

CHAPTER III

FIXATION

The term "Fixation" means to immobilize. The chief object of fixation is to immobilize the cell structures, while maintaining their morphological identity. The tissue is fixed after being taken out from the surgical specimens, fresh or dead, to avoid rapidly setting postmortem changes. Fixatives are the agents that prevent autolysis. Fixation renders proteins of the tissue insoluble and converts living or fixed natural jelly-like condition of the cell into fine granular spongy mass. Fats and carbohydrates may not be leached out during this process. How they are affected, depends upon the composition of the fixing fluid. The change that occurs during the fixation is denaturation of proteins, meaning that although the proteins are still proteins, they have been made unnatural. Natural proteins occur in the colloidal state in the living system. They tend to be natural even after the death or after removal from the living system. Denaturation changes the texture and reactivity of the tissue. Thus the cell membrane, which before fixation shows ability to regulate water in the cell contents, loses this property and hence becomes insensitive to variations of the osmotic pressure. Unfixed cells swell and burst when put in water. Fixed tissues can be washed for a long time without any change.

The immobilization and denaturation of the proteins in a tissue are done in two ways:—

- (1) Fixation by physical agents.
- (2) Fixation by chemical agents.

1. Fixation by physical agents

This process involves use of heat, drying at room temperature and drying at low temperature (Freezing-drying or cryo-desiccation) and techniques in which cooling of objects is followed by the use of liquid fixatives. Of these in the physical fixation-agents, the use of heat is the only method that ensures

fixation.

genuine fixation, that is, denaturation of proteins in a cell. The other methods involve the use of chemical agents for denaturation and hence cannot be strictly called as physical agents of fixation.

2. Fixation by chemical agents

The fixation agent used for fixation of tissues chemically should possess the following properties:—

- (a) It should stop all the metabolic activities in a cell.
- (b) It should coagulate the cell contents.
- (c) It should conserve the cytological and histological architecture.
- (d) It should conserve the original form of tissue.
- (e) It should support the tissue by hardening.
- (f) It should prevent desiccation and putrefaction in the tissue.
- (g) It should impart selective staining by dyes to the different parts of the cell.

There is no single fixation agent, which would satisfy all the above mentioned criteria. Some fixatives may preserve one element and dissolve the other or may interfere during the staining procedures. The following are some of the important fixation-chemicals, that are commonly used as ingredients of the fixatives.

- (1) Methanol, ethanol, acetone.
- (2) Hydrochloric acid, nitric acid and sulphuric acid.
- (3) Trichloroacetic acid.
- (4) Picric acid and chloroplatinic acid.
- (5) Mercuric acid.
- (6) Acetic acid.
- (7) Chromium trioxide.
- (8) Osmium tetroxide.
- (9) Formaldehyde.
- (10) Other aldehydes.
- (11) Potassium dichromate.

Fixing agents commonly used alone are called as simple fixatives, e.g., formalin, methanol, ethanol are used without an addition of other compounds.

The other types of fixatives are the mixtures of several fixing agents in a liquid form. Such fixatives are called compound

These compound fixatives include a great majority of those that are used in practice. The chemical compatibility, or incompatibility of the fixing agents that constitute a mixture, is important while choosing the fixative, for specific purpose.

Besides chemical compatibility or compatibility, nature of the solvents, frequency of their use, acid solutions, rate of penetration, thickness of the tissue are to be considered, while choosing a suitable fixative (Refer the table). It is necessary to stop fixation after definite period. This time is not only fixed by the above mentioned factors but also by the chemicals used in the fixation mixture. Thus at cellular level, fixation by osmium tetroxide is instantaneous. Prolonged period is advised if further reaction is needed. Formaldehyde has opposite effect. It is much slower in penetration and thus prolonged contact will improve the fixation of the tissue.

Modes of fixation

- (1) Fixation by vapours.
- (2) Fixation by liquids.
 - (a) Fixation by immersion.
 - (b) Fixation by injection into body cavity.
 - (c) Fixation by perfusion.

Fixatives are classified as:—

- (1) Simple fixatives.
- (2) Common fixatives.
- (3) Other fixatives.

Simple fixatives

- (1) Formalin
- (2) Ethyl alcohol
- (3) Acetone
- (4) Formalin acetic acid (FAA)

(1) Formalin (Formaldehyde):—It is a well known non-irritant, obtained commercially in an aqueous solution as formal (which is a formalin containing 35% to 40% of native formaldehyde). It is diluted generally to make 4% formaldehyde. (1 Vol. formalin and 9 Vols water).

