

## **Fixation in tissue processing**

**Hamed Ganjali<sup>1\*</sup> and Mahla Ganjali<sup>2</sup>**

1. Phd student in department of basical sciences ,. Faculty of veterinary medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. M.Sc Student of Biotechnology. Bangalor university. Brindavan college of India

***Corresponding author:*** Hamed Ganjali

**ABSTRACT:** The present study is about Fixation of tissue. It is done for several reasons. One reason is to kill the tissue so that postmortem decay (autolysis and putrefaction) is prevented (Carson and Hladik, 2009). Fixation preserves a sample of biological material (tissue or cells) as close to its natural state as possible in the process of preparing tissue for examination. To achieve this, several conditions usually must be met. In the fields of histology, pathology, and cell biology, fixation is a chemical process by which biological tissues are preserved from decay, thereby preventing autolysis or putrefaction. Fixation terminates any on going biochemical reactions, and may also increase the mechanical strength or stability of the treated tissues. First, a fixative usually acts to disable intrinsic biomolecules particularly proteolytic enzymes—which otherwise digests or damages the sample. Second, a fixative typically protects a sample from extrinsic damage. Fixatives are toxic to most common microorganisms (bacteria in particular) that might exist in a tissue sample or which might otherwise colonise the fixed tissue. In addition, many fixatives chemically alter the fixed material to make it less palatable (either indigestible or toxic) to opportunistic microorganisms. Finally, fixatives often alter the cells or tissues on a molecular level to increase their mechanical strength or stability. This increased strength and rigidity can help preserve the morphology (shape and structure) of the sample as it is processed for further analysis.

**Keywords:** Fixative, Tissue processing, Chemical process, Autolysis, Putrefaction, Morphology

### **INTRODUCTION**

#### ***Types of fixatives***

The purpose of fixation is to preserve tissues permanently in as life-like a state as possible. There is no perfect fixative, though formaldehyde comes the closest. Therefore, variety fixatives are available for use, depending on the type of tissue present and features to be demonstrated. There are five major groups of fixatives, classified according to mechanism of action:

- Aldehydes
- Mercurials
- Alcohols
- Oxidizing agents
- Picrates

#### ***Aldehydes***

Aldehydes include formaldehyde (formalin) and glutaraldehyde. Tissue is fixed by cross linkages formed in the proteins, particularly between lysine residues. This cross-linkage does not harm the structure of proteins greatly, so that antigenicity is not lost. Therefore, formaldehyde is good for immunoperoxidase techniques. Formalin

penetrates tissue well, but is relatively slow. The standard solution is 10% neutral buffered formalin (Osha et al., 2011).

**Glutaraldehyde**

Glutaraldehyde causes deformation of alpha-helix structure in proteins so is not good for immunoperoxidase staining. However, it fixes very quickly so is good for electron microscopy. It penetrates very poorly, but gives best overall cytoplasmic and nuclear detail. The standard solution is a 2% buffered glutaraldehyde. (Eltoum et al., 2001).

**Mercurials**

Mercurials fix tissue by an unknown mechanism. They contain mercuric chloride and include such well-known fixatives as B-5 and Zenker's. These fixatives penetrate relatively poorly and cause some tissue hardness, but are fast and give excellent nuclear detail.

Their best application is for fixation of hematopoietic and reticuloendothelial tissues (Eltoum et al., 2001).

**Alcohols**

Alcohols, including methyl alcohol (methanol) and ethyl alcohol (ethanol), are protein denaturants and are not used routinely for tissues because they cause too much brittleness and hardness. However, they are very good for cytologic smears because they act quickly and give good nuclear detail. Spray cans of alcohol fixatives are marketed to physicians doing PAP smears, but cheap hairsprays do just as well (Eltoum et al., 2001).

**Oxidizing agents**

Oxidizing agents include permanganate fixatives (potassium permanganate), dichromate fixatives (potassium dichromate), and osmium tetroxide. They cross-link proteins, but cause extensive denaturation. Some of them have specialized applications, but are used very seldom include fixatives with picric acid. Foremost among these is Bouin's solution. It has an unknown mechanism of action. It does almost as well as mercurials with nuclear detail but does not cause as much hardness. Picric acid is an explosion hazard in dry form as a solution; it stains everything it touches yellow, including skin (Table 1).

