

A Comparison of Fixatives and Media: From a Pathologists' Assistant's Perspective

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Figure 1. Neutral Buffered Formalin⁵



Figure 2. Alcohol⁵



Figure 3. Glutaraldehyde⁵



Figure 4. Bouin's⁵



Figure 5. B-5⁵



Figure 6. B-Plus⁵



Figure 7. Strong acid decal⁵



Figure 8. Weak acid decal Immunocal⁵



Figure 9. Versenate-EDTA⁵

Background

Most specimens that come across the bench will require routine neutral buffered formalin. Although formalin is normally sufficient and preferred, certain circumstances require alternative fixatives, and it's essential to understand when formalin is inappropriate. Once a specimen has been inadequately or inappropriately fixed, remedial changes may no longer be possible.

- Purpose of Fixation: stabilize structures, retain constituents, prevent autolysis and decay, improve contrast, harden tissue
- Fixative Classification: Based on mechanism of action → Physical/Mechanical or Chemical
- Physical fixation: Heating or Freezing
- Heating/Microwaving:
 - Not typically recommended, as it often damages tissue and requires specialized equipment that will produce potentially explosive vapors²
 - Correct equipment can dramatically reduce fixation time
- Freezing/Cryosectioning:
 - Cryosectioning is a rapid microscopic analysis to determine factors that may affect the course of a surgical procedure
 - Valid frozen specimens: Margin assessment, sufficient diagnostic tissue, lymph node analysis, confirmation of tissue type⁴
 - Tip: When picking up a frozen section, a warm microscopic slide can induce better adherence and partially fix the tissue via heat and dehydration²
- Chemical fixation: Two of the most commonly used are perfusion and immersion
 - Perfusion: Produces the highest-quality histology by evenly circulating fixative throughout the vascular system; however, may be impractical in a high-volume institution
 - Immersion: Most common method, in which fixatives slowly diffuse into the specimen

Formalin

- Neutral Buffered Formalin (NBF): Working solution of 4% formaldehyde diluted in water or in a buffered solution
- Most studied and commonly used fixative in diagnostic pathology²
- Compatible with many stains, immunohistochemistry, and molecular analysis methods
- Fixative volume should be 15-20x the volume of tissue due to slow penetration

Alcohol

- Coagulative fixatives that denature proteins and destroy ultrastructure by replacing water and disrupting hydrophobic and hydrogen bonding¹
- Slowly penetrates tissue, hardening and shrinking them efficiently
- Special uses: Cytology specimens and fresh tissue to visualize Gout-associated crystals²
- Methanol is the most commonly used fixative for hematology specimens such as blood films or smears³

Glutaraldehyde

- Slow penetrating, crosslinking fixative used for electron microscopy (EM) for ultrastructure analysis
- Bifunctional aldehyde that requires storage at 4 °C and approximate pH of 5 for stability
- Crosslinking inhibits enzyme activity, resulting in ineffective immunohistochemical methods¹
- Specimens must be small for good results, with a 0.5 mm maximum size

Bouins

- Composition: picric acid, formaldehyde, acetic acid
- Most beneficial for small biopsies
- Special uses: Testicular biopsies for infertility studies or oligospermia detection, lymph node searches, and biopsies of the GI tract and endocrine glands¹
- Its acidic composition will slowly remove small calcium/iron deposits³
- Advantages: Great retention of tissue antigenicity, excellent glycogen preservation, excellent quality of connective tissue stains
- Disadvantages: Destroys ultrastructure and nuclear membrane integrity², overuse results in yellow discoloration and brittle tissue, dry picric acid is explosive

Zenkers, B-5, B-Plus

- Recommended for lymphoid and hematopoietic tissues

Zenkers and B-5

- Zenkers Composition: mercuric chloride, potassium dichromate, glacial acetic acid, distilled water³
- B-5 Composition: mercuric chloride, sodium acetate anhydrous, 40% formaldehyde, distilled water³
- Advantages: High retention of tissue antigenicity, nuclear content preservation, good results for PTAH and trichrome stains
- Lyses red blood cells due to acetic acid presence³
- Will require washing to remove mercury and dichromate pigment before staining^{1,3}

B-Plus

- Modern version of Zenkers and B-5 fixatives
- Mercuric chloride is replaced with zinc salts and formaldehyde
- Same outcomes as Zenkers and B-5, but without the hazard and need for washing⁵
- Most optimal fixative for bone marrows, lymph nodes, and other lymphoid/hematopoietic tissues⁵

Decalcification

- Technique used to remove minerals from bone and calcified tissues
- Specimens must be fixed prior to decalcification
- Poorly fixed tissue will be macerated during the decal process and stain poorly
- Poor fixation is most commonly visualized in bone marrow specimens, resulting in many institutions extending bone marrow fixation times³
- There are 3 types of decalcification agents
 - Strong mineral acids: Hydrochloric or Nitric acid at 10% concentration
 - Most rapid decalcifying agents and should be used conservatively
 - Weaker organic acids: Formic acid and Trichloroacetic acid (TCA)
 - Most popular decal due to their gentle nature and likelihood to interfere with nuclear staining³
 - Chelating agents: EDTA (Ethylenediaminetetraacetic acid)
 - Very slow and gentle decal agents
- Stop decalcification once all the calcium is removed to prevent tissue damage
- Testing can be done via physical or chemical methods
 - Physical: Specimen manipulation - bending, probing, trimming
 - Chemical: Ammonium Oxalate is added to the final decalcified specimen, and if calcium is still present, a calcium oxalate precipitate will form³

References

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