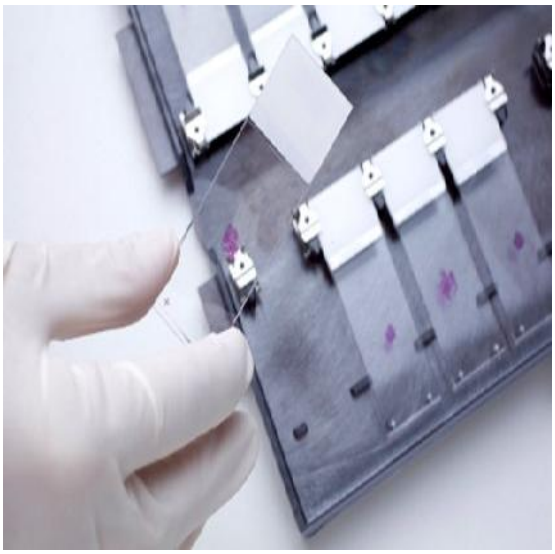
H.S.C. (VOCATIONAL)

MEDICAL LABORATORY TECHNICIAN

STD: XII (PAPER-2)

HISTOTECHNOLOGY

PRACTICALS



INDEX

Week no.	1
Practical No.	1
Title/ Aim	Collection, labeling and preparation of specimen.
Objective	Students shall be able to prepare the tissue for histological examination.
Requirements	1. 10% formalin 2. Normal saline 3. Dissection box 4. Wide mouth bottles 5. Hand gloves 6. Specimen.
Environment	MLT laboratory
Procedure	1. According to the requisition slip, enter following data in log book a) patient's name b) doctor's name c) operation date and time d) age e) description of specimen. 2. Now wash with normal saline and examine the specimen for a) weight b) size (length, breadth and width) c) shape d) color e) visible abnormality f) internal abnormality. 3. Cut a part of about 5x5x5 ml thick size portion of the specimen. 4. Put in a wide mouth bottle and add about 50-60 ml of 10% formalin. 5. Label the bottle with identification details.
Observations	


Fig. 1.1 Labeling of specimen

Result	After registering specimen its grossing is done by pathologist. Then it is properly fixed and labeled.
Clinical significance	• Proper registration and labeling helps in identification of tissue in further processing and histological diagnosis. • Adequate fixation helps to keep tissue in original condition.
Skills to be achieved	Students can maintain record of tissue received in laboratory.
Skill evaluating criteria	• Proper preparation of tissue- 4 Marks • Registration of tissue- 3 Marks • Labeling of tissue- 3 Marks
FAQs	1. How specimen is received and registered? 2. How it is labeled?
Assignment	Label 10 different specimens received in laboratory.
Reference	Chapter 3 Hand book of Histotechnology

Week no.	2
Practical No.	**2**
Title/ Aim	**Fixation of tissue-** To prepare 10% formalin.
Objective	Student shall prepare 10% formalin
Principle	10% formalin is prepared in laboratory by using commercial formalin and distilled water.
Requirements	1. Commercial formalin, 2. Distilled water.
Environment	MLT laboratory
Procedure	1. Take a clean & dry pipette (10 ml) & measuring cylinder. Keep the measuring cylinder on the table which is not disturbed. 2. Pipette 10 ml of full strength formalin with no air bubbles into measuring cylinder. 3. Add distilled water from the side of measuring cylinder slowly till 95 marks. 4. With the help of pipette add distilled water slowly & exactly till 100 ml mark. 5. The 100 ml mark & eye level should same.
Observations	10% formalin composition:- • Commercial formalin 10 ml • Distilled water 90ml
Result	10% formalin prepared.
Clinical significance	• 10% formalin is commonly used fixative for majority of tissues to be processed for histological diagnosis.
Skills to be achieved	Students can prepare 10% formalin and fix the tissue properly.
Skill evaluating criteria	• Pipetting exact quantity of constituents- 5Marks

	• Labeling- 5Marks
FAQs	1. Give composition of 10% formalin. 2. What are uses of fixation?
Assignment	Fix 10 different specimens received in laboratory.
Reference	Chapter 4 Hand book of Histotechnology

Week no.	
Practical No.	**3**
Title/ Aim	**Decalcification-** To prepare 5% formic acid.
Objectives	At the end of practical students shall be able to prepare 5% formic acid.
Principle	Acid method is the most widely used method for decalcification of large amounts of bony tissue.
Requirements	1. Formic acid 2. Distilled water
Environment	MLT laboratory
Observations- 5% formic acid composition • Formic acid - 5 ml • Distilled water - 95 ml	
Result	5% formic acid prepared.
Clinical significance	5% formic acid is commonly used as decalcifying fluid for bones and other pathological hard tissue sent for histopathological examination.
Skills to be achieved	Students can prepare 5% formic acid solution and can decalcify the tissue.
Skill evaluating criteria	Measurement of required quantity of ingradients correctly- 5 Marks Labeling- 5 Marks
FAQs	1. How 5% formic acid is prepared?
Reference	Chapter 5 Decalcification- Hand book of Histotechnology

