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

الحقيبة التدريسية لمادة

Microbial preparation/practical

First stage

فصل الدراسي الاول

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Syllabus for histological and cytological techniques

Practical lecture	Week
Introduction to histological and cytological techniques,Instruments,Tools and glass wares use in the lab.	2_1
Preparation of solutions	3
Processing of tissue by paraffin wax method	5_4
Blocking and embedding	7_6
Trimming and test for blocking and Trimming	8
Sectioning and errors during sectioning	11_10_9
Test for sectioning	12
First term examination	14_13
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الهدف من دراسة مادة تحضيرات مجهريه (الهدف العام):

تهدف دراسة مادة تحضيرات مجهريه للصف الاول الى:

1) اعداد الطلبه لتحضير سلايدات نسيجيه وخلويه
٢)يمكن للطالب تحضيرشرائح دائمه لاعضاء الجسم المختلفه

:Target group

Students of First stage/techniques of medical laboratory

التقنيات التربوية المستخدمة:

1. سبورة واقلام
2. السبورة التفاعلية
3. عارض البيانات Data Show
4. جهاز حاسوب محمول Laptop
5. ملزمة منهاج تحضيرات مجهريه

First and second week

Histological techniques are processes done on tissues which are collected from human or animal bodies.

The pathological studies on the tissue begin from the operating room & the autopsy room. Both biopsies & autopsies are delivered quickly to the laboratory.

The technician begins to:

1. Give an identification number to the specimen.
2. Carefully examine the tissue.
3. From this examination a full description of general findings is recorded.
4. According to the surgeon notes, small blocks are dissected from the tissue.
5. These specimen (blocks) immediately placed in the fixative (solution for fixation), with a piece of paper written carrying the number of the specimen & enclosed in a piece of gauze.

The histological technician should be:

1. Having a good knowledge of treating & processing the tissue specimen.
2. Good caring of producing the best possible slides that improve accuracy in diagnosis.
3. Has a strong sense of responsibility toward the patient

Glass wares needed:

Glass beakers (different sizes), conical flasks (different sizes)

Volumetric flasks (different sizes)

Droppers

Coplin jars

Staining dishes

Funnels

Slides & cover slips

Tools needed:

Dissecting scissors (large & small), Needle, Forceps, Scalpel, Spatula, L-shaped moulds, Alcoholic lamps, Wax pencils

Handling slides

Cleaning & storing slides (new slides):

1. Soak in 1% acid alcohol.
2. Wash with water.
3. Place in 95% alcohol for few minutes.
4. Wipe out with a piece of lent less cotton cloth.
5. Keep in :
    a) Cardboard trays that hold up to 20 slides.
    b) Cardboard, wood, or plastic boxes which have slots that could hold from 12-100 slides.
    c) Metal cabinets with drawers capable of holding up to thousands of slides.

Cleaning slides after use:

a) If slides have remains of wax or stains:

1. Soak in gas oil.
2. Clean with water & soap.
3. Wash with hot water.
4. Soak in the *cleaning* solution for two hours.
5. Wash with running tap water, wipe dry and keep.

Cleaning solution

Potassium dichromate	20gm
Distilled water	100ml
Concentrated sulfuric acid	20ml

Note: This solution is used for all glass wares, it is important to wear thick plastic gloves while handling glass wares.

b) If slides have cover slide stuck on it:

1. Soak in xylol at 50C (in the oven).
2. After the cover slide has dropped, brush the slides with a mild detergent.
3. Wash with water.
4. Wipe dry and keep.

Instruments

Light microscope

An electric instrument used to enlarge the minute details of the tissue section under study. It helps regulating staining procedures. The mostly used type is the compound microscope which consists of:

1. **The tube that carries**

a) Eye lenses (10X, 12X).

b) Revolving nose piece which carries the objective lenses (low power of 4X & 10x, high power of 40X, and oil immersion of 100X).

2. The arm

At the base of the arm there are the coarse & fine adjustments and the condenser knob.

3. The stage

Beneath it, the condenser & the diaphragm which control the amount of light that goes through a hole in the stage and through the slide.

4. Base (foot)

It has a hole in the center that allows the light to move up through the condenser

<u>The use of microscope</u>

1. Instrument must be clean, the lenses cleaned by alcohol& xylol (1:2).

2. Move the revolving nose piece, the low power objective should be above the center of the stage (hear a click)

3. Put the slide on the stage (cover slide upward) and the tissue section is over the light opening.

4. Rotate the coarse adjustment to move the sage up until the section is clearly seen.

5. Move the revolving nose piece to have the high power objective above the section.

6. Gently rotate the fine adjustment to magnify & clear the section.

****Note**

Do not use the coarse adjustment with high power objective to avoid the break of the slide.

