Comparative analysis of 10% Neutral Buffered Formalin (NBF) absorption in pathological tissue specimens using immersion and sponge-assisted methods.

Heather A. Hansen PathA/HTL and Mark Evans PhD LUMEA

Abstract:

Objective evaluation of the efficacy of immersion and sponge absorption fixation methods was made using time-lapse photography to monitor penetration of the fixation process in standardized 5mm thick porcine tissue explants. Tissue samples were either fully immersed in 10% neutral buffered formalin (NBF) or placed on NBF-saturated sponges. Digital images were captured at regular intervals to assess changes in tissue morphology, translucency, and overall stability over a defined period. Quantitative analysis of tissue shrinkage and changes in optical density were performed to compare the rate and uniformity of fixation between the two methods.

Sponges absorb water due to the attraction between water molecules, causing swelling until equilibrium. This attraction also prevents water from easily dripping out. When a wet sponge contacts a wet surface, water moves to the sponge, enhancing its absorption. Similarly, a sponge filled with aqueous formaldehyde placed on wet tissue will transfer the formalin solution as water molecules move until equilibrium is reached across both surfaces.

Background:

Problems during tissue fixation and processing can lead to suboptimal preservation and diagnostic errors despite protocol adherence. Common issues include autolysis (enzymatic degradation), putrefaction (bacterial decomposition), formalin pigment (dark deposits from unbuffered formalin), tissue shrinkage or swelling (distortion from fixative or duration), poor penetration (incomplete fixative action in dense tissues), crush artifacts (mechanical damage), and drying artifacts (distortion from desiccation). Prevention involves prompt and proper fixation, neutral buffered formalin, optimized fixative properties and duration, sectioning large samples, gentle handling, and keeping tissues moist. Addressing these requires attention to detail, standardized protocols, appropriate fixatives and buffers, adequate fixation times, proper processing, equipment maintenance, and thorough staff training for quality assurance in histology and pathology.

This study provides visual and quantitative evidence supporting the efficacy of sponge absorption fixation as an equivalent or potentially superior method to immersion fixation for small tissue specimens. The rapid penetration, even fixation, and enhanced tissue stability observed with the sponge method highlight its advantages, particularly in bedside and clinic biopsy collection and transport where timely and artifact-free fixation is critical. Further studies are warranted to investigate the applicability of sponge absorption fixation for a wider range of tissue types and sizes, as well as its impact on various downstream histological and molecular analyses.

Rationale and Hypothesis:

Choosing a tissue fixation method hinges on tissue size and density, desired fixation time, fixative volume and cost, and downstream analysis needs. Sponge absorption fixation is a faster, cheaper, and simpler option for small biopsies, minimizing distortion. Limited fixative or disposal concerns favor sponge absorption due to lower consumption. Rapid fixation needs or preservation of sensitive components in small samples may also necessitate sponge absorption. Immersion fixation is best for larger, denser tissues to ensure even penetration, despite longer times and more fixative. Ultimately, downstream analysis requirements guide fixative and method selection for optimal preservation of targets.

Methodology:

Study 1: Comparative Analysis of Fixation Methods

To objectively assess immersion versus sponge absorption fixation, 5mm thick porcine tissue explants were subjected to time-lapse photography. One group of samples was fully immersed in 10% neutral buffered formalin (NBF), while the other was placed on NBF-saturated sponges. Digital images captured at regular intervals allowed for the monitoring of tissue morphology, translucency, and stability. Subsequent quantitative analysis measured tissue shrinkage and optical density changes to compare the rate and uniformity of fixation between the two methods.

Study 2: Retrospective Review of Needle Core Biopsies

A post-analytical review was conducted on 28,309 single needle core biopsies collected on ‘Prostate Needle 6-Core Biopsy’ Kits. This workflow involves laying the cores on a 10% NBF soaked sponge immediately after collection. The overall quality of fixation, as observed on H&E and PIN4 stained slides, was evaluated for each case. These cases were initially reviewed by multiple pathologists from independent US laboratories.

Results:

Sponge Absorption Fixation

Principles:

- Utilizes a fixative-saturated sponge for sustained high fixative concentration.
- Accelerates fixative penetration through capillary action and direct contact.
- Relies on diffusion driven by concentration gradients.
- Absorption maintains gradients and facilitates fixative uptake.
- Unlikely spillage of fixative during transport to the lab.