Week no.	
Practical No.	**4**
Title/ Aim	**Decalcification-** To detect end point of decalcification.
Objectives	At the end of practical students shall understand detection of end point of decalcification.
Principle	The acid present in the decalcifying fluid removes the calcium salt present in the hard tissue and makes the tissue soft enough for sectioning.
Requirements	1. Blue litmus paper 2. Strong ammonia 3. Saturated ammonium oxalate 4. Acid decalcifying fluid in which tissue was kept
Environment	MLT laboratory
Procedure	1. Take about 5ml of used decalcifying fluid (i.e. in which tissue was kept for decalcification) into a clean test tube and add a small piece of blue litmus paper. 2. Add strong ammonia drop by drop until the litmus paper just turns blue indicating alkalinity. 3. If the solution becomes turbid at this stage, calcium is present and the tissue should be transferred to fresh decalcifying fluid for further decalcification. 4. If the solution remains clear, add 0.5 ml of saturated ammonium oxalate to confirm absence of calcium. Mix well. 5. Keep at RT for half an hour. 6. After addition of ammonium oxalate, if calcium is present, the solution l becomes turbid. Then re-immersion of tissue in fresh decalcifying fluid is

	necessary for further decalcification of the tissue. 7. If there is no turbidity even after addition of ammonium oxalate, the specimen is now free of calcium and ready for further processing. 8. Wash the decalcified specimen in running tap water for 24- 48 hours for complete removal of decalcifying solution.
Observations	Tissue becomes soft.
Result	End point of decalcification is studied.
Clinical significance	Decalcification is important for tissues like bones, teeth, etc which are to be cut on microtome.
Skills to be achieved	Students can detect end point of decalcification.
Skill evaluating criteria	Proper use of litmus paper- 2 Marks Proper technique - 5 Marks End point detection- 3 Marks
FAQs	1. How end point of decalcification is detected?
Reference	Chapter 5 Decalcification- Hand book of Histotechnology

Week no.	
Practical No.	**5**
Title/ Aim	Tissue processing- To study the manual tissue processing using paraffin wax.
Objective	Student shall understand the manual processing of tissue before it is cut on microtome.
Principle	Tissue processing is preparatory treatment given to the tissue before being sectioned to provide suitable consistency and support.
Requirements	1. Stainless steel or plastic perforated containers (Cassettes or capsules) 2. Fixed tissue 3. Different grades of ethyl alcohol- 70%, 80%, 95% and 100% (Absolute alcohol) 4. Copper sulphate (Anhydrous) 5. Xylene 6. Paraffin wax 7. Embedding oven (Temperature 50^0 to 60^0C) 8. Lead pencil and paper
Environment	MLT laboratory

Procedure

Different steps of manual processing are as follows-

1. <u>Dehydration</u>
   - Keep tissue blocks in capsules.
   - Write identification number with lead pencil on a piece of paper.
   - Pass the tissue through ascending grades of alcohol.
   - Detect end point of dehydration in last batch of alcohol with anhydrous copper sulphate. If it turns blue indicates incomplete dehydration. Continue the dehydration.

2. Clearing

- Now keep the tissue in xylene.
- Xylene removes alcohol and makes tissue more transparent.

3. Infiltration and Impregnation

- Keep tissue in paraffin oven.
- Temperature of it should be 50^0C to 56^0C.
- Do not expose the tissue to high temperature.

Steps	Treatment	Time (Hours)
1	70% Alcohol	3-8
2	90% Alcohol	16
3	100% Alcohol	2
4	100% Alcohol	3
5	100% Alcohol	3
6	Xylene	Overnight 16 hrs
7	Paraffin wax	1
8	Paraffin wax	1
9	Paraffin wax	1

Observations-Properly processed tissue gets suitable consistency for microtomy.

Fig. 5.1 Tissue processing cassettes

Fig 5.2 Casette basket

Result	Tissue is ready for embedding.
Clinical significance	1. Proper processing of tissue helps in thin section cutting which is required in histopathologic

	diagnosis. 2. Paraffin wax is most commonly used embedding medium for majority of tissues to be processed for histological diagnosis.
Skills to be achieved	• Students will understand manual tissue processing using paraffin wax. • They know advantages and disadvantages of it.
Skill evaluating criteria	• Preparation of grades of alcohol- 6 Marks • Organisation and fixing the schedule of different steps- 4 Marks
FAQs	1. What is tissue processing? 2. Name different steps involved in it.
Assignment	Visit to histotechnique laboratory.
Reference	Chapter 6 Tissue Processing-Hand book of Histotechnology.

Week no.	
Practical No.	**6**
Title/ Aim	**Automatic Tissue processor** To study the functioning of automatic tissue processor.
Objective	Student shall understand the automatic processing of tissue before it is cut on microtome.
Principle	Tissue processing is preparatory treatment given to the tissue before being sectioned to provide suitable consistency and support.
Requirements	• Alcohol solutions of various concentrations e.g. 80 %, 95 %, absolute alcohol • Xylene • Paraffin wax • Automatic tissue processor
Environment	Histotechnique laboratory

Procedure

- Cut the pieces of tissue and place them in the tissue receptacles or basket with an identifying number.
- Place the alcohol in ascending grade, xylene and paraffin wax in respective containers of the machine.
- Set the time level at zero and start the machine.
- The basket with the cassettes automatically changes position and travel clockwise to take a bath in different reagents kept in the container in order to accomplish
    - Dehydration- Ascending grades of alcohol
    - Clearing- Xylene
    - Impregnation- Warm paraffin

No	Timing	Reagent	Interval
1	Starting point	80% alcohol	0 hours
2	4.30 - 6.30 pm	80% alcohol	2 hours
3	6.30 - 7.30 pm	95% alcohol	1 hour
4	7.30 - 8.30 pm	95% alcohol	1 hour
5	8.30 - 9.30 pm	100% alcohol	1 hour
6	9.30 - 10.30 pm	100% alcohol	1 hour
7	10.30 -11.30 pm	100% alcohol	1 hour
8	11.30 -12.30 am	Xylene	1 hour
9	12.30 - 2.30 am	Xylene	2 hours
10	2.30 - 4.30 am	Molten paraffin	2 hours
11	4.30 - 6.30 am	Molten paraffin	2 hours
12	6.30 - 8.30 am	Molten paraffin	2 hours

Observations

- Tissue gets suitable (firm) consistency for microtomy.
- The time required for processing decreases due to the constant agitation.
- Due to raised temperature penetration of reagents in the tissue increases and produces more consistent result.