Care of microscope

1. Use the microscope with care.
2. When the microscope is not in use, it must be covered with a plastic bag to prevent dust.
3. Lenses & condenser are cleaned with lens paper.
4. Oil immersion is cleaned by lens paper moistened with xylol or benzene, immediately wiped with dry lens paper.
5. Once a year the microscope must be adjusted.

Paraffin wax oven

This type of ovens is mostly used in histological studies, with 80C is the highest temperature with ventilation. These ovens should be large enough to serve these functions:

1. Melting down of paraffin wax blocks.
2. Infiltration of tissue.
3. Drying glass wares.
4. Warming some solutions and stains.
5. Staining some tissues which stained in temperature higher than room temperature.
6. To accelerate de waxing & clearing of tissue sections during staining.

Microtome

Is a machine specifically designed to cut very thin sections of the processed tissue specimen; there are many types of microtome & each one is specified to cut tissues, depending on the embedding medium.

1. Rotary microtome is used to cut paraffin impregnated tissues.
2. Freezing microtome is used to cut frozen tissues.
3. Ultra microtome is used to cut tissues embedded in plastic materials.

Sharpener

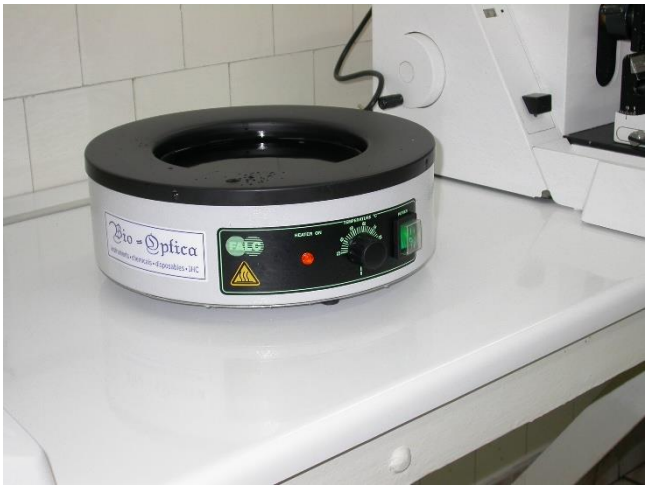
Floating (Water) bath

An electric instrument, which is thermostatically controlled to maintain the proper temperature, this will help to flat the paraffin ribbon.

The water container has a dull black color to allow high visibility of the floating paraffin ribbon.

The temperature of the bath should be 10C below the melting point of the paraffin to be sectioned. Care should be taken to prevent water bubbles from being trapped under the section; this can be accomplished by using distilled water in the bath.

After use power out the water & wipe thoroughly to remove adherent bits of paraffin wax while they are still soft. Cover with plastic bag to prevent dust.



Balance

A small balance of capacity to weigh up to 100 gm with 1-2 m gm sensitivity is required in the histological lab.

After use, wipe the pan free from the corrosive materials that are placed or dropped on it.

Magnetic stirrer

An equipment that is helpful in the preparation of fixatives, reagents and dye solutions. It employs Teflon-coated magnetic spinning bars in a variety of sizes. The stirrer could be supplied with a hot plate, thermostatically controlled. A beaker or flask is placed on the plate form, the motor supplies the impetus, and the magnetic rods do the mixing.

Refrigerator

An electric instrument, thermostatically controlled, used to:

1. To store some acids, alcohol, and some dye solutions.
2. Store tissue embedded in paraffin wax.
3. Cooling water for casting by adding ice cubes.

مدة المحاضرة: ساعه ونصف لكل شعبة ولكل اسبوع

الأنشطة المستخدمة:

1. Q1 .Enumerate the parts of ligh microscope.
2. Q2.أfunction of the oven wax

أساليب التقويم:

1. قيام الطلبه استخدام الاجهزه التي تستخدم وتصحيح اخطائهم.

Third week: preparation of solution

Tissue processing needs different kinds of solutions and dye stains, some of that are used in bulk.

These solutions are of two types:

1. Weight / volume
2. Volume / volume

A) Weight / volume

These types of solutions are prepared by adding certain weight of a chemical material (stain, salt, alkali, etc.) to a solvent which is water or alcohol; make the solution up to the needed volume.

e.g. (1) Prepare a liter of 2% sodium hydroxide.

