Histotechnology

Unit-I

Introduction: Histology is the branch of medical science which deals with study of tissue which may be diseased or not.

Histopathology: It is the branch of medical science which deals with diseased tissue.

Histotechnology: It is the branch of medical science which deals with the techniques involved in studying the tissue.

Histotechnician: The person who knows the technique involved in studying the tissue.

The Histopathology helps in diagnosis of disease mostly the cancer patients.

Unit-II: Tissue Processing

Tissue Processing: It is the process by which tissue is made firm (hard) so that thin sections can be cut and studied under microscope. It can be done either manually or with the help of a machine known as automatic tissue processor. It involves the following steps.

1. Reception and logging
2. Grossing
3. Fixation
4. Decalcification (if required)
5. Dehydration
6. Cleaning
7. Impregnation
8. Embedding
9. Section cutting
10. Staining
11. Mounting
12. Microscopy

1. Reception and logging : The specimen sent for histopathological examination is collected in plastic or glass contain. Once specimen is received in the laboratory ensure that
   (a) Specimen is accompanied by a requisition form with relevant details of the patient i.e. name, age, sex, hospital number, diagnosis history site of biopsy.
   (b) The specimen and the requisition form are of the same patient.
   (c) Ascertain the presence of the tissue in the bottle.
   (d) In case more than one specimen check whether the specimen number consider with that on the requisition form.

   Providing proper number to the tissue for its proper identification is known as logging or labelling. It helps in removing errors in pre analytical and post analytical stage.

Number is given as S/108/19 in this number –S- represents the Sr. No. of the tissue while 19 indicates the year in which sample has come this number remains with the tissue throughout the tissue processing.

2. Grossing : Grossing means gross examination of the tissue. It includes the macroscopic examination of tissue that is to observe its shape, size, colour texture etc. and the tissue in thin slices of 3 to 5 mm.
After grossing the tissue is placed in tissue cassette along with the label on which number is written with the help of the pencil. The tissue cassette helps in prevent change in morphology of tissue while transferring it from one container to another container, its another advantage is that we can process more than one tissue in a single container.

3. Fixation : To preserve the tissue from any physical or chemical change after it is removed from patient body is known as fixation. The physical change involves change in morphology i.e shape and size of tissue the tissue should not shrink or there should not be any swelling in the tissue. While chemical change involves Autolysis and purification.
Autolysis : Self destruction or damaging of tissue by the enzymes which is secreted by cell itself, name of the enzyme is hydrolytic enzyme and it is secreted by lysosome.

Putrefaction: Destruction or damaging of tissue by the bacteria which are present in surrounding atmosphere.

When fixation is done these changes do not occur.

Fixation is done with the help of chemicals known as fixatives the most commonly used fixative is 10% Formalin. Other fixatives are formal calcium, Zenker's fluid, Bouin's fluid, Heidenhains susa etc.

4. Decalcification: Removal of calcium from the tissue is known as decalcification. It is done in order to make the tissue soft so that its proper section cutting can be done and microtome knife do not damage. It is done only in those tissues which contains calcium i.e bones and cartilage. If the given sample does not contain calcium thin step is not required for example skin, intestine liver etc. Decalcification is done with the help of chemicals known as decalcifying fluids some examples are 5 to 10% nitric acid, 10% Formic acid, Gooding Stewart reagents, formal nitric acid etc.

5. Dehydration: Removal of water from the tissue is known as dehydration. It is done because in further tissue processing there is a step of impregnation in which we have to introduce paraffin wax inside the tissue for proper section cutting. As paraffin wax and water are not soluble with each other. In order to enter paraffin wax inside the tissue we will have to remove water from the tissue. Dehydration is done with the help of dehydrating agents e.g Graded ethyl alcohol, Acetone, isopropyl alcohol, dioxane etc. Most commonly used dehydrating agent is graded ethyl alcohol. We take seven containers of ethyl alcohol i.e 70%, 80%, 95%, 95% and then three containers of absolute alcohol and keep the tissue for one hour in each container in this way water is removed from the tissue and alcohol enter inside the tissue.

