

Ex.No:1 Learning the cytogenetics laboratory, various chemicals to be used for fixation, dehydration, embedding, staining, cleaning etc.

The process of killing and fixing brings about the sudden death of cells or tissues in such a manner that the morphological organization and chemical composition of the cells remain unaltered greatly. Though both are distinct processes, both are usually obtained by means of a single fluid, which in turn is commonly a mixture of various chemical reagents, which are compatible. Killing means the sudden and permanent termination of the life processes.

Killing invariably precedes fixation.

Fixation is a process by which the cellular and structural elements are preserved in nearly the natural living condition.

Properties of good fixative:

i) It should penetrate rapidly ii) It should change the soluble cell contents to insoluble substances that will remain in their original position. iii) It should protect tissues against shrinkage and distortion during subsequent treatment. iv) It should make the cell constituents stainable.

Type of fixation:

Fixation images obtained by the action of fixing agents are of two types

- i) Acid fixation image : By this method of fixation, chromatin, nucleoli and spindle fibres are preserved. Cytoplasm is fixed strongly while nucleoplasm and mitochondria are dissolved.
- ii) Basic fixation image : By this method, resting chromatin and spindle fibres are dissolved. Nucleoplasm and mitochondria are preserved. Cytoplasm is fixed as hyaloplasm and vacuoles are more or less preserved.

Types of fixatives

Fixatives are broadly classified into two types.

- i) **Non metallic:** Ethanol, Chloroform, Formalin, Glacial acetic acid, Methanol and Propionic acid.
- ii) **Metallic fixatives :** Chromic acid, Mercuric chloride and Uranium nitrate.

Apart from the above solution, combinations of several of the above chemicals are also employed. Among the solution, Carnoys fluid I and II finds wide application for cytogenetical studies.

Carnoys fluid I:

It consists of Glacial acetic acid 1 part and Absolute ethanol 3 parts. It is effective for fixing all plant, animal and human materials. The period of fixation varies from 15 minutes to 24 hours. After fixation, the specimen should be washed in 95% ethanol 2-3 times to remove the acetic acid since it may interfere with staining process. The constituents of Farmer's solution are same as Carnoy's fluid I.

Carnoy's fluid II:

It is composed of Glacial acetic acid 1 part Chloroform 3 parts and Absolute ethanol 6 parts

Formalin – Aceto – Alcohol (FAA) fixative: This fixative is widely employed for botanical studies. The standard proportion of chemicals for this fixative is 70% Ethanol - 90 ml, Glacial acetic acid - 5 ml and Formalin - 5 ml.

Staining

Preparation of stains: The process of colouring the cells by employing certain inorganic or organic dyes is known as staining. The selection of dye or stain for a particular material depends on its chemical nature, the pH value of the fixative used and the chemical reactivity of stain to the material. Most cytological stains are solutions of dyes of aromatic organic compounds which have two kinds of active chemical groups viz., chromophoric and auxochromic groups. The chromophoric group gives colour to the dye while the auxochromic group gives to the dye the ability to adhere to the tissue or material.

Dyes are broadly classified into three groups

i) The natural dyes ii) Coal tar dyes and iii) Other compounds.

Natural Dyes: Three natural dyes are used. One is derived from Cochineal insects while the other two are derived from plants belonging to Caesalpiniaceae.

i) **Brazillin:** The dye is obtained from *Caesalpinia crista* and *C. echinata*. It is employed as 0.5% solution in 70% alcohol, which is allowed to ripen for a week in a dark place in a tightly stopped bottle. It imparts colour only after mordenting with Ferric ammonium sulphate. ii) **Hematoxylin:** Hematoxylin is derived from log wood (*Hematoxylin campuchianum*). The stain is made up in combination with metallic salts of iron (always in the ferric form) Aluminum and Copper. In presence of acid medium, it produce red colour and in presence of alkaline medium it produce blue colour. iii) **Cochineal and derivatives :** Cochineal is a yellowish- red powder obtained by grinding the dried bodies of the female cochineal insects and extracting the colouring matter. These are not employed for botanical studies to grate extent. iv) **Carmin:** It is a bright red colour stain obtained by adding alum to cochineal. It is extensively used in iron acetocarmin smear technique by botanists.

The coal tar dyes: Coal tar dyes have been classified into six groups consisting of several dyes. The important dyes are Aniline blue, Congo red, Picric acid and Safranin.

Other substances acting as stains

Iodine: Iodine is well known as a specific colour indicator for starch when made upon combination with potassium iodide. It produces blue colour with starch, brown with cellulose and yellow with pectin and callose. Some of the important stains employed for cytological studies are Aceto carmine, Propiono carmine, Aceto orcein and Feulgen stain.

Preparation of Aceto carmine: Aceto carmine stain is prepared by dissolving carmine stain in Acetic acid. Forty five percent acetic acid (45ml glacial acetic acid + 55 ml distilled water) is boiled gently. A quantity of 1g of carmine powder is added to 45% acetic acid at about boiling point and allowed to boil for a few minutes. After boiling, the solution is removed from the flame and allowed to cool at room temperature. The solution is then filtered with a Whatman No.1 filter paper in to a clear bottle. The filtrate is light red coloured. If necessary, ferric chloride and ferric acetate may be added for deep staining and preservation.

Preparation of Propino carmine: One gram of carmine powder is dissolved in boiling 45% Propionic acid and the procedure is similar to the preparation of aceto carmine. Propionic acid gives better fixation and staining properties than Glacial acetic acid. It also dissolves more carmine and gives more intensive reaction together with great clarity.

Preparation of Aceto orcein: The procedure for preparation of Aceto orcein is the same as Aceto carmine. One gram of pure orcein is devolved in 45% of Glacial acetic acid and heated to boiling point for a few minutes. The contents are allowed to cool. Later this solution is filtered through Whatman No.1 double filter paper and stored in a clean bottle.

Preparation of crystal violet: It is also referred as Gentian violet. The disadvantage with this stain is over staining of cytoplasm. Distilled water (100ml) is heated to boiling point in a clean beaker. Gentian violet (1g) is added to boiling water and cooled down to room temperature. Then the solution is filtered to give 1% crystal violet solution.

Feulgen stain: Feulgen stain is employed for biological studies because of its specificity to DNA. It is prepared by dissolving 0.5g of basic Fuchsin in 100 ml boiling distilled water. The contents are shaken thoroughly, cooled to 50°C and filtered. To the filtrate about 10ml of 1N HCL and 0.5g potassium meta sulphate are added, shaken thoroughly in a tightly closed container and keep in a dark cupboard for about 18 hours. The colorless solution obtained is stored in a coloured bottle in a cool place away from sunlight. (1N Hcl can be obtained by adding 1 part of concentrate Hcl to 11 parts of distilled water).