Fig. 6.1

Result	Completely processed tissue remains in last paraffin bath which is ready for embedding.
Clinical significance	1. Proper processing of tissue helps in thin section cutting required in histopathologic diagnosis. 2. Paraffin wax is most commonly used embedding medium for majority of tissues to be processed for histological diagnosis. 3. As time required for processing is less than that of manual processing, histopathological diagnosis can be given earlier which helps in further line of treatment for patient.
Skills to be achieved	• Students will understand structure and working of automatic tissue processor. • They know advantages and disadvantages of it.
Skill evaluating criteria	• Preparation of reagents and solutions- 5 Marks • Programming and execution of the schedule- 5 Marks
FAQs	1. What is tissue processing? 2. Explain structure of automatic tissue processor? 3. What are advantages of automatic tissue processor?
Assignment	Visit to histotechnique laboratory
Reference	Chapter 6 Tissue processing - Hand book of Histotechnology.

Week no.	
Practical No.	**7**
Title/ Aim	To embed the given impregnated tissue.
Objectives	The student shall be able to do embedding of tissue using moulds.
Principle	Placing the impregnated tissue in warm liquid paraffin that solidifies into a firm block when it cools at room temperature.
Requirements	1. The leuckhart mould consists of 2 L shaped pieces of metal 2. Glycerin 3. Wax impregnated tissue 4. Molten wax
Environment	MLT laboratory
Procedure	1. Take two L moulds and join them to form the sides of rectangular box. 2. Place the mould on a sheet of heavy metal or glass. 3. Apply glycerin from inside of mould. 4. Fresh molten wax is poured into mould. 5. Lift the tissue from the final wax bath with forcep & press to the bottom with the cutting surface facing downwards. 6. Fix the label bearing number of the specimen to the corner of the solidifying wax. 7. When the block has cooled sufficiently immerse it in cold water to prevent crystallization of wax. 8. When the block becomes hard remove moulds. 9. Fix a fresh label with right number to the side of the block by pressing a hot scalpel. 10. The block is now ready for either cutting or storing.
Observations	

Fig 7.2 L-mould joined to form square

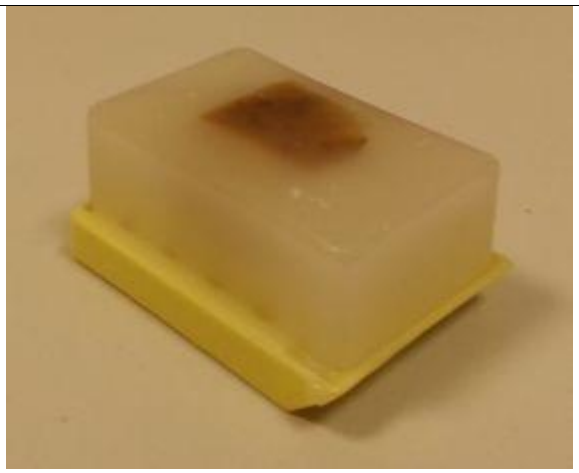


fig 7.3- Paraffin block

Fig. 7.1 L-mould

Result	Paraffin tissue block is prepared.
Clinical significance	• Paraffin wax is used for embedding different types of tissues which provides support during microtomy.
Skills to be achieved	Students can embed tissue perfectly.
Skill evaluating criteria	• Use of moulds- 2 Marks • Temperature of paraffin wax- 2 Marks • Correct method proper orientation of tissue- 4 Marks • Labeling- 2 Marks
FAQs	1. What is embedding? 2. How embedding is done?
Assignment	• Prepare block from given tissue. • Visit to histotechniques laboratory.
Reference	Chapter 6 Hand book of Histotechnology

Week no.	
Practical No.	**8**
Title/ Aim	To identify different parts of rotary microtome and study their functions.
Objectives	Students shall be able to identify different parts of microtome.
Principle	Microtome is an instrument used for obtaining thin sections of tissue for microscopy.
Requirements	1. Rotary Microtome
Environment	MLT laboratory
Procedure	1. Observe the microtome. 2. Study various parts of microtome. 3. Place the knife in the knife holder. 4. Place the block in the block holder. 5. Turn the wheel.

Observations -

1. The tissue block is moved up & down in a flat plane across the knife edge.
2. The movement is effected by the turning of a wheel.
3. The block moves forward.

1- knife holder 4- knife carrier base

2- knife clamp screw 5- Tilt adjustment screw

3- knife adjustment screw 6- Block holder

6-7-8-9- Block adjustment screws

10- Operating wheel 11-Thickness gauge

12- Operating wheel lock

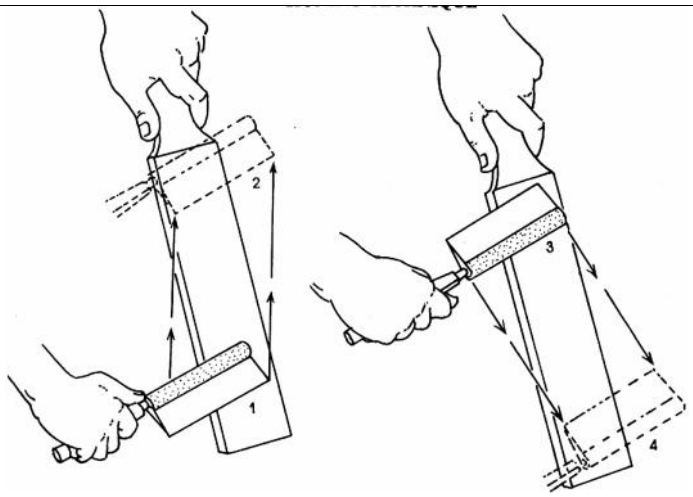
Parts of microtome

- Block holder-in this tissue holds the position.
- Knife carrier& knife-it holds knife.
- Knife clamp screw-to adjusts the knife.
- Thickness gauge-to adjusts thickness. It varies from 1 to60 micron.
- Block adjustment screw-to adjust the block.
- Angle of tilt adjustment-to adjust angle of knife
- Operating wheel-to rotate the block in forward &backward direction.

