National University-Sudan

Faculty of Medical Laboratory Sciences

Student Practical Manual
Histopathology and Cytology Department

Second Year, Semester (4)
Basic Histology and Histological Techniques
(MLS-HIST-222)

Student Name: ……………………………………………………………...

ID: ………………………………………**Batch**……………………......…...

Practical No (1)

Work flow in histopathology laboratory

Aim:

To discuss the work flow in histopathology laboratory and to list the different steps of handling specimens in histopathology laboratory.

Steps of specimens handling in histopathology laboratory:

1. **Reception**

   This step include receiving the specimens with completed information in request form and insuring that the sample is preserved in fixative solution.

   Request form :-important to demonstrate type of biopsy and the data of patient and history,

   - diagram is important for any remark eg to indicate right and left for eg in histroctomy for fallopian tube.
   - Date of collection,Name of patient, Type of specimen
   - Type of procedure,Physition,Sergune
   - Clinical history ,previous surgery,treatment

2. **Fixation**

   In this step the samples remains in the fixative solution (time varies according to the type of fixative used) until selection.

3. **Selection (grossing)**

   After insuring complete fixation of the sample, small piece/pieces of sample is/are selected by histopathologist by special set of tools for tissue processing and hence examination.

4. Tissue processing

Tissue is treated in a group of chemical substances;

Dehydration (alcohol): to remove the water from tissues.

Clearing: to remove the dehydrating agent from tissue so as to be replaced later by embedding media.

Impregnation: this step involves filling the tissue spaces with a media (e.g wax) to support the tissue for sectioning

5. **<u>Embedding</u>**

   This step involves surrounding the tissue with embedding media (e.g paraffin wax) to give it a frame for sectioning.

   Orientation of tissue biopsy is the critical step in embedding cause it is effect the diagnosis

   <u>Embeding unit:-</u>

   Instrumentation used to embedded tissue in mould with specific orientation.

6. **Sectioning (microtomy)**

Thin sections are cut to be further stained and examined under microscope.

The instrument used is called microtome.

7. **Staining (H&E and special staining)**

Tissue sections are colored to facilitate examination and distinction between different tissue constituents.

8. **Microscopic examination**

Stained sections are examined under microscope.

9. **Issuing result**

This involves writing detailed report about macroscopic and microscopic observation.

Practical No (2)

Preparation of fixatives

<u>Aim :</u>

To prepare different types of simple and compound fixatives

Simple fixative:

10% formalin

Distilled water……………………………90 ml

Formaldehyde…………………………...10ml

Routine fixative in histopathology laboratory

Compound fixative

(i) **Carnoy's fixative**

Absolute alcohol …………………………. 60ml

Chloroform………………………. …….30ml

Acetic acid………………………. ……..10ml

Used for urgent biopsies.

(ii) **Bouin's fixative**

Saturated aqueous picric acid ……………75 ml

Formalin …………………………………25ml

Acetic acid …………………………………5ml

Best fixative for glycogen

Practical No (3)

Tissue processing

Tissue processing:-

Aim:-

- It involves treating the tissue in different type of chemical substances to prepare it for microtome sectioning
- To replace tissue water and fixative by wax to enhance & facilitate the cutting of tissue.
- To infiltrate the tissue in a solid medium firm enough to support the tissue, give it sufficient rigidity and suitable consistency to enable thin sections to be cut without damaging knife or tissue.

1. Dehydration in ascending grades of alcohol(70%, 90%, 100%, 100%)
2. Clearing in clearing agents (e.g xyelene)
3. Impregnation (e.g paraffin wax)
   Duration of each step varies according to the specimen schedules used

Routine manual process (blocks 3-5 mm)

1. Fixation-first beaker for complete fixation 1hour
2. Washing in water if necessary(to prevent crystals formation)
3. 50% alcohol……….1 hour
4. 50% alcohol……….1 hour
5. 70% alcohol……….1 hour
6. 70% alcohol……….1 hour
7. 80%alcahole………..1hour
8. 90% alcohol………1 (hour)
9. 100% alcohol (I)………1 hour
10. 100% alcohol (II)………1 hour
11. 100% alcohol (III)………1 hour
12. Xyelene……………..1 hour
13. Xyelene……………..1 hour
14. Xyelene……………..1 hour
15. Paraffin wax (I)……….1hour
16. Paraffin wax (II)……….1hour
17. Paraffin wax (III)……….1hour

Practical No (4)

Embedding

Aim

To obtain a solid block containing the tissue specimen.