   In order to check the complete dehydration we place the anhydrous copper sulphate in last container of absolute alcohol the anhydrous copper sulphate is hygroscopic in nature and white in colour, if water will be there it will be absorbed by copper sulphate and it will change into hydrated copper sulphate which is blue in colour. So if colour of anhydrous copper sulphate changes from white to blue means dehydration is incomplete and if it remains white means dehydration is complete.

6. Clearing : Removal of dehydrating agent from the tissue is known as clearing, it is done because the dehydrating agent alcohol is also not soluble with wax so we have to remove the alcohol. It is done with the help of clearing agents which are soluble both in alcohol and wax. Most commonly used clearing agents is xylene. We take two containers of xylene and place the tissue for one hour in each container. Other examples of dehydrating agents are Benzene, tolerance cidar wood oil, clove oil, chloroform phenol etc.

7. Impregnation: Entering of paraffin wax inside the tissue and removal of clearing agent is known as impregnation. It is done in order to provide proper hardness to the tissue for proper section cutting. The impregnation media should be solid and room temperature and its melting point should not be too high. Example of impregnation media or supporting media are paraffin wax, paraplast, paraplast plus, celliodine, tissue mat ester wax etc. It is done in paraffin oven.

8. Embedding or Blocking : Embedding is the process of placing the impregnated tissue is precisely arranged position into a mould containing the embedding media and causing

this medium to solidify. Embedding is done to give the tissue proper shape and size so that it can be attached to microtome for proper section cutting. Moulds used in embedding are Leuckhart's piece or L-piece which are made up of grass (an alloy of copper and zinc) or tissue tek.

Technique of embedding:

(a) The mould is placed on a tray, tile or a plain surface.
(b) Fresh molten wax is poured from stock jug into the mould.
(c) The tissue is lifted from final wax bath and placed at the bottom of the mould.
(d) The surface to be cut is pressed gently against the solid layer with no trapped air bubbles.
(e) The labelled bearing number of the specimen is fixed to the corner of the solidifying wax.
(f) When block has cooled to form a skin on the surface, it should be immersed in cold water.
(g) When the block hardens, remove the mould.
(h) A fresh labelled with typed number is fixed to the side of the block by pressing hot forceps. The block is now ready for cutting or storing.
(i) A block has to be cut, the surface of the block away from the surface to be cut is pressed against chuck. Chuck helps in attachment of a embedded block to the microtome.

9. Section cutting or microtome : In order to view the structure of the cell, a tissue is properly cut in very thin sections of the order of the microns. It is done with the help of a machine known as microtome. The most commonly used microtome is rotary microtome. When sections are thin, it can be stained in better way and viewed properly in the microscope generally thickness of the tissue is 3 to 5 microns or advised by the pathologist. Technique of cutting paraffin embedded sections.

Technique of cutting paraffin embedded sections :

(a) Trim the block to remove the surplus paraffin on the surface.
(b) The cutting surface should be even and parallel.
(c) Attach the block to the chuck and seal the sides of the block with warm forceps.

(d) Attach the block to the microtome.

(e) Turn back the feed mechanism as far as it goes.

(f) Insert the knife in the knife holder with slight tilt to produce the clearance angle.

(g) Adjust the knife holder or move the block holder, so that wax block just touches the knife.

(h) Block should move parallel to the knife edge.

(i) If not minimized earlier set the gauge to 15 microns and cut till complete sections are obtained.

(j) If it is already trimmed set the gauge to required thickness.

(k) Operate the microtome till complete sections are cut and ribbon is formed.

(l) With forceps hold the ribbon at its free margin and separate the sections attached to the knife by brush.

(m) Float the sections on tissue floatation water bath maintain at temperature 5 to 6° below melting point of wax.

(n) Immerse the albumenized slide in the water and bring the section gently into the center of the slide.

(o) Keep the slide with section vertically on the rack till the water is drained.

(p) Write the login number on the glass slide with the help of diamond pencil.

(q) Place the slide on slide warming table maintained at temperature 1 to 2°C above melting point of the wax. Then remove moisture, left out wrinkles and make attachment of tissue better.

