Microtomy: Preparation of permanent slides of Goat/Fish tissues

For studying histological, histopathological and histochemical details of the organs and tissues thin paraffin sections are cut with a **Microtome**. The process is called as **Microtomy**. For proper diagnosis of certain diseases, microtomy is very helpful. Rather some diseases can only be diagnosed properly if thin well-stained paraffin sections are available. Most of the biopsy tests (examination of tissues) can be performed through **microtomy**. By this method tissue is made fit for microscopic examination.

Microtomy includes the following steps :

(1) Animals and tissues for microtomy.
(2) Narcotisation of animals.
(3) Fixing of tissues.
(4) Washing of tissues.
(5) Dehydration of tissues.
(6) Clearing or dealcoholization of tissues.
(7) Embedding of tissues.
(8) Block making.
(9) Triming of blocks.
(10) Section cutting.
(11) Double staining, dehydration, clearing and mounting.
(12) Microscopic study.
(13) Microphotography (if desired).

1. Animals and tissues for microtomy. Frog, black rats and white or albino rats are favourite animals for microtomy. Various tissues of these animals are used for histological studies. Earthworms can also be used to cut sections in order to study invertebrate histology.

2. Narcotisation of animals. Take a medium size frog. Chloroform it in a large jar. Wet some cotton in chloroform and put in the jar containing frog. Close the mouth of jar with glass cover. After 15 to 20 minutes, when fully anaesthetized, take out the frog and keep it in a dissecting tray. Make a longitudinal incision in abdomen to expose viscera.

3. Fixing of tissues. Take cleaned 100 c.c. wide-mouthed glass-stoppered bottles. In each bottle keep about 50 cc of aqueous Bouin's fluid. Now take out liver, lung, tongue, stomach and intestine and keep them in separate bottles for 30 minutes. From each bottle, after half an hour, make 2 to 3 cm rectangular pieces of each organ, and again keep them in their respective bottles for 24 hours.

The above tissues can be fixed in alcoholic Bouin's fluid also. Mostly for research purposes, tissues are fixed in alcoholic Bouin's fluid.

It is very essential to fix the tissues in fixative agent. Fixation serves 3 functions :

(1) Fixative renders **hardness** to tissues to resist further **postmortem changes**.
(2) Fixative agent **coagulates and renders** the elements of tissues insoluble so that cellular substances may not be washed away.
(3) Fixative agent alters the **refractive indices** of tissues and makes them **optically differentiated** under the microscope.

4. Washing of tissues. After 24 hours take out the pieces of tissues and keep in a beaker. Tie the mouth of the beaker with a thin cloth and keep it under slow running tap water. Keep on washing under tap water till all the picric acid is removed. The indication of complete removal of picric acid comes when no yellowish water is seen. Normally, it takes 24 hours for perfect washing. Tissues fixed in aqueous Bouin's fluid are washed with tap water. While those fixed in alcoholic Bouin's fluid are washed with 70% alcohol. While washing with 70% alcohol, change it frequently till the yellow colour disappears.

5. Dehydration of tissues. Take one or two pieces of any organ in a staining tube and dehydrate through distilled water →30% alcohol →50% alcohol →70% alcohol →90% alcohol →100% alcohol. In each grade of alcohol keep tissues for 5 minutes with 2 changes. Dehydration removes water to prevent putrefaction. The graded alcohol gradually replaces water in tissues.

6. Dealcoholization or clearing of tissues. Clearing or dealcoholizing agents are **cedar wood oil** or **xylene**. Although cedar wood oil is better but because of its high cost, xylene is used.

Removal of alcohol from tissues is done through clearing agent. Take xylene in a staining tube and transfer tissue in it from 100% alcohol. Keep in xylene for 15 minutes till the tissue becomes transparent. Don't leave tissue in xylene for longer period otherwise it would become fragile. Now tissues are ready for embedding.