To solve this problem, use this equation:

$$W1 \times V2 = W2 \times V1$$

$$2 \times 1000 = W2 \times 100$$

W2 = 20gm sodium hydroxide

This weight is dissolved in amount of distilled water, complete up to a liter.

e.g. (2) Prepare 1% aqueous eosin

In this example, the volume is 100ml, take 1gm of eosin and dissolve it in amount of distilled water, complete up to 100.

B) **Volume / Volume**

This type of solutions, are the different concentrations of alcohol, are used in dehydration of tissue and staining of tissue sections.

For calculations, use this equation

C1 x V1 = C2 x V2

Where:
C1 = the higher conc. Of alcohol
C2 = the wanted conc. Of alcohol
V1 = the volume of alcohol of higher conc.
V2 = the wanted volume

e.g. (1) Prepare 20 ml of 20% alcohol, from 50% alcohol

C1 x V1 = C2 x V2

50 x V1 = 20 x 20

V1 = 20 x 20 / 50

V1 = 8ml of 50% alcohol

20 – 8 = 12ml of distilled water added to the(8ml) of 50% alcohol.

e.g. (2) Prepare 70% alcohol

Simply take 70ml of absolute alcohol, complete up to 100ml with distilled water.

مدة المحاضرة ساعتان لكل شعبه
الانشطه:

Q1 Prepare 1 liter of 4%sodium hydroxideof

Q2. Prepare 40ml of 20%alcohol from 50%alcohol?

يقوم الطلبه بتحضير أنواع المحاليل مع تصحيح الأخطاء أن وجدت.

Fourth &fifth week

Processing of tissue by paraffin method

1. Sample collections:

Consist of 2 kinds biopsy and autopsy .

2. Fixation:

Tissue specimen under study must be fixed immediately after cutting to prevent postpartum decomposition & to preserve the tissue. The solutions that are used for fixation are called fixatives which are classified as "routine" and "special" fixatives.

Period of time for fixation depends on: type of fixative, size of tissue specimen, and the temperature of the laboratory.

3- Washing:

At the end of fixation the tissue must be washed to remove the excess of the fixative. Choosing the washing solution depends on the type of the fixative as follows:

1. Tissue that have been fixed in a fixative containing water (10% formalin, Helly's solution), the fixative should be washed out by using running tap water, with minimum time of one hour.
2. Tissue that have been fixed in a fixative containing alcohol (Bouin's fluid), the fixative should be washed out in several changes of 50% or 70% alcohol until the yellow color disappears. If there is still a yellow color seen in the sections, this could be washed out by leaving the sections longer time in 70% alcohol during staining (hydration).

3. If the tissue have been fixed in alcoholic fixative (Carnoy's fluid), the tissue transferred to absolute alcohol to complete dehydration.

4- Dehydration

It is the complete removal of water from the washed tissue. This is done by:

1. The tissue is transferred into a series of ascending concentrations of alcohol beginning from 70% alcohol up to 100% alcohol, (two changes for 100% alcohol) the time for each change is 1 1/2 – 2 hours.
2. The tissue goes through three changes of acetone, 1/2 hour for each (to save time).
3. Using dioxane, which is an excellent reagent to dehydrate and clear the tissue, in a period of time 2-4 hours depending on the type of the tissue.

5- Clearing

At the end of dehydration, the tissue is immersed in the clearing agent for 10-45 min depending on tissue thickness. The tissue will be transparent pale yellow. Some of the clearing agents are xylol, toluene, & benzene.

***** Note**

If a white cloud appears when the tissue is transferred into the clearing agent, this means that the tissue is still contains water, hence, the tissue must be returned to absolute alcohol and left for one hour, then transferred into a new clearing agent.

Infiltration

The tissue cells and the intercellular spaces should be supported by paraffin wax; this is done by immersing the tissue in melted paraffin wax inside the oven, temperature 2 – 4C higher than the melting point of the used paraffin wax.

The tissue is transferred through 2 or 3 melted paraffin changes, of 3 hours period of time. At the end of infiltration, paraffin wax is removed out from the oven, kept until casting.

****Note**

1. Alcohol does not miscible with paraffin wax.
2. Alcohol miscible with water & xylol.
3. Xylol does not miscible with water.
4. Xylol miscible with alcohol & paraffin wax.

مدة المحاضره ساعه ونصف لكل شعبة للأسبوع الواحد

Q1. How can be choose the washing solution depends on the type of the fixative?

Sixth and seventh week:

مده المحاضرة ساعة ونصف لكل شعبة لكل اسبوع

Blocking & Embedding

It is the enclosing of tissue with a solid mass of embedding medium. Hard paraffin wax is preferred in casting. Tissue can be embedded in metal L-shaped moulds, paper boats, or plastic moulds.

Orientation of tissue is according to which section is needed (longitudinal or transverse section).

