**Popular fixative solutions:**

1. **Formalin Solution (10%, buffered neutral)**

40% formaldehyde: 100 ml

Distilled water: 900 ml

NaH2PO4: 4.0 g

Na2HPO4 (anhydrous): 6.5 g

The most widely used formaldehyde-based fixative for routine histopathology. The buffer tends to prevent the formation of formalin pigment. Many epitopes require antigen retrieval for successful IHC following its use. Most pathologists feel comfortable interpreting the morphology produced with this type of fixative.

1. **Formal calcium**

40% formaldehyde: 100 ml

Calcium chloride: 10 g

Distilled water: 900 ml

Recommended for the preservation of lipids especially phospholipids.

1. **Zinc formalin (unbuffered)**

Zinc sulphate: 1 g

Distilled water: 900 ml

40% formaldehyde: 100 ml

Zinc formalin solutions were devised as alternatives to mercuric chloride formulations. They are said to give improved results with IHC. There are a number of alternative formulas available.

1. **Alcohollic formalin**

40% Formaldehyde: 100 ml

95% Ethanol: 900 ml

0.5 g calcium acetate can be added to ensure neutrality

Combines a denaturing fixative with the additive and cross-linking effects of formalin. It is sometimes used during processing to complete fixation following incomplete primary formalin fixation. Can be used for fixation or post-fixation of large fatty specimens (particularly breast), because it will allow lymph nodes to be more easily detected as it clears and extracts lipids. If used for primary fixation specimens can be placed directly into 95% ethanol for processing.

1. **Bouin’s solution**

Picric acid saturated aqueous soln. (2.1%): 750 ml

40% formaldehyde: 250 ml

Acetic acid glacial: 50 ml

Bouin’s gives very good results with tissue that is subsequently trichrome stained. Bouin’s also preserves glycogen well but usually lyses erythrocytes. Bouin’s fixative is sometimes recommended for gastro-intestinal tract biopsies, animal embryos, testes and endocrine gland tissue.

1. **Glutaraldehyde**

50% glutaraldehyde: 10ml

100 mM phosphate buffer at pH 7.0: 240ml

Glutaraldehyde is most frequently used for the fixation of specimens for electron microscopy because it preserved ultrastructure the best of any of the aldehydes. But it is not good for immunohistochemical staining.

**7. Ethanol and Methanol**

Ethanol and methanol are considered to be coagulants that denature proteins. They replace water in the tissue environment disrupting hydrophobic and hydrogen bonding thus exposing the internal hydrophobic groups of proteins and altering their tertiary structure and their solubility in water. They are not used routinely for tissues, but commonly used to fix frozen sections and smears. Fixation commences at a concentration of 50 – 60% for ethanol and >80% for methanol.

**8. Acetic Acid**

Acetic acid is coagulant in action with nucleic acids but generally does not fix proteins. It is incorporated in compound fixatives to help prevent the loss of nucleic acids and, because it swells collagen, to counter the shrinkage caused by other ingredients such as ethanol. Combining the two may result in better preservation of tissue morphology.