The tissue may remain in this fixative indefinitely. This fixative is prepared as under:—

Commercial formalin (35% to 40%)	= 10 ml.
Distilled water	= 90 ml.

Osmium tetroxide

It is miscible with water. It is a reducing agent. It has no staining effect. It has great hardening effect.

*(2) Dehydrating agent
(3) boiling Point 78.3
(4) In formalin
(5) reducing agent
(6) fixative
(7) fixative
(8) fixative*

- (1) Ethyl Alcohol (Period of fixation 12 to 24 hours)

Ethyl alcohol	= 75 ml.
Distilled water	= 25 ml.

Absolute alcohol (Ethyl): This is a good fixative for enzyme histochemistry, such as alkaline phosphatase, and also for glycogen, but does not fix lipids and carbohydrates.

(8) if it is miscible with water

- (c) Acetone: (Period of fixation 1 to 3 days).

Cold acetone at 5°C is useful as a fixative for lipase and acid phosphatase. Dilute acetone is useful as a fixative for lipids. It has slow penetrative action and hence it should be used to fix small pieces of tissue.

- (d) Formalin acetic acid (Period of fixation 1 to 24 hours)

This is a good fixative for parasitological material such as intestinal worms, and also for insects and crustaceans. After the period of fixation, transfer the material to 90% alcohol directly.

70% alcohol	= 90 ml.
Commercial formalin	= 8 ml.
Glacial acetic acid	= 2 ml.

Common fixatives

- (1) Aqueous Bouin's fluid (Period of fixation 12 to 24 hours)

Saturated solution of picric acid	= 75 ml.
Formaldehyde	= 20 ml.
Acetic acid	= 5 ml.

- (2) Alcoholic Bouin's fluid (Period of fixation 1 to 3 days)

Picric acid	= 1 gram
Formaldehyde	= 60 ml.
Alcohol 80%	= 150 ml.
Acetic acid	= 15 ml (to be added at the time of use)

The tissue should be fixed in freshly prepared fixative and after the period of fixation, it should be transferred directly to

And make the home distilled by using sulphuric acid and water

(3) Allen's Bouin's fluid (Period of fixation 12 to 24 hours)	
Bouin's solution	= 10 ml.
Chromic acid crystals	= 1.5 grams
Urea crystals	= 2 grams

This fixative is commonly used for chromium fixation. In all laboratories, Bouin's fluid is widely used, as it is an excellent protein coagulant. The time of fixation with this solution depends upon the thickness of the tissue. It may range from 12 to 24 hours or more. After proper fixation, the tissue may be washed thoroughly in water or 50% or 70% alcohol. The yellow colour of Bouin's fluid must disappear prior to staining and it is removed during dehydration. If the colour does not disappear ordinarily, treat the material or the sections with 70% alcohol having few drops of lithium carbonate.

(4) Zenker's fluid (Period of fixation 8 to 24 hours).

Potassium dichromate	= 2.5 grams
Mercuric chloride	= 5 grams
Distilled water	= 100 ml
	(Stock solution)
Glacial acetic acid	= 5 ml (to be added just before use)

To prepare this fixative, dissolve first the salts of potassium dichromate and mercuric chloride in water and add glacial acetic acid just before use. Do not keep the tissue in the fixative for a long time. After fixation, wash the tissue in running water overnight.

(5) Helley's fixative (Period of fixation 24 hours).

This fixative is prepared from Zenker's stock solution.

Zenker's stock solution	= 100 ml
Commercial formalin 40%	= 0.5 ml. (added just before use)

With Zenker's or Helley's fixative, a precipitate of mercuric chloride is left in the tissue, which causes difficulties while staining and hence it must be removed. It is removed by treating

the cut sections with Lugol's Iodine soln. Lugol's Iodine soln is prepared as follows:

- (i) Lugol's Iodine (5%) Iodine in 1% aqueous potassium iodide.
 (ii) 5% aqueous soln of sodium metabisulphite.
 procedure:—(i) Place the hydrated sections in Lugol's iodine soln for 1 to 5 mts.
 (ii) Wash with tap water.
 (iii) Place these sections in 5% sodium metabisulphite soln till the sections become colourless.
 (iv) Wash the sections with tap water.