Principle

Surrounding the tissue with embedding media

Embedding steps

1. Different size of mould according to the size of specimen Eg:-

a- Endoscopic biopsy (gastric,sigmoid,….

b-Core needle biopsy(trephine BM,liver ,renal,….

c-skin biopsy.

Tissue orientation:-

2. Orienting the tissue in the mould to insure it will be cut in the right plane.

3. Cooling the mass to solidify.

Practical No (5)

Chemical test of end point of decalcification

Calcium oxalate test

Aim

To test the end point of decalcification and ensure complete removal of calcium from tissue.

Principle

In an alkaline medium calcium in the decalcifying fluid reacts with ammonium oxalate to produce ca oxalate which appear as turbidity (cloudiness) or precipitate.

Procedure

1. In a test tube add 5 ml of the decalcifying fluid.
2. Add a piece of litmus paper to the fluid.
3. Add concentrated ammonia drop by drop until the fluid become alkaline (litmus paper becomes blue).
4. Add 0.5 ml of ammonium oxalate and wait for 15-30 minutes.

Result interpretation

Cloudiness or turbidity………………………………calcium presence

Clearness…………………………………………calcium absence

In the presence of calcium the specimen is placed in a fresh solution of decalcifying fluid and the test is repeated interval until the calcium is absent.

Note:-

If there is a present of turbidity after ammonium oxalate that mean we most renewing the decalcification fluid.

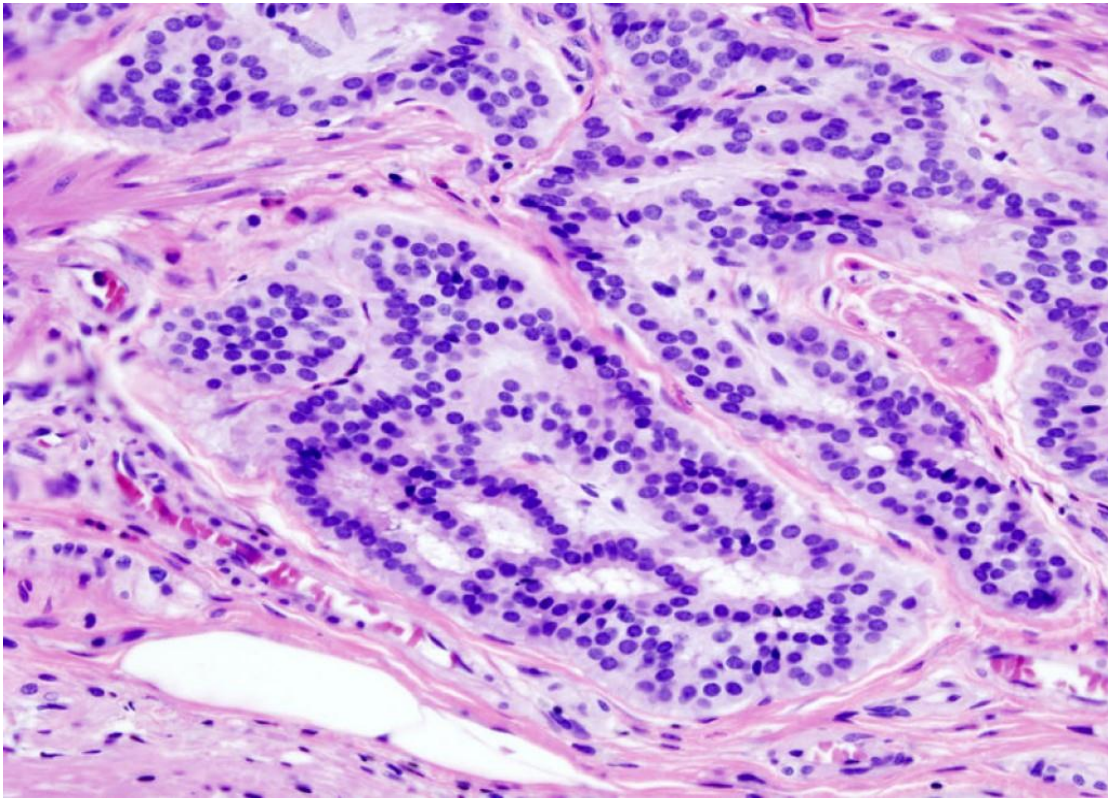
Practical no (6)

Hematoxylin and eosin staining

H&E

Alum hematoxylins

Mayer's hematoxylin



Aim

To give contrast by altering the tissue (nucleus and background) colors to facilitate differentiation and microscopic examination.