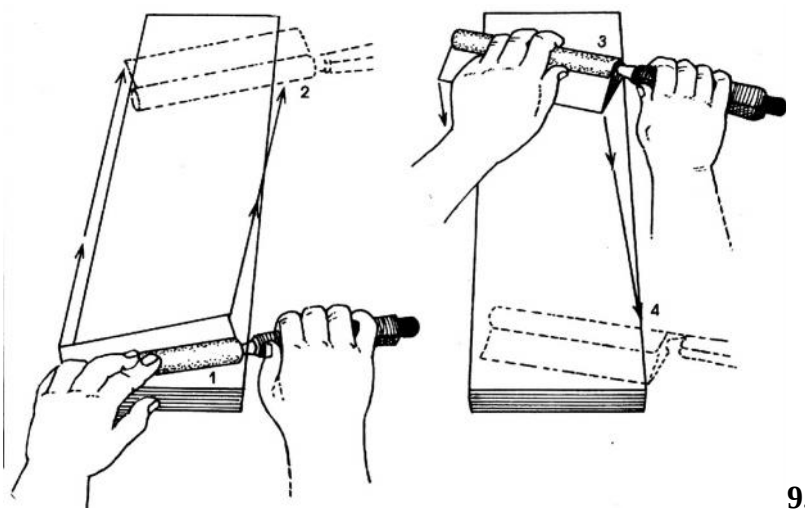
Result	Different parts are identified.
Clinical significance	Rotary microtome is used to cut thin sections of tissue for microscopic examination.
Skills to be achieved	Students can identify different parts of microtome.
Skill evaluating criteria	• Proper identification of microtome parts- 6 Marks • Proper adjustment and operation of microtome according to tissue- 4 Marks
FAQs	1. What is microtome? 2. Name different parts of microtome and state their uses.
Assignment	• Observe different parts of microtome. • Visit to histotechniques laboratory..
Reference	Chapter 7 Section Cutting -Hand book of Histotechnology

Week no.	
Practical No.	**9**
Title/ Aim	To sharpen the given microtome knife.
Objectives	The student shall be able to do sharpening (honing and stropping) of microtome knife.
Principle **9.1** **9.2**	 **Honing** is a process to remove all nicks and sharp pieces of metal and make the edge straight and sharp. The knife is moved on hard surface. It is known as hone (Sharpening stone). **Stropping** is the process of polishing the knife in order to remove buff formed during honing. It is carried out on a softer surface such as leather.
Requirements **9.1** **9.2**	 1. A microtome knife 2. Hone 3. Honing back 4. Lubricating oil 1. A microtome knife which has undergone honing 2. Strop
Environment	MLT laboratory
Procedure **9.1**	**Honing** 1. Clean the knife with soft cloth. 2. Put the proper honing back. 3. Keep hone on a bench of suitable height. Keep a damp cloth under the hone to prevent it from slipping. 4. Clean the surface of hone and lubricate with oil or soapy water.

9.2	5. Place the knife at one end of hone with the edge facing away from operator. 6. Push the knife diagonally forward with cutting edge leading so that whole edge is equally ground by giving light but sufficient pressure. This is required to maintain edge of knife in contact with hone. 7. Just before the edge reaches the end of hone, turn over the knife on its back without lifting it. Then pull back the knife with cutting edge along the hone in a diagonal fashion. This is **Heel to Toe** movement. 8. Examine the knife for its sharpness. **Stropping** 1. Thoroughly clean the knife and dry it completely. 2. Place the rigid strop on the bench or if hanging strop is there, it is pulled taut. 3. Place the knife at one end of strop and polish it in a direction opposite to honing by giving sufficient pressure. This is **Toe to Heel** Movement. 4. Examine the knife for its sharpness. 5. Knives should be thoroughly cleaned after honing and stropping.
Observations	



9.2-Stropping



9.1-Honing

Result	Knife is sharpened by honing and stropping.
Clinical significance	Properly sharpened knife easily cut the tissue of required thickness (5µ).
Skills to be achieved	Students can sharp the microtome knife perfectly.
Skill evaluating criteria	**1. Honing** ➢ Correct technique- 3 Marks ➢ Assessment of sharpness- 2 Marks **2. Stropping** ➢ Correct technique- 3 Marks ➢ Proper assessment of sharpness of knife- 2 marks

FAQs	1. What is honing/ stropping? 2. What are indications? 3. How it is carried out?
Assignment	• Sharp the given microtome knife. • Visit to histotechniques laboratory.
Reference	Chapter 7 Hand book of Histotechnology

Week no.	
Practical No.	**10**
Title/ Aim	To Prepare Mayer's egg albumin- an adhesive mixture.
Objective	At the end of practical students shall be able to prepare Mayer's egg albumin.
Principle	An adhesive is used to keep the section firmly attached to the slide so that it will not get washed away during staining.
Requirements	1. Egg white 2. Glycerol 3. Sodium salicylate
Environment	MLT laboratory
Procedure	1. Separate the whites from the yolks of fresh chicken eggs. Discard the yolks. 2. Beat the egg white until they are homogenous but still fluid. Add the glycerol to the egg white and mix well. 3. Add the sodium salicylate and mix well to dissolve. 4. Filter through a few layers of gauze to remove any membranes *etc*. 5. Keep a small quantity in a capped container for use. (Blood bank antisera bottles are suitable) 6. Refrigerate the stock. (Egg albumen adhesives are in common use. To inhibit bacterial and mold growth, a small crystal of thymol can be used instead of sodium salicylate,as a preservative.)
Observations	Mayer's egg albumin composition • Egg white 50 ml • Glycerol 50ml