For casting you need:

1. Two forceps
2. L-shaped moulds
3. Needle
4. Alcoholic lamp
5. The infiltrated tissue (inside paraffin wax)

6. Melted paraffin wax
7. Glycerol
8. Label (number or name of tissue)

Technique

1. Moist the L-shaped moulds with glycerol.
2. Arrange the moulds as a square or rectangle.
3. Warm the wax that contains tissue.
4. Pour the molten wax inside the moulds to the top.
5. With a warm forceps pick a tissue specimen up & orient it inside the wax.
6. With a warm needle affix the label in the wax, at one side of the mould.
7. Gently blow parallel to the surface of the wax to harden it, put in cold water to cool quickly, avoiding the formation of large crystals that brake during sectioning.
8. After solidifying of wax, remove the block from the mould.
9. Keep in refrigerator until sectioning.
10. Clean tools & keep.

****Note:**

a. Pouring molting wax should be continuous to avoid the formation of paraffin layers that break during sectioning.
b. Transferring of tissue into the molten wax should be quick to avoid the formation of a solid layer of wax covering tissue; hence the tissue will fall off the block during sectioning.

Q1. How can be avoid the formation of paraffin layer that break during sectioning?
يقوم الطلبه بصب القوالب الشمعية وطمر الانسجه المعالجه

Eighth week:

مدة المحاضرة ساعه ونصف لكل شعبه

Trimming and test for blocking and Trimming

It is necessary to remove the excess of paraffin wax around the tissue, having a cube-shaped paraffin block that will help to get a straight paraffin ribbon.

To trim the block you need:

1. Scalpel
2. Alcoholic lamp
3. Card board (to work on)

Technique:

1. Hold the block upside down
2. Draw a square around the tissue (2-3mm)
3. Warm the scalpel and cut longitudinally for all four sides
4. Turn the block to remove the wax from the opposite side to the tissue (surface tension)
5. The upper & lower edges of the block must be parallel.

   Q. Why the trimming necessary to the paraffin block?

Eighth week

Blocking and embedding, trimming Evaluation for students.

Nineth and tenth and eleventh week:

Sectioning and errors during sectioning

After paraffin processing, tissue sections could be gotten by using microtome. These instruments produce thin sections of 4-8 microns, less or more thickness is needed, depending on tissue kind.

(micron = 1/ 1000 of mm)

To have sections of paraffin-embedded tissue, rotary microtome is used, it consists of:

1. **Body**: which has a strong cover, it carries on the right the fly wheel, that moves block holder up and down in front of the knife. Lock of this fly wheel is underneath it at the base of the microtome. On the left side of microtome, there is a small fly wheel that moves block holder back and forth. There is a small screen showing tissue thickness.
2. **Base**: It carries knife holder which moves back and forth on 2 metal bars, underneath there is a lock that moves the bars up to lock the knife holder. Knife holder holds the knife inside a movable groove to control the angle of tilting.

Important roles for good sectioning:

1. Laboratory temperature should be cool in summer, not very cold in winter.
2. Large thickness for soft paraffin embedded tissue & small thickness for hard paraffin embedded ones.
3. A well trimmed paraffin block.
4. The knife must be evenly sharpened.
5. Tissue should be well processed.
6. Microtome should be in good condition.

For sectioning you need:

1. Large forceps with folded cotton on end.
2. 2 fine brushes.
3. Knife of microtome
4. Vial containing xylol
5. Card box or card board to put ribbon on

Technique

1. Be sure that large fly wheel is locked.
2. Adjust paraffin block in block holder, tissue in front of you.
3. Revolve small fly wheel, the block should be behind the knife.
4. Fix the knife in proper position.
5. Tilt the knife so the clearance of angle is 5 to 10 degrees.
6. Lower the block, lock fly wheel, move knife holder forward & touch the block, lock knife holder.
7. Start sectioning to remove to remove the outer surface of the block until the tissue is completely in touch with the knife.(thickness is 25-30 micron)
8. Stop sectioning, reduce thickness to 5 micron; continue cutting to have ribbon.
9. Having a ribbon of several centimeters long, stop, put a brush under the first section, with the second brush detach the last section from the knife.
10. Carefully place the ribbon on card board, keep until mounting.

11. After finishing, all accumulated paraffin & tissue must be brushed away with a soft brush.

12. Carefully remove knife holder, brush away broken paraffin ribbons, wipe clean with xylol underneath block holder and base of microtome. Dry carefully.