General Principle of Hematoxylin :-

Hematoxylin oxidation product haematein ,haematein is an acid dye have a poor affinity for tissue, so mordant substance is added to form mordanted dye, mordanted dye react with the tissue to form tissue mordant dye complex(acid base reaction)

Preparation

Hematoxylin Powder………. 1 g (dye)

D.W……………………….. 1000 ml (solvent)

Potassium alum…………… 50 g (mordant)

Sodium iodate……………… 0.2 g (oxidizing agent)

Citric acid………………… 1 g (accentuator)

Chloral hydrate……………. 50 g (preservative)

Procedure

1. Take section to water:
   (i) Dewaxation in xyelene for 10 minutes
   (ii) Rehydration in descending grades of alcohol
   100% alcohol………….3 minutes
   100% alcohol………….3 minutes
   90% alcohol…………...3 minutes
   70% alcohol…………..2 minutes
   D.W……………………2 minutes
2. Nuclear Staining in Mayer's Hematoxylin for 10 minutes
3. Bluing in running tap water for 10 minutes
4. Background staining in eosin for 3 minutes
5. Wash in water
6. Dehydrate in alcohol
   70%.. 1minute
   90%..1 minute
   100%..2 minutes
   100%..2 minutes

7. Clear in xyelene
8. Mount in DPX

Result

Nucleus……………………………………………Blue

Cytoplasm and background…………………….Red

H&E is the routine stain in histopathology laboratory and is used to demonstrate the general structure of tissues.

Practical No (7)

Hematoxylin and eosin staining

Alum hematoxylins

Harris hematoxylin

Cytological µanatomical staining

Aim

To give contrast by altering the tissue (nucleus and background) colors to facilitate differentiation and microscopic examination.

Principle

Tissue dye mordant complex

Preparation

Hematoxylin powder ……..5 g (dye)

Absolute ethanol …………50 ml (solvent),

D.W……………………... 950 ml (solvent)

Potassium or ammonium alum……….. 100 g (mordant)

Sodium iodate……………………….. 1 g (oxidizing agent)

Glacial Acetic Acid…………………. 40 ml (accentuator)

Procedure

9. Take section to water:
   (iii) Dewaxation in xyelene for 10 minutes
   (iv) Rehydration in descending grades of alcohol
   100% alcohol………….3 minutes
   100% alcohol………….3 minutes
   90% alcohol…………...3 minutes
   70% alcohol…………..2 minutes
   D.W……………………2 minutes
10. Nuclear Staining in harris Hematoxylin for 10 minutes
11. Differentiate in 1% acid alcohol for few seconds.
12. Background staining in eosin for 3 minutes
13. Wash in water
14. Dehydrate in alcohol
    70%.. 1minute
    90%..1 minute
    100%..2 minutes
    100%..2 minutes
15. Clear in xyelene
16. Mount in DPX

Result

Nucleus……………………………………………Blue

Cytoplasm and background…………………….Red

Harris hematoxylin is the common used type in cytology because it gives good nuclear details.

Practical No (8)

Hematoxylin and eosin staining

Iron hematoxylins

Weigert's hematoxylin

Micro anatomical stiaing

Aim

Use usually for connective tissue and nerve cell nucleus when using acid background staining .

To give contrast by altering the tissue (nucleus and background) colors to facilitate differentiation and microscopic examination.

Principle

Tissue dye mordant complex

Preparation

haematoxylin solution (A)

Hematoxylin powder…………..1g (dye)

Absolute alcohol…………….100 ml (solvent)

Iron Solution (B)

30% $FeCl_3$………………. 4ml (mordant and oxidizing agent)

conc HCl………………... 1ml

D.W……………………… 95ml

Working solution

Add Equal volumes of solution A&B prior to staining.

Procedure

1. Take section to water:
   (v) Dewaxation in xyelene for 10 minutes
   (vi) Rehydration in descending grades of alcohol
   100% alcohol…………3 minutes
   100% alcohol…………3 minutes
   90% alcohol…………...3 minutes
   70% alcohol…………..2 minutes
   D.W……………………2 minutes
2. Nuclear Staining in weigert's Hematoxylin for 15 minutes
3. Differentiate in 1% acid alcohol for few seconds.
4. Wash in water.
5. Background staining in eosin for 3 minutes
6. Wash in water
7. Dehydrate in alcohol
   70%... 1minute
   90%...1 minute
   100%..2 minutes
   100%..2 minutes
8. Clear in xyelene
9. Mount in DPX

Result

Nucleus…………………………………………Black

Cytoplasm and background…………………….Red

Iron hematoxylins are used when subsequent acidic dyes will be applied.