10. Staining : Colouring a dying the tissue to known as staining. Staining of tissue sections enables us to study the characteristics of the tissues and their constituent cells. The most commonly used stain is hematoxylin and Eosin stain it is also known as routine stain.

Composition of Hematoxylin stain

Harris Hematoxylin

Hematoxylin - 2.5 gm

Absolute alcohol - 25 ml

Potash alum - 50 gms
Dist. Water - 500 ml
Mercuric oxide - 1.25 gm
Or
Sodium Iodate - 0.5 gm
Eosin - Eosin alcoholic
Stock solution : - Eosin - 1 gm
-Dist water – 20 ml

Dissolve eosin in water by heating it gently. Cool the mixture and add 80 ml of 95% ethyl alcohol.

Working Solution :

Stock Solution : 25 ml

80 % ethyl alcohol = 75 ml

Adding 15 ml glacial acetic acid to 100 ml of stain gives deeper shades of red colour.

Procedure :

(a) Deparaffination : Removal of paraffin wax is known as deparaffiniation. It is done with three container of xylene for 5.5 and two minutes.

(b) Hydration : Bringing of water inside the tissue is known as hydration. It is done as the stains are soluble in water. It is done with the help of decreasing con of alcohol i.e 100% alcohol, 95% alc, 80% alc, 70% alc, 50% alcohol than in water for one minute each.

(c) Primary Stain : Now slide is placed in Harris hematoxylin for 4 to 5 minutes.

(d) Give one washing with water.

(e) Differentiation : In this step selective removal of stain is done. It is done with the help of 0.5% Hu or 1% acid alcohol in this step the stain is removed from cytoplasm and retained by the nucleus but due to acidic pH blue colour of hematoxylin changes to red.

(f) Bluing : Regaining of original blue colour of hematoxylin which was changed to red during acidic pH is known as bluing. It is done with the help of Lithium carbonate solution or dilute ammonia for one minute. It can also be done by tap water for five minute.

(g) Give one washing with water.

(h) Counter stain Eosin : Now slide is placed in eosin stain for 2 minutes in order to stain cytoplasm.

(I) Give one washing with water.

(j) Dehydration : It is done with increasing concentration of alcohol or three container of acetone.

(k) Clearing : Removal of dehydrating agent, it is done with the help of xylene for 5, 5 and 2 minutes.

11. Mounting : Covering the stained slide with the help of mounting media (DPX) and cover slip is known as mounting. It is done in order to

(a) Protect the tissue section from physical injury or deterioration of stain due to oxidation.

(b) Fix the slide to cover slip.

(c) Fill the tissue space and cavities.

(d) Remove and trapped air bubbles.

(e) Avoid distortion of tissue and loss of stain or long periods.

(f) Visibility becomes better.

12. Microscopy: Viewing the stained slide under microscope is known as microscopy after staining following results are seen.

Nucleus = Blue

Cytoplasm = Pink

RBC = Red

Unit-III: Instruments Used in Histotechnology

1. Microtome : It is a type of machine which is used to cut thin sections of the tissues of the order of microns. There are 5 classes of microtome.

(a) Rotary microtome.
(b) Rocking microtome
(c) Base sledge or sliding microtome
(d) Freezing microtome
(e) Ultra thin section microtome for electron-microscopy work.

The most commonly used microtome is Rotary Microtome.

Basic Principle : The pawl or panel is brought in contact with ratchet wheel which is connected to mill head micrometer screw. This action turns the wheel and rotate the screw. As a result the block is moved towards the knife at a predetermined thickness.

Parts of Rotary Microtome :

(a) Block holder , (b) Knife holder (c) knife clamp, (d) knife clamp screw, (e) Block adjustment screw, (f) Thickness gauges, (g) Thickness adjustment screw, (h) Operating handle, (i) Operating wheel, (j) Reverse wheel, (k) Angle of tilt adjustment

Mode of Action : It is the most popular microtome, a rotary action of hand wheel starts the cutting movements block is mounted on the steel carriage the fly wheel is turn towards the top of upwards stroke. The pawl comes in connected with ratchet wheel which in turn rotates the micrometer screw. The block moves forward and block hold moves up and down vertically in front of knife.

