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Medical Lab Tech. Department

Tissue Preparations

First semester

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Lab1

Microtechnique:-

Is the preparation and staining of tissue sections from surgical biopsy or autopsy or materials to study the shape and structure of cells.

TOOLS and instruments used in pathological laboratory.

The tools glasses war and instruments used in pathological laboratory
There are a lot of types of glasses

1.Reagent potles: used to store the samples or reagents, ranging From50-200mlin volume.

2. Staining jars:

There are two types .

A: Staining dish and rack

Used to hold the slides horizontally during staining process.

B:Cuplin jars:-used to hold the slides vertically during staining process fixation.

3. Cylinder: Used to measure the volume of fluid.
4. Flasks : used to store pigments and iliquids.
5. Pipetes: used to transport specific aamunts of fluids from one place to another.
6. Beakers: simplu containers used to hold samples and reagents.
7. Funnels: used in casting fluids from narrow sllote .
8. Slides : Athin glass piece used to hold the tissue sections.

The tools lab

1. Forceps.
2. Scalpel.
3. Spatula.
4. Discecting needles.
5. scissor
6. Casttel.

The laboratory
instrument

1-Compound microscope.
2-Refrlgerator.
3-Water bath.
4-Ovens.
5-Mlcrotome.
6-Sharpping hife.
7-Center fuge.

There are many supplies that we need in labory work

1-Filter-paper they are of various sizes used to absorbexcess pigments from slides.
3-A pencll for labeling samples because it does not dissolve in water and other liquids.
4-Wax – pencll for writing on glass.
5-Diamond – pencil special for writing on the glass slide.

Lab2

Dilution is the addition of a new amount of solvent without a new addition to the solute.

Diluent :is the medium or solvent added to a concentrated to solution in order to dilute it

1:x dilution means your concentrated solution should be diluted to 1/x. Add1 volume of concentrate to (x-1) volumes of diluent to create volume equal to x.

1:100 means 1 part concn.,99part diluents
1:14 means 1 part concn.,13 part diluents
1:2 means 1 part concn., 1 part diluents
1:1 means straight concn.

Dilution law

v1c1=v2c2

v1n1=v2n2

V1m1=v2m2

C:- concentration

M:-molarity

Volume/volume (v/v) :-the amount of fluid diluted in volume of diluent

Ex:-

1% aqueous 10ml/L

Weight/volume(w/v):- Is based on a convention which is the mass/volume of pure water10%NaCl=100mg/ml

1%NaCl=10mg/ml

Ex:- 95%alcohol means that there is 95ml alcohol+5ml D.W.

Ex:-

Calculate the volume of water that should be added to 50ml of NaOH solution of 0.5molar to get a solution of 0.2molar of NaOH?

V1=50ml c1=0.5M

V2=? C20.2M

V1×c1=v2×c2

50ml×0.5=v2×0.2M

V2=125

Volume of added water=v2-v1

125-50=75ml from the water.

Lab3

Process to prepare the slide:-

There are many steps in the pathological lab.

1-The first and important step begin when the technician receive the Sample, he must be patient when he deals with this sample by

Checking the name of patient on the type of sample and the site of biopsy

2-Give the number to the sample and write this number on the request form and sample container.

All these above steps are very important to prevent the mixing of the sample between different patients.

Fixation:-

It is the process by which the components of the cells and tissues are fixed in physical and chemical stats and prevent the contents of cells from change by treated them with special reagents {ex 10/ formalin ,

Bounis solution ,zenker}

Properties of an ideal fixative:-
1-prevents autolysis and bacterial decomposition.

2-preserves tissue in their natural state.
3-penetrate rapidly and kill the tissue to prevent post mortem changes.
4-Harden the tissues.
5-should not be very expensive.
Temperature..
The fixation can be carried out at room temperature.
Amount of fixative fluid..
Should be approximately 10-20 times the volume of the specimen.
To obtain a good fixative tissue the following steps must be followed

1-The fixative material should be cover all the tissue sample.
2-choice the suite fixative{Ex formalin for the soft tissue Bounis' solution for bones}.
Fixation process
After receiving the sample in pathological room and the technician gave number to the sample.
The pathologist start to describe the sample grossly and recorder the notes and take some pieces from these sample, put it in casttels and transfer it to fixation jar.
The process and fixation takes about 16-48hrs.

Dehydration..

It is the process that occur on tissue to eliminate the water from tissue sample to allow the penetration of wax to inside tissue, this done by processing the sample in alcohol gradually

70%	1-1.5hr
80%	1-1.5hr
90%	1-1.5hr
100 %	1-1.5hr

When the dehydration process completed, then sample
transferred to other steps called clearing.