	• Sodium salicylate 1gm
Result	Mayer's egg albumin is prepared.
Clinical significance	Mayer's egg albumin is commonly used adhesive for all the tissues to be taken on slide before staining to keep them attached to slide.
Skills to be achieved	Students can prepare Mayer's egg albumin and can take the section on slide after microtomy.
Skill evaluating criteria	• Separation of white of egg- 2 Marks • Exact quantity of constituents- 5 Marks • Preservation and labeling of an adhesive- 3 Marks.
FAQs	1. Give composition of Mayer's egg albumin.
Assignment	Prepare an adhesive- Mayer's egg albumin.
Reference	Chapter 7 Section Cutting- Hand book of Histotechnology

Week no.	
Practical No.	**11**
Title/ Aim	To obtain thin sections of properly processed tissue using microtome.
Objective	At the end of practical students shall be able to cut the given wax impregnated tissue and can mount it on slide.
Principle	Microtomy is the process of cutting thin sections of tissue with the help of microtome.
Requirements	1. Properly processed and embedded tissue (Paraffin block) 2. Suitable microtome in good condition 3. A sharp knife
Environment	MLT laboratory
Procedure	**1. Attaching paraffin blocks to microtome-** • Attach paraffin block properly to microtome head by using object holder or carrier. • Allow the paraffin wax to melt on the back of the block, fix it to object carrier and clamp it to microtome head. • First fix the knife in knife clamp and draw the tissue block across the knife edge. Position the knife edge. **2. Orientation of block-** • Now orient the block with the help of screws provided, which help to change the block position as per the requirement. • The orientation should be such that the top and bottom of the block are parallel and horizontal to the edge of the knife at the moment of impact. • The block face should be as small as possible. • There should be 3mm of paraffin around the tissue.

	Somewhat more paraffin should be available at the back of block. **3. Trimming-** It is the removal of surplus wax present in front of tissue and exposure of complete surface area of specimen. • After attaching and orienting the block, set the gauze controlling the thickness of a section to 15µm. • Use the extreme ends of knife or another knife to trim the block. • Trim the block. • Once the surface is exposed stop the trimming. **4. Cutting the sections-** In properly trimmed block face, the top and bottom edges will be parallel; the block face touches the knife. • Now replace the old knife with one which is reserved for section cutting. • Adjust the thickness to 5µ. • Raise the object just short of knife and begin the cutting. • Maintain a regular cutting rhythm. • With the act of cutting sections lie on knife. A ribbon of section is produced. • When a ribbon of 10-15 cm is formed, stop cutting and take the sections on slide. **5. Attaching section to microscope slides-** • After obtaining a ribbon of section, grasp the first section with a pair of fine forceps held in the left hand. A small camel hair paint brush held in the right

	hand can be used to brush away the last section from the knife. • Lay them on a piece of black paper before mounting on a slide. • Immerse slides in 1% HCl in 70% alcohol and clean it with fluff free cloth. • Apply an adhesive mixture thinly on a slide before taking section on a slide. • Strip of individual sections are laid on surface of warm water taken in a beaker, the temperature of water should be 5-10^0 below melting point of wax. • The sections will float and expand slightly and become flatten. Remove wrinkles by gentle stretching. • Dip the slide obliquely in a beaker. • Draw the section towards slide with forceps or needle. • As the selected edge of section touches slide, remove the slide along with section with upward and forward movement. • Keep the slide in upright position to drain. • Allow the slide to dry at room temperature but if quick drying is required it is done in an oven at 45-50^0C for 2 hours or more. Then slide is ready for staining.
Observations	

Fig. 11.1 Ribbon of sections

Result	A ribbon of section is obtained.
Clinical significance	Thin sections cut on microtome are used for histopathological diagnosis.
Skills to be achieved	Students can cut thin sections of tissue on microtome and take the section on slide.
Skill evaluating criteria	• Proper attachment of tissue block to microtome head- 2 Marks • Orientation of block in relation with edge of knife- 3 Marks • Thickness selection- 1 Mark • Maintenance of regular cutting rhythm- 1 Mark • Taking section on slide- 3 Marks
FAQs	1. What is microtomy? 2. What is trimming? 3. How sections are taken on slide?
Assignment	Cut the 10 different tissues embedded in wax on rotary microtome.
Reference	Chapter 7 Section cutting- Hand book of Histotechnology

Week no.	
Practical No.	**12**
Title/ Aim	To stain the tissue with Haematoxylene and Eosin.
Objectives	At the end of practical Students shall be able to do haematoxylin and eosin staining of the given tissue section.
Principle	Haematoxylin and Eosin are the principal stains used for demonstration of nucleus and cytoplasmic inclusions. Alum acts as a mordant. Haematoxylene containing alum stains nucleus. Eosin acts as counter stain which imparts pink color to the cytoplasm.
Requirements **Reagents**	1. Coplin jars with lid - holds 5-10 slides or small jars which can hold single slide 2. Staining racks –Slotted racks with handles 3. Unstained section 4. Cover slips 5. A Bunsen burner, 37^0C incubator, 60^0C oven 6. Polythene wash bottles for distilled water and reagents. **Harris's Alum Haematoxylin –** Haematoxylin crystals 1gm Absolute alcohol 95% 10 ml Ammonium or potassium alum 20 gm Distilled water 200 ml Mercuric oxide 0.5 gm Dissolve haematoxylin in 95% alcohol and alum in water. Mix haematoxylene solution with alum solution while alum solution is hot. Bring it to boil. Add mercuric oxide and cool rapidly in bath with running tap water or