Table 1. Target and chemical fixative do's and don'ts

Target	Fixative of Choice	Fixative to Avoid
Proteins	Neutral Buffered Formalin, Paraformaldehyde	Osmium Tetroxide
Enzymes	Frozen Sections	Chemical Fixatives
Lipids	Frozen Sections, Glutaraldehyde/Osmium Tetroxide	Alcoholic fixatives, Neutral Buffered Formalin
Nucleic Acids	Alcoholic fixatives, HOPE	Aldehyde fixatives
Mucopolysaccharides	Frozen Sections	Chemical fixatives
Biogenic Amines	Bouin Solution~, Neutral Buffered Formalin	
Glycogen	Alcoholic based fixatives	Osmium Tetroxide

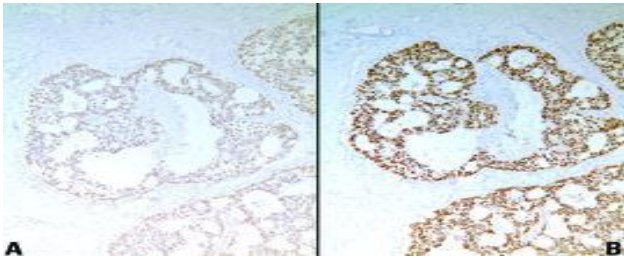


Figure 1. Estrogen receptor (ER) staining of breast carcinoma. A. Tissue block fixed for 3 hours in 10% formal saline showing weak demonstration of ER positive cells. B. Tissue block fixed 8 hours in 10% formal saline showing strong demonstration of ER positive cells

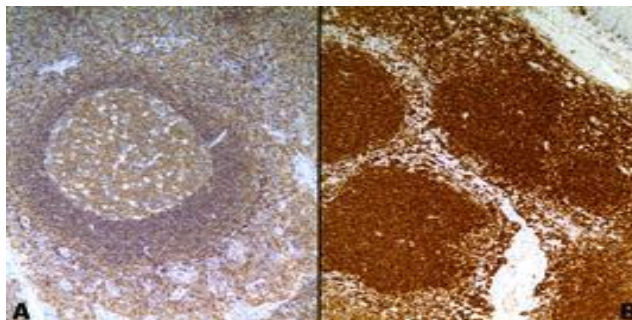


Figure 2. LCA (CD45) staining on reactive tonsil. A. Block fixed for 3 hours in 10% formal saline showing weak demonstration of lymphocytes. B. Block fixed for 8 hours in 10% formal saline showing strong staining of lymphocytes

### **Factors affecting fixation**

There are a number of factors that will affect the fixation process:

- 1-buffering
- 2-penetration
- 3-volume
- 4-temperature
- 5-concentration
- 6- time interval.

Fixation is best carried out close to neutral pH, in the range of 6-8. Hypoxia of tissues lowers the pH, so there must be buffering capacity in the fixative to prevent excessive acidity. Acidity favors formation of formalin-heme pigment that appears as black. Polarizable deposits in tissue. Common buffers in clued phosphate, bicarbonate, cacodylate, and veronal. Commercial formalin is buffered whit phosphate at a pH of 7.

Penetration of tissues depends upon the diffuse ability of each individual fixative, which is constant formalin and alcohol penetrate the best, and glutaraldehyde the worst. Mercurials and others are somewhere in between. One way to get around this problem is sectioning the tissues thinly (2 to 3mm).penetration into a thin section will occur more rapidly than for a thick section.

The volume of fixation is important. there should be a 10:1 ratio of fixative to tissue. Obviously, we often get away with less than this, but may not get ideal fixation.one way to partially solve the problem is to change the fixative at intervals to avoid exhaustion of the fixative. Agitation of the specimen in the fixative will also enhance fixation (Leong, 1994).

Increasing the temperature as with all chemical reactions, will increase the speed of fixation,as long as you don't cook tissue. Hot formalin will fix tissues faster,and this is often the first step on an automated tissue processor. However, care is required to avoid cooking the specimen. Fixation is routinely carried out at room temperature.