Clearing:-

Is the process thae makes tissue permanent to allow
the light to pass through out the tissue under microscope during exam

the sample , and this done by xylole solution

1-xylole 1-1.5hr

2-xylole 1-1.5hr

Clearing of tissue is achieved by any of the foll owing reagents:

1-xylene 3-benzene

2-chloroform

Infiltration:-

The penetration of wax to tissue sample and removing of xylole , to facilitate the sectioning of sample.
The infiltration don by..
1-Paraffin wax.
2-Gelatinous wax.
3-Other material, like plastics etc.
paraffin wax: is used routinely in the pathological lab.
The process done by putting the sample in wax {2 changes} in the oven 56-60C {molting temperature for wax} 1 hr. for each change

Causes of infiltration:

1.fixed tissues are not firm or adhesive enough to allow thin sectioning.
2.to hold the cells and intracellular structures in proper relation to each other.

Blocking:-

{embedding}

Treated tissues are placed on a black with their label and fresh melt wax is add on these tissue and allowed to settle and solidify.

Types of blocking

1-L-shap block
2-Net .work block or paper block

Process and blocking
1-Wax must be melted in oven{65C}
2-Arrange tissue sample on suitable surface and put the L- shape each opposite.
3-Add the wax carefully ; avoid burning the tissue sample by
Adding Avery hot wax.
4-Let block cooled at room temperature and then transfer it characters

of embedding media

1.must be capable of being converted readily from liquid to solid form .

2-in the liquid form the embedding media must penetrate easily into the interstices of the tissue, and then is converted into solid.

The types embedding media:

1-paraffim

2-celloidin

3-plastis.

Trimming:-

A margin of paraffin in excess of a few millimeters around the tissue is unnecessary.
Trimming done by microtome on the thickness 20 micron

Test:-

1-what is blocking ?
2-what is the benefit of trimming

Lab4

Sectioning:-

it is process for cutting the tissue to thin ribbon 3-4 micron in thickness to staining and exam it under microscope.
After trimming on 20 micron thickness and the sample will be appear on the ribbon change the thickness and the sample will be appear on the ribbon, change the thickness of knife and to 4 micron and start sectioning ,the angle of knife must be on 45 degree to get a good ribbons without folding.

Overview of processing of embedding tissue

2-Dehydration
70%alcohol 1-1.5hr
1-Fixation 10% formalin over night
80% alcohol 1-1.5hr
90% alcohol 1-1.5hr
100% alcohol 1-1.5hr
3-clearing
Xylol 1-1.5hr
Xylole 1-1.5hr
4-Infilteration
-paraffin wax 1 hr.
-paraffin wax 1 hr.

5-blocking

Microtome:- ls a machine specifically designed to cut very thin sections of the processed tissue. There are many types of microtome and each on is specified to cut tissues , depending on the embedding media.

1- Rotary microtome for paraffin impregnated tissues.
2- Freezing microtome for frozen tissues
3- Ultra microtome for tissues embedded in plastic materials

Steps of sectioning

1- Cheack the angle of microtomes knife (45)
2- put the paraffin block in microtome holder and let the knife attached the block.
3- Adjust the thickness on 20 micron
4- Trim the excess paraffin until tissue appears.
5- Change the knife thickness to 3 or 4 micron.
6- Sectioning the block to obtain a good ribbons hold it by discection needle ,put l drop of alcohol 70% to let the ribbon to extended.
7- Put it in water bath (56 C) to allow the ribbon to extended more and prevent the folding in ribbon.
8- By using a clean slide hold the ribbon from water bath, put the slide horizontally in water bath and pull the ribbons gently.
9- Recorde the number of sample on the slide label by wax-pencil
10- Transfer the slide to oven to de-waxing.

Lab 5

Difficulties most commonly encountered during cutting ribbon

1-fall of block to ribbon	a-block not parallel to knife edge b-knife dull c-knife tilted too much d-paraffin too hard sections
2-un even and crocked ribbon	a-wedge-shaped or irregularly trimmed block b-edge of block not parallel to knife edge c-irregularity in knife edge d-paraffin not homogenous
3-compressed Wrinkled sections	a-knife dull b-paraffin block too worm c-paraffin on knife edge d-sections too thin
	e-microtme screws are loose
4-tearing crumbling	a-incomplete fixation of tissue b-incomplete dehydration or clearing of tissue
Sections	c-incomplete infiltration of tissue with paraffin d-paraffin too hot for bath and or embedding
5-split ribbon or Lengthwise Scratches in ribbon	a-nicks in knife edge b-knife edge dirty c-too much knife tilt d-grit, dirt, mercuric chloride crystals, calcium sutures, or foreign bodies in paraffin or tissue

6-lifring of sections From knife on upstroke	a-too vertical knife tilt b-knife dull c-paraffin too soft or room too worm
7-section clinging to knife	a-static electricity b-knife edge dirty c-knife dull d-knife tilt too vertical
8-varying thickness of sections	a-screws on block or knife holder are not tight b-knife not tilted enough to clear bevel c-block too large d-block too hard e-microtome not adjusted correctly

Lab6

Flatting the sections of slide

Transferring of sectioning (Ribbon) and put it on slide to staining the tissue and exam it

Steps of flatting:-

1-Transffer the ribbons to water bath (56c) by slide and let it lying in water (this step occur by rinsing the slide in water bath gradually and left the ribbon in water, before rinsing the slide put few drops of alcohl on ribbon).