	in ice cold water. **Eosin** Eosin Y (Water soluble) 1gm Distilled water 80ml 95% alcohol 320ml Glacial acetic acid 0.4ml Dissolve eosin in water and then add this to 95% alcohol (1 part of eosin solution with 4 parts of alcohol). To the final mixture add 0.4ml of Glacial acetic acid. When ready to use the stain should be cloudy. **Dilute aq. HCl (0.5%)** Conc. hydrochloric acid- 2.5ml Dist. Water 500ml **Dil. Ammonia water** Strong Ammonia 1.5ml Distilled water 500ml • Xylene • Different grades of alcohol. • DPX mounting media
Environment	MLT laboratory

Procedure

Prestaining steps

1. Place the slide in a incubator or hot plate for at least 1 hour to get the section thoroughly dried (50^{o}c) (Drying)
2. Treat the section immediately with xylene (5 minutes each). (Deparaffinisation)
3. Take the section to water through descending grades of alcohol (100%, 90%, 80%, 70%, water) 30-60 seconds each. (Hydration)
4. Wash in tap water, rinse in distilled water drain well.

Staining

5. Stain with Harris haematoxylin for three to five minutes. (Staining)
6. Wash in running tap water.

7. Quickly dip slide in and out of 0.5%HCl. Good differentiation is present when the nuclei are dark purple and the rest of the section is very pale if nuclei are not dark enough rinse in distilled water and repeat the staining. (Differentiation)
8. Wash briefly in water (30 to 60 seconds).
9. Dip several times in dilute ammonia water or lithium carbonate; the section will change to a blue color. (Bluing)
10. Wash in water.
11. Rinse in 95% alcohol.
12. Agitate in eosin staining solution for 10 to 60 seconds. (Counter staining)
13. Drain the stain.

Post staining

14. Place in 70% alcohol - 30 to 60 seconds.
15. Place in 95% alcohol - 30 to 60 seconds.
16. Place in absolute alcohol 2 changes - 30 to 60 seconds each. (Dehydration)
17. Clear in xylene 2 changes - 30 to 60seconds in each. (Clearing)
18. Allow the excess xylene to drain.

Mounting

19. Take a cover slip and put a drop of DPX on it.
20. The cover slip with DPX is gently lowered on the section.
21. DPX spreads evenly. The cover slip is slightly pressed to remove the air bubbles.
22. Excess DPX around the cover slip is wiped off with gauze soaked in xylene.
23. Label the slide.
24. Keep slide at RT for firm adhesion between section and cover slip.

Observations

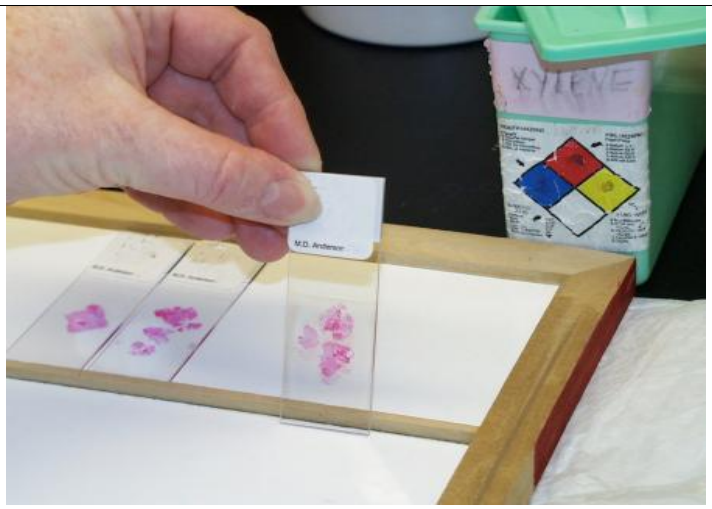


Fig. 12.1 H and E stained section

Result	Cell nuclei blue Erythrocytes red Muscle and connective tissue and cytoplasm are in varying shades of pink.
Clinical significance	• Unstained sections are of little use to study the internal structure of tissues because cellular components cannot be differentiated clearly. • Staining helps to study the physical characteristics and relationships of tissues and their constituent cells. • Haematoxylene and Eosin is the standard stain used to examine various types' tissues for histopathological diagnosis.
Skills to be achieved	1. Students can stain the tissue using H and E staining method. 2. Students can prepare various reagents used in staining process.
Skill evaluating criteria	• Prestaining technique- 3 Marks • Staining- 3 Marks • Post staining- 2 Marks

	• Mounting- 2 Marks
FAQs	1. Give composition of haematoxylene and eosin. 2. Give significance of permanent mounting of stained section. 3. Describe various steps of H & E staining.
Assignment	1. Perform H and E staining of 10 different given tissues. 2. Visit to histotechnique laboratory.
Reference	Chapter 9 Staining- Hand book of Histotechnology

Week no.	
Practical No.	**13**
Title/ Aim	**Exfoliative cytology** To prepare smear for cytology
Objective	Students should prepare smear from different samples sent for cytological examination.
Principle	The technical quality of the smear for cytological investigations determines the accurate evaluation and diagnosis.
Requirements	1. Glass slides 2. Wire loop or an applicator stick 3. Centrifuge 4. Pooled serum or plasma 5. Different samples like urine, sputum. Vaginal smear, cervical smear, gastric contents,etc
Environment	MLT laboratory

Procedure

1. Preparation of specimen

- Specimens like cervical smear or breast secretions are examined unconcentrated by making direct smears.
- Watery specimens like urine, serous fluids require concentration before preparation of smear. It is carried out as

1) <u>Thick specimen</u>
   a. Mix equal quantities of specimen and 95% alcohol.
   b. Centrifuge at 3000 RPM for 20 minutes.
   c. Prepare smear from sediment.
2) <u>Watery specimen</u>
   a. Transfer the specimen in a centrifuge tube.
   b. Add serum or pooled plasma (quantity greater than specimen).
   c. Centrifuge at 3000 rpm for 20 minutes.
   d. Prepare smear from sediment.