13. Wipe clean the knife with xylol, from base up to the honed edge, keep inside knife box.

14. Cover microtome when not in use.

Faults & Remedies in paraffin sectioning

1. Failure of block to ribbon

Cause**:**

a) Paraffin wax is too hard for sectioning.

b) Clearance angle is incorrect.

c) Wax on knife edge.

Remedy:

a) Breathe on block to warm or re-embed in low melting point wax.

b) Adjust knife to optimal angle.

c) Clean knife with xylol.

2. Varying thickness of sections

Cause:

a) Wax too soft for tissue.

b) Block or knife loose.

c) Small slop angle.

d) Mechanical faults.

Remedy

a) Cool block or re-embed in higher melting point wax.

b) Tighten block or knife.

c) Slightly increase slop angle.

d) Adjust microtome.

3. Compressed or wrinkled sections

Cause:

a) Blunt knife.

b) Warm block.

c) Cutting too fast.

d) Clearance angle too great.

e) Poor quality wax.

Remedy:

a) Sharpen knife.

b) Cool block.

c) Adjust clearance angle.

d) Use good quality wax.

4. **Splitting of sections longitudinally (or) knife lines**

Cause:

a) Nick in knife.

b) Hard particles in wax.

c) Hard particles in tissue (calcium).

d) Buffer salts precipitation in specimen.

e) Poor processing.

Remedy:

a) Use different part of knife or re-sharpen knife.

b) Re-embed in fresh filtered wax.

c) If calcium deposit, decalcify; if mineral or dirt, remove with fine sharp pointed scalpel.

d) Use well processed specimen.

5. Ribbon is curved

Cause:

a) Block & knife edges are not parallel.

b) Knife blunt in some area.

c) Tissue varying in consistency.

d) Excessive paraffin on one side.

Remedy:

a) Trim until parallel.

b) Sharpen knife.

c) Re-orient block.

d) Trim away excess wax.

6. Sections attached to block

Cause:

a) Small slop angle.

b) Wax debris on knife edge.

c) Static electric charge on ribbon.

d) Debris on block edge.

Remedy:

a) Increase slop angle.

b) Clean knife with xylol.

c) Humidify air with moist breath or water bath near microtome.

d) Trim block edge.

7. Section roll into a tight coil

Cause:

a) Knife dull.

b) Section thickness too high for used wax.

Remedy:

a) Sharpen knife.

b) Reduce section thickness.

8. Sections clinging to knife

Cause:

a) Static electricity.

b) Wax on knife edge.

c) Knife dull.

d) Knife tilted vertically.

Remedy:

a) Moist block with breath.

b) Clean knife with xylol.

c) Sharpen knife.

d) Tilt knife to optimal angle.

مدة المحاضره ساعه ونصف لكل شعبة ولكل اسبوع

Q. Give two faults and Remedies in paraffin sectioning?

يقوم الطلبه استخدام جهاز تقطيع الأنسجة وتقطيع القوالب الشمعية

Twelfth week

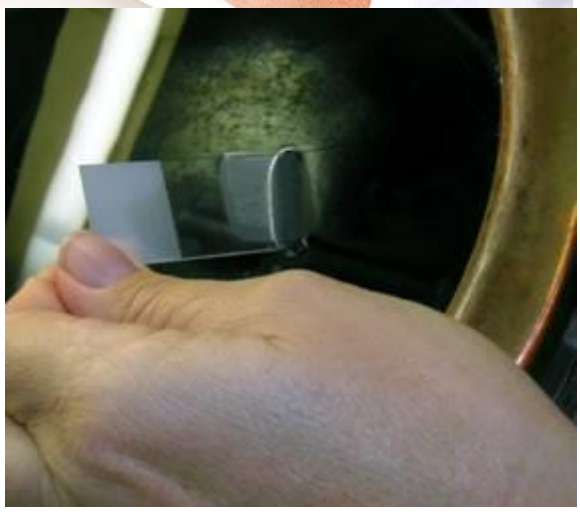
مدة المحاضرة ساعه ونص لكل شعبه

Sectioning evaluation for students

Floating out sections:

The floating out of the ribbon must be smooth, with a slight drag on the surface of the water; this dragging will remove most, if not all, of the folds that occur in the ribbon.

Sections are floated on the water bath, shiny side down. Approximately 30seconds should be long enough for a ribbon to flatten; prolonged time on the water causes excessive expansion, distorting the tissue. Using 30% alcohol for floating out ribbon will set up diffusion currents that help to flatten the tissue sections in the water bath.



: References

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and-1
.Practice of Histological Techniques (8th ed.). Elsevier
Carson, F. L., & Hladik, C. (2014). Histotechnology: A Self-Instructional -2
Text (3rd
.ed.). ASCP Press

Thirteenth and fourteenth week

First term examination