2-drag the ribbon by slide :- Rinse the slide in water bath
Vertically and begin to drag the end of ribbon on slide carefully.

3-Drag the slide from water by air.

4-Write the number of sample on the slide.

5-Arrange the slide on the staining basket and put it in oven (56- 60c for 15 min.) to make de-waxing (Remove of wax from tissue to allow stain to penetrate the tissue).

Lab7

Over view to previous steps of processing tissue blocking .

Lab8

Staining :- it is the process that make tissue takes color by rinsing Slide in difficult stains to facilitate the examination of tissue contains.

Hematoxylin and Eosin staining .

-Hematoxylin :- it is a blue color stain that is stain the nucleus

Eosin:-it is a red color stain used to stain the cytoplasm and the wall of

H and E staining

1- De-wax the slide

2- xylole (1) 5 min } clearing

3- xylole (2) 5min }

4- 100% Alcohol(ethanol) 5min

5- 90%Alcohol (ethanol) 3 min

6- 80% Alcohol (ethanol) 3min

7- 70% Alcohol (ethanol) 3 min 5 min

8- D.W

9- Skim the surface of hematoxylin by filter-paper to remove Oxidized particles

10- Rinse slide in hematoxylin 1-2min

If The satin is old prolong the time of hamatoxylin.

11- D.W rinse

12- Tap water 5min (to allow stain to develop)

13- Fast dip in acid ethanol (to de -stain)

14- Tap water

15- Eosin 30 seconds.

16- Tap water

17- 70% ethanol

18- 90% ethanol

19- 100% ethanol

20- Xylole (1)

21- Xylole (2)

22- mounting by Canada balsam or DPX
By place on drop of DPX on the cover slide
Angle the slide on cover slide and left to fall it on the cover slide gently.

Lab 9

Preparation of solution
-10% formalin from 40% formation
-prepare different dilution from absolute alcohol

Lab 10

Sectioning of block fixed by Zinker solution

Lab11

Special stain

-Verhoff' s stain (van gieson (vvG):-

This stain used for identifying elastic fiber in tissues such as skin, Aorta, etc. on formalin fixed ,paraffin – embedded sections and may be used for froezen sections

Solution and reagents:-

1-5%alcoholic hematoxlin:-
Hematoxy lin 5gm
100%alcohol 100ml
Mixed to dissolute with gentle heat. Filter
2-10% Aqueous ferric chloride:-
Dissolve 10 gm of ferric chloride in 100 ml D.W
3-Weigertis iodine solution
Potassium iodide 2 gm
Iodine 1 gm
D.W 100 ml
Dissolve potassium iodide in 4 ml D.W, and then add iodine. Once iodine is dissolved, dilute this solution by adding 96 ml of D.W this solution can be prepared fresh as needed or can made in larger quantities and store in brown bottle in the dark at room temperature (RT).

4-Verhffis working solution:-

This solution should be made up freshly for best result. It will not satisfactorily if it is kept more than one working day prepare the working solution by add the following reagents:-

5% alcoholic hematoxylin 20ml

10%aqueous ferric chloride 8ml

Weigert's iodine solution 8ml

Solution should be jet black. Use immediately and discard after use .

5-2%aqueous ferric chronicle:-

Mixed 10 ml of 10% FERRIC CHLORIDLE (Above solution),

With 50 ML D.W

6-5% aqueous Sodium thiosulfate :-

Dissolved 5mg of Sodium thiosulfate in 100 ml . D.W

7-Van Gieson's COUNTERSTAIN:-

Mixed 1% aqueous acid fuchsin (5ml) with 100 ml of saturated aqueous picric acid.

For Nerve tissue the counterstain can be prepared as following:-

1%aqueous acid fuchsin 15ml

Saturated aqueous picric acid 50ml

D.W 50ml

Staining :-

1- Dewax and hydrate working slide to D.W

2- Stain in Verhoff's working solution for 1hr tissue should to be completely black

3- Rinse in tap water 2-3 changes

4- Rinse in 2% aqueous ferric chloride 1-2 mines (differentiate)

5- Rinse in tap water (several changes)

Check under microscope black for elastic fiber staining and gray Background.