2. Preparation of smear

- Depending on type of specimen the smear is prepared as follows-

I. Streaking (For sputum, vaginal secretions, gastric contents)

a. By using wire loop or an applicator stick transfer the fixed specimen or sediment on a clean and prelabeled slide.
b. Prepare smear by spreading specimen uniformly covering about 2/3 area of the slide.
c. Keep smear in a fixative immediately.

II. Spreading (For sputum, bronchial aspirate)

a. Transfer selected portion of specimen to a clean glass slide.
b. Remove excess material with an applicator stick.
c. Spread the mucoid secretion using teasing process.
d. The film should be moderately thick.

III. Pull-apart (For urinary sediment, breast secretions, serous fluids)

a. Place a drop of specimen in the middle of the clean glass slide.
b. Place another slide over this drop holding the edges of the both slides.
c. The material will disperse evenly over the surfaces of the two slides by slight movement of two slides in opposite direction to initiate the flow of the material.
d. The two slides are then pulled apart with a single uninterrupted motion.
e. Place them immediately in the fixative.

Observations- The technique of smear preparation

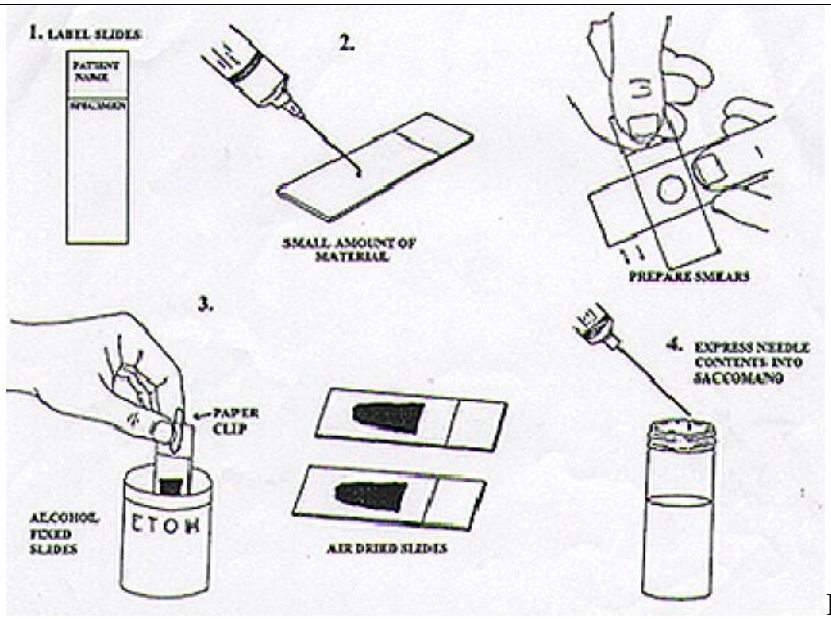


Fig 13.1

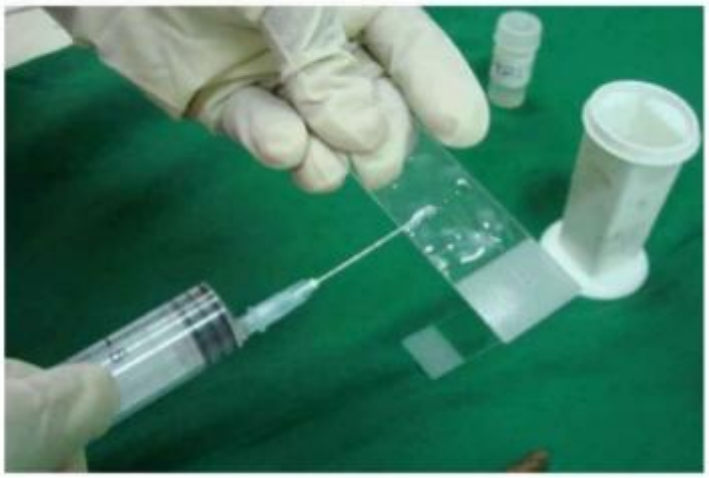


Fig 13.2

Clinical significance	Proper preparation and wet fixing of smear is the foundation of cytology techniques.
Skills to be achieved	Students can prepare smear for cytology as per the type of specimen perfectly.
Skill evaluating criteria	• Specimen registration- 2 Marks • Preparation of specimen- 4 Marks • Choice of method and smear preparation- 4 Marks
FAQs	1. What is exfoliative cytology? 2. Name different samples received in cytology laboratory.