Advantage :

(a) The microtome is heavy and stable
(b) Knife angle is adjustable
(c) Knife holder is moveable
(d) Sections are cut flat and plain
(e) Good serial sections are obtain
(f) Its is useful for routine and research purpose

Disadvantage :

(a) It is very heavy, so difficult to move

(b) Microtome knife is costly, so needs extra care

Automatic Tissue Processor (Autotechnichon)

The automatic tissue processor is a excellent device which can perform the different steps of tissue processing automatically. It is electrically operated. However, it create serious problem, if there is interception in the supply of electricity, in addition if there is no proper maintenance of the instrument it is a matter of trouble for most of the technician. It consist of main body having beaker platform with beaker, a transfer arm to shift the basket containing tissue capsule through different reagents . It has a metal disc know as timer disc which is used to set the time schedule for tissue processing. It has a plastic cover plate which covers all the baskets to prevent evaporation of reagents. It also contains thermostatically controlled wax bath for impregnation some time delay timer facility also available which helps inn delaying the time if required. It is also known as Autotechnician.

Mode of Action :

(a) The transfer arm moves enroller which is responsible for shifting tissue basket from one container to another container.

(b) The transfer arm moves the tissue through processing reagents by lifting it in and out of beaker.

(c) The timer unit consist of an electric clock which is attested to melt disc in which slots are cut at require intervals with the help of metal cutter.

(d) The disc rotates against the spring loaded liver, which when slips into slots caused the transfer arm to lift and tuning mechanism is set in motion, shifting the tissue to the next position in the cycle.

(e) Delay mechanism is available to process the tissue latter on.

Bone Decalcifier : It is a machine with the help of which tissue is decalcified. It is electrically operated. It reduces the time of decalcification by 1/3 of that which is taken when decalcification is done manually.

Mode of Operation :

(a) assemble the base plate with vertical pillar

(b) Mount the rotor motor and clamp it on horizontal pillar

(c) Keep the tissue with calcium in the stainless steel perforated basket

(d) Suspend the basket in pins of the clamps

(e) Place the beaker with decalcifying fluid on heating unit

(f) By unscrewing the knob on the pillar of the stand lower the rotor so that basket is immersed in the beaker fluid know clamp the rotor at this position by tightening the knob of the pillar.

(g) Connect the rotor motor lead wire plug to the socket of the control box.

(h) Connect the control box wire to the socket.

(i) Switch on the main supply to control box.

(j) Set the digital temp. controller temperature generally maintained is 37°C.

(k) The basket along with tissue will be provided rotary and translator motion by the motor rotor. It rotates at 30 rpm.

(l) Run the process for required time.

(m) After desired time, switch off the rotor and heating mantle by switching off the main supply.

(n) The process is now complete.

(o) Raise the motor and basket, basket will come out of the beaker.

(p) Remove the tissue from the basket for further processing.

Microtome Knife Sharpener

It is a machine with the help of which we sharpen the microtome knife.

Mode of Operation :

(a) Clean the plate thoroughly to provide the surface completely free from dust and dirt clean it with solution of calcium carbonate and water if necessary.

(b) After washing the glass plate thoroughly with the solution, rub the glass surface vigorously with cotton. Presence of water on glass surface indicates that glass place is not clean. The glass plate must always be free from dirt, oil or grease.

(c) Insert knife carefully and tight it with the help of screw provided with the knife holder.

(d) The knife should not be removed from the holder, until the sharpening operation is completed. To remove the knife from the holder, carefully release the screw and remove the knife.

Unit-IV

Autopsy

An Autopsy is also known as postmortem examination, is a specialized surgical procedure used to determine the cause and manner of death. The cause of death is the medical reason explaining why patient passed. The manner of death is the circumstances surrounding the death. It recognizes the following manner of death:

Natural, accident, homicide, suicide and unknown

Autopsy is continually advance our understand of disease. What we learn from Autopsies allows clinicians to better understand disease process, accurately diagnose the disease improve therapy and potentially aid other patients who are currently suffering from similar disease.

Sometime a forensic expert is also present if death takes place in ambiguous circumstances.