

Ex.No: Principles of killing and fixing,

Killing and Fixing: 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, Carnoy's fluid I and II finds wide application for cytogenetical studies.

Carnoy's 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 classic plant 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

Formaldehyde - 5 ml