	3. What are different methods of preparation of smears for cytological examination?
Assignment	Visit cytology laboratory.
Reference	Chapter 10 Exfoliative cytology Hand book of Histotechnology

Week no.	
Practical No.	**14**
Title/ Aim	To prepare cytological fixatives- Ether Alcohol
Objective	Student shall understand preparation of ether- alcohol a good cytological fixative.
Requirements	Absolute ethyl alcohol Ether
Environment	MLT laboratory
Procedure	1. Mix the equal quantities of ether and alcohol. 2. Place it in a jar with a tight stopper. 3. Fix smear for 30 minutes or more, rinse in alcohol, then distilled water and then do staining.
Observations	Composition of fixative • Absolute ethyl alcohol 50ml • Ether 50ml
Clinical significance	Ether alcohol is mainly used with the Papanicolaou staining method but also for other types of cytology smears.
Skills to be achieved	Proper preparation of fixative.
Skill evaluating criteria	• Correct quantity- 5 Marks • Preparation and labeling- 5 Marks
FAQs	1. Name different cytological fixatives. 2. Give composition of ether alcohol.
Reference	Chapter 10 Exfoliative cytology- Hand book of Histotechnology

Week no.	
Practical No.	**15**
Title/ Aim	To stain smear with Papanicolaou stain.
Objective	Students shall perform Papanicolaou staining of given smear.
Principle	Pap method is designed to give sharp nuclear staining, transparency of cytoplasm and good differential coloring of acidophilic & basophilic cells.

Requirements

1) Coplin jars with lids - holds 5-10 slides or small jars which can hold single slide
2) Staining racks –Slotted racks with handles
3) Fixed smear
4) Cover slips

Reagents

Harris's Alum Hematoxylin –

- Haematoxylin crystals 1gm
- Absolute alcohol 95% 10 ml
- Ammonium or potassium alum 20 gm
- Distilled water 200 ml
- Mercuric oxide 0.5 gm

Dissolve haematoxylin in 95% alcohol and alum in water. Mix haematoxylene solution with alum solution while alum solution is hot. Bring it to boil. Add mercuric oxide and cool rapidly in bath with running tap water or in ice cold water.

Orange G

- Orange G stock solution
  (0.5% solution in ethyl alcohol) 100ml
- Phosphotungstic acid 0.015gm

Polychrome stain EA 36

- Light green SF (yellowish)
  (0.1% Solution in 95% alcohol) 45ml

- Bismark brown
  (0.5% solution in 95% alcohol) 10ml
- Eosin yellowish
  (0.5% solution in 95% alcohol) 45ml
- Phosphotungstic acid 0.2gm
- Lithium carbonate
  (saturated aqueous solution) 1drop

Fig. 15.1

Environment	MLT laboratory

Procedure

1. Remove slides from fixing jar.
2. Pass through descending grades of alcohol to water 6 dips each
   (Hydration of smear)
3. Rinse in distilled water.
4. Stain with Harris's haematoxylene 2-3 minutes
5. Rinse in tap water.
6. Differentiate in 0.25% aq. HCl
   (Until the cytoplasm is decolorized and nuclei retain the stain).
7. Rinse in tap water.

8. Blue in ammoniated water.
   (Until the desired staining density is reached).
9. Wash in running tap water 5 minutes
10. Pass through ascending grades of alcohol
    (Complete dehydration).
11. Stain in OG 6 2 minutes
12. Rinse in two changes of 95% alcohol.
13. Stain in EA 36 2-4 minutes
    (Until the desired intensity of color has been reached).
13. Rinse in two changes of 95% alcohol.
14. Pass through 100% alcohol.
15. Clear in xylol (3changes).
16. Mount and examine under microscope.

Observations

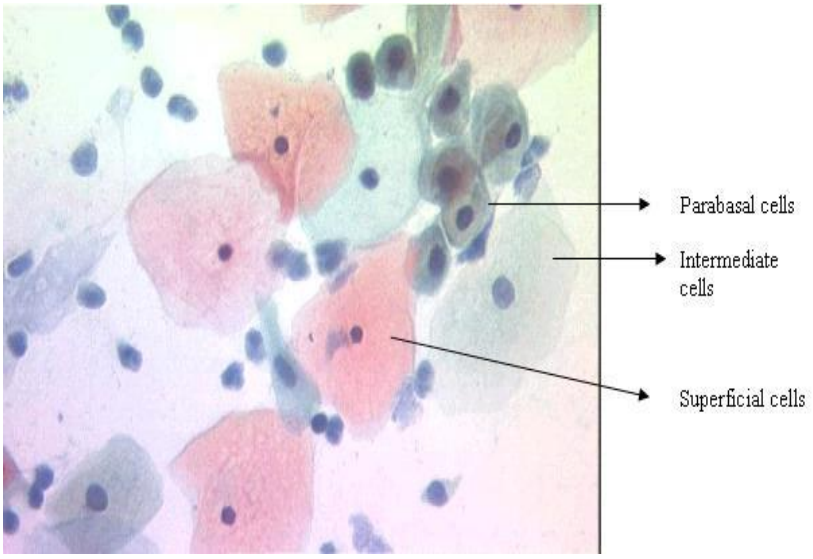


fig 15.2

Result	Nuclei Dark blue Cytoplasm of superficial cells pink Cytoplasm of intermediate cells pale green Cytoplasm of parabasal cells deep green Red blood cells Bright red Leucocytes- Pale blue with dark blue-black nuclei

	Bacteria Grey Mucus pale blue or pinkish strands
Clinical significance	• This is the most commonly used staining procedure in cytopathology laboratory. • It is devised for the optimal visualization of cells exfoliated from epithelial surfaces of the body. • It is a popular method for the cytological examination of smears from female genital tract.
Skills to be achieved	1. Students can stain the tissue using Papanicolaou staining method. 2. Students can prepare various reagents used in staining process.
Skill evaluating criteria	• Proper fixation- 2 Marks • Proper hydration technique- 2 Marks • Staining steps- 4 Marks • Mounting- 2 Marks
FAQs	1. Give composition of haematoxylene, OG 6 and EA 36. 2. Describe various steps of pap staining.
Assignment	Stain 10 different smears with Pap stain.
Reference	Chapter 10 Exfoliative cytology Hand book of Histotechnology