7. Embedding of tissues. Depending on melting point of wax, adjust the oven at 58°C or 60°C. Take wax with ceresin instead of plain wax. Keep flakes of wax in a beaker of 100 cc in oven 4 to 5 hours before embedding. In another beaker keep some wax plus xylene. Now take the tissue from xylene and first keep it in the beaker containing xylene + wax for 30 minutes. Then transfer the tissue in pure melted wax for embedding for 1 to 2 hours. Normally double time is given for embedding than the time required by tissue to sink at bottom in xylene.

8. Block making. Make blocks either in metal L-shaped angles or in paper boat or in cavity blocks. 'L' pieces are preferred. First apply little glycerine on their internal surface. Pour melted wax at bottom of rectangular cavity formed by two 'L' pieces. Then add tissue and more melted wax to fully cover the tissue. Keep L pieces in a trough. Add water around them. As the melted wax is solidified, flood it with tap water. After cooling, the block comes out from 'L' pieces or remove L pieces. Because of glycerine 'L' pieces don't stick with wax. During block-making, see that no air bubble comes. If there are some air bubbles remove them with hot spatula.

9. Trimming of blocks. Trim the wax around the embedded material and make a perfect rectangular block. On one side keep sufficient space in the block for fixing it on a block holder. Apply half an inch wax layer over block holder.

10. Section cutting. The blocks are cut either by **rocking microtome** or **rotatory microtome** at 6 microns (μ) thickness. Ribbons are kept over Mayer's albumen coated slides. Keep clean slides ready. Apply pin head Mayer's albumen over the slide and rub it by last finger. Mayer's albumen helps in sticking the sections over glass surface. Keep 2 to 4 rows of sections depending upon the breadth of the sections. Ribbons should be kept upto more than half of the slide. Space should be left for putting labels over the slide. Flatten the section over a hot brass plate. The temperature of the hot plate should be nearly 40-45 degree. Add few drops of water below ribbons. As the water is heated ribbons become expanded by semi-melting of the wax. Sections should not be separated. Never do flattening over spirit lamp as in most of the cases wax melts and sections are burnt. After all the sections and ribbons become flattened, drain off water and leave the slide at room temperature overnight for drying the ribbons. Mark the ribbon side by glass marking pencil.

11. Double staining, dehydration, clearing and mounting. Double staining is applied. Haematoxylin

and eosin stain nucleus and cytoplasm of the cells respectively. Take individual slide and first keep it in xylol to remove wax for 5-10 minutes. Wax is dissolved in xylene and sections are left free. Now pass the slide in **descending series** of alcohols 100% →90% →70% →50% →30% →distilled water. After rinsing in distilled water stain the slide in **haematoxylin** for 2-5 minutes. Take out side and again dip in distilled water. Then immerse the slide in a beaker containing tap water. The sections turn blue because of alkalinity of water. Observe the slide under microscope. If stain is dark, then immerse the slide in acid water and quickly immerse in tap water. Now dehydrate the slide through **ascending series** of 30, 50, 70 and 90% alcohols. After 90%, immerse the slide in alcoholic eosin for 30 seconds. Wash eosin in 90% alcohol. Then keep slide in 100% alcohol for 5 minutes and then in xylol for 15 minutes. Mount the slide in D.P.X. Keep ready the following descending and ascending series of stains and grades of alcohols in separate coupling jars.

12. Microscopic study. A good stained slide reveals pinkish colour of cytoplasm and blue colour of nuclei.

13. Microphotography. The slides meant for research may be microphotographed. For microphotography slide must be nicely stained.

Descending series

Slide
↓
Xylene
↓
100% alcohol
↓
90% alcohol
↓
70% alcohol
↓
50% alcohol
↓
30% alcohol
↓
Distilled water
↓
Ascending series

Ascending series

Distilled water
↓
Haematoxylin
↓
Distilled water
↓
Tap water
↓
Acid water
↓
30% alcohol
↓
50% alcohol
↓
70% alcohol
↓
90% alcohol
↓
alcoholiceosin
↓
90% alcohol
↓
100% alcohol
↓
Xylene
↓
Mount in D.P.X.