(6) Rossman's fluid (Period of fixation 6 to 48 hours).
 Saturated soln picric acid in absolute alcohol

	= 90 ml
Commercial formalin	= 10 ml
(7) <u>Formal sublimate acetic</u> (Lillie, 1958).	
Mercuric chloride	= 5 grams
Sodium acetate (anhydrous)	= 2 grams
Formalin 10%	= 100 ml

(Prepared from commercial formalin)

(8) Susa Fixative (Period of fixation 24 hours)

Saturated mercuric chloride soln in 6% sodium chloride soln	= 50 ml.
Trichloroacetic acid	= 2 ml.
Glacial acetic acid	= 4 ml.
Distilled water	= 30 ml.

This is a good substitute for Zenker's fluid, provided potassium dichromate is not required. It penetrates rapidly in tissues. After fixation, the tissue is directly put in 50% alcohol.

(9) <u>Regaud's fluid</u> (Fixation period 12 hours)	
3% potassium dichr. mate soln.	= 80 ml.
Commercial formalin.	= 20 ml.

These solns are to be mixed just before use. This is a good fixative for mitochondria and other cytoplasmic inclusions. Wash the material in running water overnight.

(10) Glison's fluid (Period of fixation 24 hours to several days)

Conc. nitric acid	= 2 ml.
Glacial acetic acid	= 4 ml.
Mercuric chloride	= 20 grams.
Ethyl alcohol	= 100 ml.
Distilled water	= 339 ml.

This is a good fixative for invertebrates. This does not harden the tissues. Wash the material directly in 50% alcohol.

(11) Formol Nitric acid (Period of fixation from 10 minutes to 1/2 an hour).

- (a) Formalin: 25 parts of 40% formalin
and 75 parts of D.W. = 3 parts
- (b) Nitric acid conc. 10% = 1 part

Mix 3 parts of (a) soln with 1 part of (b) soln. This a good fixative for chick embryos. Cut the egg shell and make a small window in it. Through the window, put this fixative by a dropper on the embryo.

Other fixatives

- (i) Preservation of lipids (Period of fixation 24 hours)
- | | |
|--------------------------|----------|
| Cadmium chloride | = 1 gram |
| Commercial formalin | = 10 ml. |
| Aqueous calcium chloride | = 10 ml. |
| Distilled water | = 80 ml. |

The material fixed in this fixative should be stained with Sudan Black B.

- (ii) Preservation of polysaccharides (Period of fixation 1-4 hours).

90% alcoholic saturated picric acid	= 85 ml.
Commercial formalin	= 10 ml.
Glacial acetic acid	= 5 ml.

After fixative, wash the material directly with 90% alcohol.

- (iii) Preservation of enzymes (Period of fixation 24 hours).

Cold absolute acetone at 5°C is good for most, but not for all enzymes. Fix the tissue for 24 hours and then wash it with absolute alcohol directly.

- (iv) Preservation of proteins (Period of fixation 24 to 72 hours).

All fixatives having mercuric chloride in solution are good for proteins. Neutral buffered formalin is best fixative for proteins.

Commercial formalin 40%	= 100 ml.
Distilled water	= 900 ml.
Sodium acid phosphate monohydrate	= 4 grams.
Sodium phosphate anhydrous	= 6.5 grams.

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CHAPTER IV

DEHYDRATION

After the period of fixation of tissue is over, the fixed-tissues have to be dehydrated to remove water from the tissues. Dehydration is a process of gradual or stepwise removal and replacement of water from the tissues, small specimens and other biological material by the graded dehydrating agents such as ethyl alcohol, cellosolve (ethylene glycol monoethyl ether), dioxane (diethylene dioxide) and isopropyl alcohol. We generally use the progressive higher grades of alcohol, prepared invariably from rectified spirit, for dehydrating the tissues. The grades used for dehydration are 30%, 50%, 70%, 90% and 100% (absolute alcohol). Progressive increase in the volume of rectified spirit and similar reduction in the volume of distilled water—the mixture of these two ingredients gives us the above grades of alcohol. At the end of this chapter, a table shows the method of preparation of the alcohol grades. The grades should be prepared accordingly and stored in fairly large bottles, for instance the clean empty bottle of rectified spirit, with percentage-labels thereupon. These bottles must always be closed either by tight corks or screw-caps. These bottles containing the alcohol-grades constitute the stock, which is used for dehydrating the fixed tissues and tissue-sections during the staining process.

