STTARR Histopathology Core
University Health Network

Tissue Fixation

Lab area: Histopathology Core	Effective: October 1, 2024
Issued by: Napoleon Law	Revised:
Trainee sign & date:	File Name: Tissue fixation

Introduction:

A fixative alters tissue by stabilizing the protein so that it is resistant to further changes. Different fixative choices are used on specific purpose to demonstration tissue elements with special technique or stains. This protocol describes the purpose of tissue fixation, factors affecting proper fixation and the choices of commonly used fixative solutions in Histology.

Functions and actions of fixatives:

-Fixative kill the tissue by **denaturing proteins** so that the postmortem activities of decay and autolysis are prevented. Any enzyme activities on the tissue will rendered inactive as a result of the protein-stabilizing action.
-Fixative help maintain the proper relationship between cell and extracellular substances, rendering **better cellular structure and morphology** on the histology tissue section.
-Fixative **enhance staining** by making tissue more receptive to dye, such as acting as mordant in many staining procedures.
-Some fixative, such as the formaldehydes stabilize tissue proteins by forming **cross-linking hydrogen bonds on proteins**. This process mask the antigenic sites on the tissue, which may result on faint or negative immunohistochemistry staining.
-Fixation will **make tissue firmer**, making gross dissection and collecting thin sections easier.

Factors affecting fixation:

-Temperature: Formaldehyde fixation is recommended to be carried out at **room temperature** for formaldehyde. Higher or lower (0°C-4°C) temperatures are not recommended as it would alter the cellular morphology or slow down the fixation process.
-Size: Thickness of the tissue should be **≤ 3mm thick**.
-Volume ratio: Fixative volume should be at least **15-20x** greater than the tissue volume
-Time: Tissue should be placed immediately in fixative after cut off from blood supply. Formalin should have at least 6-8 hours to act in order for the tissue to be fixed. Routinely tissue fixed with 10% neutral buffered formalin should have a minimum of 6 hours to maximum of 72 hours of fixation time with most staining procedures.

Choice of fixative:

-Formaldehyde:

Formalin: 10% neutral buffered formalin. Routinely used in histology since it allows most tissue elements to be demonstrated. It also causes less shrinkage than other fixatives. Contains 10%-14% methanol to help prevent polymerization (forming white powder deposits)

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Paraformaldehyde: 4% PFA. Used in electronic microscopy and sometimes in IHC staining. Contains no methanol, stock solution need to be stored in 4°C or -20°C to prevent polymerization.

Zinc formalin: Preserve antigenicity by preventing crosslinking during fixation process. It's the choice of fixative for immunohistochemistry.

-**Picric acid:** Both as a fixative ingredient and as a stain. Will decalcify small calcium deposits such as breast tissue. Must be washed out before processing. Will dissolve RNA and DNA on tissue samples, avoid using for in-situ hybridization.

-**B-5 fixative:** Compounded with mercuric/zinc chloride, sodium acetate and formaldehyde. Used in hematopoietic and lymphoreticular tissue, ability to demonstrate beautiful nuclear details

-**Bouin solution:** Compounded with picric acid, formaldehyde and acetic acid. This fixative lyses RBC because of its acetic acid content. The yellow stain must be washed away with 50%-70% alcohol before processing. Maximum fixation time for bouin is ≤ 24 hours. Bouin is excellent for use on biopsy specimens from the GI tract because the nuclei are much crisper. This fixative can also decalcify small specimens of bone

-**Acetone:** Used when demonstrating enzymes, especially acid and alkaline phosphatase, where tissue is indicated to be processed for paraffin embedding. Fixation with acetone is done at refrigerator temperature. Acetone is also used for rabies diagnosis in the brain and frozen tissue section stained for cell surface antigens by IHC. This fixative is fast acting but causes extreme shrinkage, distortion and overhardening.

-**Alcohol:** Methyl alcohol is used as fixative for touch preparations and blood smear.

Transport solution

Michel transport medium: If unfixed tissue is to be held for long period of time to be transport for long distance, this solution can be used to held tissue for several days. This is usually used for kidney biopsy samples but not for muscle biopsy.

Routine formalin fixation procedure for paraffin embedding

1. Once tissue are cut off from blood supply, immediate submerge the tissue in a sample container with 10% neutral buffered formalin in a 15-20% volume ratio against the tissue volume. Do not pack tissue in a small tube, allow tissue to move freely inside the collection tube for thorough fixation.

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2. Fix the tissue in room temperature, best if the sample tubes can be placed on a shaker to facilitate the fixative penetration into the core of the tissue. It is not necessary to place the sample in the refrigerator as it would only slow down the fixation process.
3. Allow the tissue fixed in formalin for a minimum of 6-8 hours to a maximum of 72 hours for most staining procedure, including immunohistochemistry staining. Best practice is to write the starting time and date of the fixation on the sample tubes.
4. If the samples are only intended for H&E staining or other specific stains that antigenicity of the proteins are not a concern, tissue can be kept in formalin for extended period of time until it is ready to be processed.
5. For tissue sample that are planned for IHC staining, the fixation process has to be terminated by washing out the fixative with tap water after 24 hours fixation. (no longer than 72 hours.) Fixed tissue can then be transferred into 70% ethanol for long term storage until they are ready to be processed and paraffin embedded.