult to cut

Impregnation (embedding) medium:

Some tissues tend to fragment during sectioning so, to avoid this problem gelatine is used as a supporting medium, permitting easier sectioning and handling.

Gelatine impregnation:

Gelatine-glycerine solution:

Gelatine……..16gm , Glycerine ……15ml

Distilled water ……..70ml , Thymol…….. 4 crystal

Store at 4c and warm till liquid before use.

Sectioning:-

1. The tissue block is fixed on to the microtome stage.

2. Add a drop of water.

3. Blast carbon dioxide, the tissue will be frozen.

4. Sections will be cut when the block is hardened to its optimal temperature.

5. Single sections obtained & collected by finger or wet camel hair brush.

6. Staining could be done by floating sections on surface of staining solutions, or by floating on a slide and drying it by gentle flame to affix the section, then completing the staining procedure.

Staining:-

A) Rapid HE stain:-

1- Fix tissue 10% neutral buffered formalin, at room temperature for 20 sec.

2- Rinse in tap water.

3- Stain in double strength Carazzi's HX for 1min.

4- Wash well in tap water for 10-20seconds.

5- Stain in 1% aqueous eosin for 10 sec.

6- Rinse in tap water.

7- Dehydrate, clear, and mount.

B)For fat and lipoid material, oil red-o, Sudan III, Sudan black B & fat soluble stains.

The differences between Paraffin method & Freezing method

Paraffin method	**Freezing method**
1. The tissue must be fixed 10% formalin.	1. Fresh tissue or fixed with
2. It takes long time 1-2 days or more . minutes	2. It takes short time, few
3. Embedding medium is paraffin wax.	3. Embedding medium is ice.
4. Thickness of sections 4-10	4. Thickness of sections 10-20

5. It is possible to process all tissue types.	5. It is recommended for fat, lipids, and enzymes
6. Produce a ribbon of sections.	6. Produce a single sections.

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture

Q1/Mention differences between Paraffin method , Freezing method

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.

11-12 week

Examination for second term

13-14 week

Tissue slide , Freezing microtome

Educational objective:

The student should be able to :

1-Know method of frozen tissue processing

3-Know features of freezing microtome

Lecture duration:
Theory (2) hour+ practical (3) hour /weekly.

Activities Used:

1- Interactive classroom activities

2- Brainstorming questions

3- Group activities (if required)

4- Homework

5- Online homework (classroom)

6- Quick written exam

Freeze microtome:

Freezing Microtome is designed for the purpose of accurate and quick diagnosis in surgery. The microtome consists of a table clamping device. The specimen rises accurately and automatically by a handle. The CO2 freezing attachment is provided with a hard rubber non-conducting plate between the corrugated surface to which the object is frozen and the rest of the apparatus, which prevents the conduction of heat from the other parts of the apparatus to the specimen; thus saving time and CO2.

The automatic feed mechanism can be thrown out of action by turning the Index finger to the extreme left or it may be set to cut sections of any desired thickness. Each division of the graduated scale marks 5 µm, the microtome range is 5-40 µm. The sturdy Knife Holder can be tilted to a convenient angle of the Knife suitable to the object.

The Freezing Microtome

* Used for sectioning the fresh frozen tissue when the diagnosis is required urgently.
* Table clamping device
* No prior fixation required
* Bursts of CO_2 released under pressure and allowed to pass from under the stage of microtome.
* Freezing is complete when the whole tissue turns white.
* Sectioning done afterwards.

Freezing microtome

- Gives best results for cutting frozen sections.
- Machine is clamped to the edge of a bench and connected to a cylinder of CO_2 by means of a specially strengthened flexible metal tube.

Evaluation methods :

1-Immediate feedback (formative assessment).

2-Involving students in self-assessment (correcting their own mistakes).

3. Final feedback (summative assessment), which refers to answering questions given as a class activity at the end of the lecture

Q1/Mention features of freezing microtome.

References :

1- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques (8th ed.). Elsevier.

2- Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP Press.

3- Young, B., O'Dowd, G., & Woodford, P. (2013). Wheater's Functional Histology: A Text and Colour Atlas (6th ed.). Elsevier.