The optimum length of time for keeping the tissue in any particular alcohol-grade basically depends upon the thickness and nature of tissue. For stepwise dehydration, the tissue or the material should be kept in the progressive alcohol-grades for the following period or duration: in 30% and 50% grades for 5 to 10 hours; in 70% grade for 10 to 12 hours; in 90% grade for about 15 hours and in absolute alcohol, i.e., 100% grade for 6 to 8 hours. The tissue or other biological material should be kept for relatively less period in absolute alcohol, as this

hardens the tissue, causing difficulties in section cutting. After the absolute-alcohol stage of dehydration is over, the process of clearing of the tissue should be undertaken. This is described in the next chapter. Certain precautions have to be observed during dehydration.

Precautions during Dehydration

- (1) Dehydration should be done in tightly closed (with glass-stoppers) specimen tubes (3 to 4" in height and an inch in diameter) to avoid either the evaporation of alcohol or the entry of humidity into it.
- (2) Earlier alcohol-grade from the specimen tube should be drained out completely before putting the next higher grade in the tube.
- (3) The specimen tube containing the material should be closed tightly with the glass cork, immediately after the change of the next higher alcohol-grade, to avoid the possibility of the drying of tissue.
- (4) In case there is an extreme humidity in weather, the dehydration should be done either by placing the specimen tubes in closable metal box or if possible in a desiccator.
- (5) During the staining process of the sections on a slide, all the jars of the staining-set should contain appropriate quantity of the required alcohol-grades, so that the slides remain completely immersed in them of course, this point is related to the chapter of 'staining'.

Significance of using the Graded Alcohol

During the process of dehydration, the dehydration medium (*i.e.*, the alcohol-grades) enters or diffuses into the tissues and the water content of the tissues diffuses out. This diffusion is directly related to the concentration of the diffusing medium. The use of the progressive concentration of the alcohol-grades leads to gradual and controlled diffusion of water out of the tissues with the result that the tissues do not shrink. If we place the fixed-tissue in a higher grade of alcohol, by-passing the lower grades, the diffusion is so abrupt and strong that the tissue immediately shrinks. Therefore, to preserve and maintain the

original form and texture of the tissue by avoiding the shrinkage, the alcohol-grades and the graded alcohol is used for dehydrating the tissues.

The dehydration of tissues and other biological material by using the graded alcohol is very significant, as it serve the following functions:

- (1) It prevents the putrefaction of the material.
- (2) It disallows the cellular deformities in the material.
- (3) The cell-membranes of the cells of the tissues are protected from damage. The cell-membranes are prone to damage in an aqueous medium.

Alcohol as intermediary between water and clearing media

Thus, we have seen that the alcohol-grades act as intermediate steps, right from the moment the tissues are collected from the animal into the fixative (which contain much water in their composition), *i.e.*, in water, till the tissues are ready (after final dehydration by absolute alcohol) for clearing by clearing media, *viz.*, xylol, benzene, toluene, clove oil and cedar-wood oil. The use of alcohol grades as intermediaries, as above, ensures gradual and complete dehydration, preventing the shrinkage and consequent turbidity of the material in the clearing agents.

This enables the material to preserve and maintain its original form and texture to give good result in the clearing process. Similarly, this role is played by various alcohol-grades in the ascending series during the staining process of sections (slide) after haematoxylin-staining stage till the sections are cleared in xylene.

TABLE FOR THE PREPARATION OF THE ALCOHOL GRADES FROM RECTIFIED SPIRIT

Alcohol Grade—	Rectified Spirit	Distilled Water
30% Alcohol	31.5 ml.	68.5 ml.
50% Alcohol	52.6 ml.	47.4 ml.
70% Alcohol	73.6 ml.	26.4 ml.
90% Alcohol	94.7 ml.	5.3 ml.

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CHAPTER V

CLEARING AGENTS

The process of dehydration leads to the saturation of tissue with alcohol. After dehydration, the tissue is impregnated with paraffin (wax) to make it firm for the purpose of section cutting. This means, the paraffin has to remove the alcohol from the tissue to take its place. However, the diffusion of paraffin into the tissue to replace the alcohol is not possible, as the paraffin is immiscible with alcohol. Therefore, after dehydration, the tissue has to pass through an intermediate step in which it is placed in a fluid, which being miscible with alcohol and paraffin, makes the paraffin to infiltrate into the tissue. This intermediate step is called clearing.