Concentration of fixative should be adjusted down to the lowest level possible, because you will expend less money for the fixative. Formaline is best at 10%, glutaraldehyde is generally made up at 0.25% to 4%. Too high a concentration may adversely affect the tissues and produce artifact similar to excessive heat (Hopwood, 1996). Also very important is time interval from of removal of tissues to fixation. The faster you can get the tissue and fix it, the better. Artefact will be introduced by drying, so if tissue is left out, please keep it moist with saline. The longer you wait, the more cellular organelles will be lost and the more nuclear shrinkage and artefactual clumping will occur.

Fixation is a chemical process, and time must be allowed for the process to complete. Although "over fixation" can be detrimental, under-fixation has recently been appreciated as a significant problem and may be responsible for inappropriate results for some assays (Carson, 1997).

### **General usage of fixatives**

There are common usages fixatives in the pathology based upon the nature of the fixatives. The type of tissue and the histologic details to demonstrated.

Formalin is used for all routine surgical pathology and autopsy tissues when an Hand E slide is to be produced. Formalin is the most forgiving of all fixatives when conditions are not ideal, and there is no tissue that it will harm significantly. Most clinicians can understand what formalin is and does and it smells bad enough that they are careful handing it.

Zenkers fixatives are recommended for reticuloendothelial tissues including lymph nodes, spleen, thymus, and bone marrow. Zenkers fixes nuclei very well and gives good detail. However, the mercury deposits must be removed before staining or black deposits will result in the sections.

Bouins solution is sometimes recommended for fixation of testis, GI tract, and endocrine tissue. It does not do a bad job on hematopoietic tissues either, and doesn't require dezenkerizing before staining (Eltoum et al., 2001).

Glutaraldehyde is recommended for fixation of tissues for electron microscopy. The Glutaraldehyde must be cold and buffered and not more than 3 months old. The tissue must be as fresh as possible and preferably sectioned within the Glutaraldehyde at a thickness no more than 1 mm to enhance fixation.

Alcohols, specifically ethanol are used primarily for cytologic smears. Ethanol 95% is fast and cheap. Since smears are only a cell or so thick, there is no great problem for shrinkage, and since smears are not sectioned, there is no problem from induced brittleness.

For fixing frozen sections, we can use about anything though methanol and ethanol are the best (Leica, 2007).

## CONCLUSION

Fixation is the process by which the constituents of cells and tissue are fixed in a physical and partly also in a chemical state so that they will withstand subsequent treatment with various reagents with minimum loss of architecture. This is achieved by exposing the tissue to chemical compounds, called fixatives.

Most fixatives act by denaturing or precipitating proteins which then form a sponge or meshwork, tending to hold the other constituents.

Good fixative is most important factors in the production of satisfactory results in histopathology. Following factors are important:

1. Fresh tissue
2. Proper penetration of tissue by fixatives
3. Correct choice of fixatives.

No fixative will penetrate a piece of tissue.

## REFERENCES

- Carson A, Freida L, Christa H. 2009. Histotechnology: A Self-Instructional Text (3 ed.). Hong Kong: American Society for Clinical Pathology Press. p. 2. ISBN 978-0-89189-581-7.
- Eltoum I, Fredenburgh J, Myers RB, Grizzle WE. 1997. Introduction to the theory and practice of fixation of tissues. J Histotechnol 2001;24:173 - 190. Carson FL. Histotechnology. 2nd ed. Chicago: ASCP Press.
- Eltoum I, Fredenburgh J, Grizzle WE. Advanced concepts in fixation: 1. Effects of fixation on immunohistochemistry, reversibility of fixation and recovery of proteins, nucleic acids, and other molecules from fixed and processed tissues. 2. Developmental methods of fixation. J Histotechnol 2001;24:201-210.
- Hopwood D. Fixation and fixatives. In Bancroft J and Stevens A eds. 1996. Theory and practice of histological techniques. New York: Churchill Livingstone.
- Leica, Microsystems. Material Safety Data Sheet: 10% Millonig's Buffered Formalin. 2007.
- Leong ASY. 1994. Fixation and fixatives. In Woods AE and Ellis RC eds. Laboratory histopathology. New York: Churchill Livingstone; 4.1-1 - 4.1-26.
- OSHA. 2011. Occupational Safety and Health Standards. Standard Number: 1910.1048 : Formaldehyde. United States Department of Labor, Occupational Safety and Health Administration,.