6- wash with tape water

7- Treat with 5% aqueous sodium thiosulfate for 1 min. (discard solution)

8- Wash with running tap water for 5 min.

9- Counterstain in Van Gieson's solution for 3-5 mins.

10- Dehydrate quickly with 95% alcohol, 100% alcohol (2chauges).

11- Clean with Xylene (2changs, 3mins. Each change).

12- Mounting with DPX

Result :-(elastic fiber will be stained blue-black and background Will be stained yellow).

nuclei ⟶ blue to black
Collagen ⟶ red
Other tissue elements ⟶ yellow

Lab12

Best carmin's stain:

This stain used to demonstrate glycogen bond formation between OH groups on the glycogen and H^+ atoms of the Carmine acid.

Reagents:-

1-Carmin stock solution
Carmine 2gm
all above in 60 ml D.W and gentle boil in large conical flask for 5min. cool and add 20ml concentrated ammonia. Filter and store in dark
Potassium carbonate 1gm
Potassium chloride 5gm
D.W 60ml
Dissolved container at4^{o}c for 1-2 months.
2-Working
solution :-
Mixed 15ml of stock solution with 12.5 ml of concentrated ammonia solution and 12.5ml of methanol
4-Best carmine counterstanin:-
Methanol 40ml
Ethanol 80ml
D.W 100ml

Staining

1-Dewax and hydrate to water
2-Treat with carmine working solution for 10mines
3-Wash in alcohol
4-Counterstanin2-4mins
5-Wash in alcohol
6-Mounting with DPX
Result:-
Glycogen:- bright red
Neuclei:- blue

Lab13

Periodic acid Schiff (PAS)

Glycogen is a simple intracytoplasmic polysaccharide found in greatest amount in liver, cardic and skeletal muscle, with significant quantities also present in hair follicles, endometrial glands, vaginal and cervical epithelium, neutrophils and megakaryocytes.

Solutions ans reagents :-

1% aqueous periodic acid Schiff reagent
Basic fuchsin 2gm
Potassium or sodium meta-bisulphite 4gm
Decolorizing activated charcoal 0.4gm
Concn. Hydrochloric acid (HCL) 4mi
D.W 400ml

1- bring D.W to boil, remove flame and add basic fuchsin slowly and Stir until dissolve (if added too fast solution will bubble explosively out of the flask)
2- cool to 5^0 c, add meta-bisulphite. Dissolve and cool to RT
3- Addconcn HCL and mix.
4- Leave overnight in the dark at RT

5- Add charcoal and shake for 1-2 mins, filter and store at 40^0c indark bottle.

Staining

1- Dewax and hydrate to water

2- oxides with periodic acid for 5 mins.

3- Rinse in D.W

4- Treat with Schiff's reagent for 5mins.

5- Wash in running water 10mins. (intensifies the color reaction)

6- Stain the nuclei with Mayer's hematoxylin 1min.

7- Wash in water

8- Dehydrate

9- Clear

10- Monnting with DPX

Result:-

Nuclei:- Blue

PAS positive material Magenta (glycogen, cellulose).

Lab 14

PAS for Mucin :-

1-ALCIAN BLUE Solution (1gm) dissolved in 3% Acetic acid (100ml) sore in refrigerator.

2-schiffs reagent.

3-1%periodic acid.

4-3%Acetic acid.

Staining:-

1-Dewax and hydrate to water

2-Rinse in acetic acid for 2mins.

3-Drain.

4-Alcian blue solution 10mins.

5-Wash in tap water, than D.W

6-Treat with 1% periodic acid for 5mins.

7-Rinse in D.W

8-Treat with Schiff's solution 5mins.

9-Wash in running water 5-10mins.

10-Stain nuclei with Mayer's hematoxylin 90 sec

11- Wash in tap water

12- Dehydrate' clear and mount

Result:

Acid mucin ⟶ blue

Neutralz mucin ⟶ magenta

Nuclei ⟶ pale blue

Lab15

H and E stain for bone tissues.

Lab16

Microtome explanation

Lab17

Sudan staining :-

Have high affinity to fats therefore, used to demonstrate triglycerides, lipid and lipid-protein (staining for frezon section).

Reagents:-

1- sudan 3 1gm

2- acrton 5ml

3- 70%alcohol 50ml

Staining

1- Bring the frozen section

2- Rinse in Sudan stain for 5min.

3-Wash

4-Rinse in 70% alcohol

5-WashD.W

6-Stain with Mayer's hematoxylin 1min.

7-Wash.

8-put the section on slide and cover it by gelatinous material (warm)

Result:-

All parts of tissues will be coloured except the adipose tissue still colourless

Lab18

Review

Lab19

Training on electron microscope