The clearing of the tissue is done by clearing agents, viz., xylene (xylol), benzene, toluene, (all hydrocarbons), clove oil and cedar wood oil. Xylene is a commonly used clearing agent. These clearing agents not only bring about the infiltration of paraffin into the tissues but also make them transparent by removing their opacity. If turbidity appears on keeping a tissue in a clearing agent, it indicates that the tissue is not perfectly dehydrated. In such situation, the tissue should be removed from the clearing agent and placed back in absolute alcohol for the period of 2 to 3 hours to ensure complete dehydration. After this, the tissue should be kept in fresh quantity of clearing agent. The optimum time, for which the tissue should be kept in a clearing agent, is indicated by the shine or the transparency of the tissue. This time naturally varies with the nature and the thickness of the tissue. Invariably, this optimum time ranges between 2 to 4 hours. However, the tissue should not be kept for a long period in a clearing agent, as this hardens the tissue, thereby creating hindrance in the infiltration of paraffin into the tissue. A brief description of some clearing agents is given below.

(1) Xylol (Xylene) is dimethylbenzene having the formula $\text{CH}_3-\text{C}_6\text{H}_4-\text{CH}_3$. The three position isomeric namely O-xylene, M-xylene and P-xylene occur in coal tar in small amounts. They are isomeric with ethyl benzene. These three isomeric xylenes are now obtained industrially by reforming $\text{C}_{7}-\text{C}_8$ petroleum fraction of light naphtha at $400-500^\circ\text{C}$.

The xylene is a colourless mobile liquid with somewhat pleasant odour. The melting point of O-xylene, M-xylene and P-xylene is respectively -25°C , -47°C and -130°C . The boiling point respectively is 144°C , 139°C and 138°C . Xylene on oxidation with various substances gives rise to COOH groups, phthalic anhydride (commercial) and tetraphthalic acid.

In addition to its function of clearing the tissues in micro-technique, xylol (a mixture of xylenes) is used as a solvent dilutant for making lacquers. P-xylene is used in the manufacture of polyester fibres. The merits and demerits of xylene are:

Xylene, a good clearing agent, brings about the quick removal of alcohol from the tissues and speeds up the infiltration of paraffin into them. It does not impart any colour-tinge to tissue, as other clearing agents such as clove oil and cedar wood oil do. Xylene has the merit in that it does not make the tissues hard and brittle, provided the time factor is followed, as compared to benzene. However, xylol is relatively less effective in cold embedding than benzene.

(2) Benzene (C_6H_6): It leads the class of aromatic hydrocarbons. It was first isolated by Faraday (1825) from oil, condensed in cylinders. Hofmann (1849) separated benzene from coal tar. Its industrial name is benzol. Benzene is obtained from petroleum, from toluene by hydrodealkylation and by distillation of coal tar.

Benzene is a colourless, highly refractive, mobile liquid, melting point 5.5° , boiling point 80° , specific gravity 0.8790 at 20° . It has an odour which is not unpleasant. It is insoluble in water and soluble in ether ethanol and petrol. It is good solvent for fats, resins, etc. Both the liquid and vapour benzene is

highly poisonous and must be used with care. Its chemical properties show many electrophilic substitution reactions and addition reactions.

Benzene is used as a solvent for extraction of fats and oils. It is used as a starting material for the manufacture of dyes, drugs, perfumes, explosives, etc. It is used to obtain chemicals required in the manufacture of plastics. It is also used in obtaining Phenol needed in the manufacture of bakelite.

The merits of benzene are:

- (a) as it is a good solvent for many hydrocarbons, it brings about speedy infiltration of paraffin into tissues.
- (b) because of its electrophilic substitution reaction, it removes the absolute alcohol quickly from the tissues. Benzene is highly soluble in ethyl alcohol and this property enables benzene to displace absolute alcohol from the tissues.
- (c) because of the above abilities of benzene, it is often preferred for cold embedding to xylol (Small filings or pieces of paraffin are put into benzene containing the tissue).

The demerits of benzene are:

- (a) it makes the tissue or the microtomy material harder and brittle early as compared to xylene. Therefore, there is resistance in the smooth block-cutting and the sections tend to slip out of the ribbon.
- (b) It is harmful to the body.

(3) Toluene or Methylbenzene ($C_6H_5-CH_3$): It is obtained from petroleum and coal. It is a colourless, mobile liquid. It is insoluble in water, but soluble in ethanol, ether and petroleum. Toluene shows electrophilic substitution reactions and addition reactions.

Since toluene has chemical properties like benzene, it can be used for clearing. However, it is not commonly used for clearing the tissues. Its merits and demerits are almost the same as those of benzene, as a clearing agent.

(4) Cedar-Wood Oil. It is obtained by steam distillation from the heartwood of the eastern red-cedar (Juniperus virgi-

niana) and allied southern species. Chips, saw-dust, waste from the lead pencils industry, old stumps, roots, and even fence rails are utilized. It has a yellowish tinge, lesser than that of clove oil.

Cedar-wood oil is used in perfumery, soaps, deodorants, liniments, clearing and polishing preparations. It has insecticidal properties and is used in fly-sprays.

It is preferred as a clearing agent, especially in botanical preparations, because of its high refractive index. Because of its deodorant and insecticidal properties, this clearing agent may protect the tissues from external factors, in case the bottles or specimen tubes with tissues are left uncovered. However, for clearing animal tissues, cedar-wood oil is rarely used.

(5) Clove Oil. It is obtained from the clove-trees (Syzygium aromaticum) grown in the wild state in South India. These are cultivated in Nilgiris and in Kerala state. The clove trees grow abundantly in Zanzibar, from where the cloves are supplied to all parts of the world. The oil is distilled from the buds.

The clove oil is used in the manufacture of tooth pastes, soaps, perfumes, dentifrices, confectionaries and in medicines including pain balms and rubrifacients. The merits and demerits of clove oil, as a clearing agent, are:

- (a) Clove oil has a good refractive index.
- (b) It has the capacity to accommodate some moisture or negligible water-traces in the tissues, in case the tissues or sections are not perfectly dehydrated. If some turbidity is seen, remove the tissues or the section-slide from the first clove oil container and put them in fresh clove oil. Turbidity disappears.
- (c) It gives clarity and transparency to the tissues or sections relatively early.
- (d) Its demerits are a yellowish tinge to the material and making the tissues harder quite early as compared to other clearing agents. The clove oil is a costlier clearing agent and as such cannot be used widely.

Procedure

- (1) Hold posterior end of the *Drosophilla* larva (in saline solution) with forceps and with a dissecting needle pull away the mouth parts. The salivary glands will be attached to the mouth parts.
- (2) Place the glands directly into a small amount of stain on an albuminized clean slide.
- (3) Place a cover slip on the preparation, and apply a little pressure on the cover slip.
- (4) Seal the edges of the cover slip with paraffin and allow it to stand for a day.

Results

Giant chromosomes red.

METHOD OF MOUNTING

In order to make a permanent preparation of sections of any tissue, for examination and storage without deterioration, a final processing of the slide is necessary. All the traces of alcohol must be replaced by a medium, preferably toluene or xylene, that maintains the tissue in a clear and transparent condition. A mounting medium is then applied that is transparent, does not alter the colour or the intensity of the stain, and holds a cover glass permanently in place. The mounting media commonly employed are Canada balsam and DPX mountant.

Cover Slip Mounting

Place the stained and cleared slide on a plane table on a piece of blotting paper. Apply a thin streak of the mounting medium on the cover slip, turn the cover slip over the slide and rest it on one side besides the sections. Gradually rest the cover slip in place so that all the air can escape from under the cover slip. Gently press the cover slip from centre outwards to distribute the medium evenly. An alternate method is to apply mounting medium along one edge of the sections on the slide and to rest one edge of the cover slip in contact with the medium on the slide, then to gradually lower the cover slip, so as to ease out the air without any bubble formation. Finally press the cover slip gently in place and allow it to dry.

Precautions

- (1) Never allow the sections to dry after taking the slide out from xylene for mounting.

- (2) Dehydrate and clean the sections thoroughly before mounting; otherwise the permanent slide will become dull and turbid instead of being crystal clear due to traces of water in the tissue.
- (3) Never use too much of mounting medium, which otherwise may ooze out from the sides of the cover slip and spoil the slide.
- (4) Allow freshly mounted slides to become completely dried before observing it under the microscope.