Advantages:

- Faster fixation.
- Reduced fixative volume.
- Minimizes mechanical distortion of delicate tissues.
- Simplified collection and transportation.
- Improved antigen retrieval and IHC staining.
- Improved recovery of genetic material.
- Can be used with formalin gas vapors.

Disadvantages:

- Limited to small tissue specimens (generally less than 5 mm thickness).
- Inefficient fixative diffusion over larger distances leads to heterogeneous fixation in thicker tissues.

Immersion Fixation

Principles:

- Tissue specimen is submerged in fixative.
- Fixative penetrates tissue through diffusion.

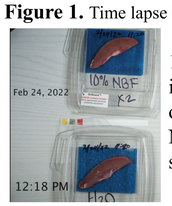
Advantages:

- Suitable for larger and more dense tissues.
- Ensures adequate fixative penetration throughout the specimen.
- Minimizes risk of uneven fixation.

Disadvantages:

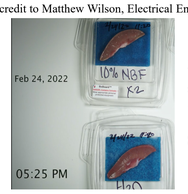
- Requires longer fixation times.
- Requires larger volume of fixative.
- Over “fixation” or over “crosslinking of proteins”.
- Uneven results of diffusion to the way the aqueous formalin penetrates the parenchyma.
- Likely spillage of fixative during transport to the lab (poor tissue exposure to fixative).

Figure 1. Time lapse images of gross tissue changes. *Cameras and Lighting credit to Matthew Wilson, Electrical Engineer



1 hour ischemic; on a 10% NBF sponge

2 hours on a 10% NBF sponge

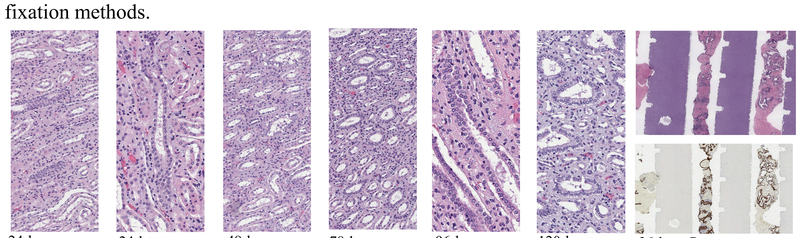


5 hours on a 10% NBF sponge

8 hours on a a 10% NBF sponge: tissue appears well fixed; “fleshy” appearance is replaced.

24 hours on a 10% NBF sponge: is fixed and nearly “rubbery” in texture; “fleshy” appearance is100% replaced.

Figure 2. Side by side images of similar tissues fixed using immersion and absorption fixation methods.



24-hour Immersion | 24-hour Absorption | 48-hour Absorption | 78-hour Absorption | 96-hour Absorption | 120-hour Absorption | 36-hour Prostate BxBoard

Discussion:

Sponge absorption fixation offers several advantages over traditional immersion fixation, especially for small tissue samples. This method utilizes a fixative-saturated sponge to provide a sustained high concentration of fixative at the tissue-sponge interface, accelerating fixative penetration through capillary action and direct contact. Key benefits include faster fixation, reduced fixative volume, minimization of mechanical distortion of delicate tissues, and simplified collection and transportation. The absorptive capacity of the sponge also helps maintain a favorable concentration gradient for diffusion by removing released cellular components. However, this method is limited to small tissue specimens (generally less than 5 mm in thickness) due to the decreasing efficiency of fixative diffusion over larger distances, which can lead to heterogeneous fixation and compromised preservation in thicker tissues. The efficacy of sponge absorption fixation relies on the principles of diffusion, driven by concentration gradients, and absorption, which supports diffusion by maintaining these gradients and facilitating fixative uptake into the tissue.

The selection of the most appropriate tissue fixation method depends on several critical factors, including the size and density of the tissue specimen, the desired fixation time, the volume of fixative available, associated cost considerations, and the specific requirements of the downstream analyses.

For larger and more dense tissues, immersion fixation remains the preferred method due to its ability to ensure adequate fixative penetration throughout the entire specimen. While it may require longer fixation times and a larger volume of fixative, the risk of uneven fixation is minimized. Conversely, for small biopsies and tissue fragments, sponge absorption fixation offers a rapid, cost-effective, and less cumbersome alternative that minimizes distortion and simplifies handling, particularly in point-of-care settings.

The availability of fixative and the associated disposal considerations can also influence the choice. Sponge absorption fixation's lower fixative consumption can be advantageous in situations where fixative volumes are limited, or disposal costs are a significant concern. Furthermore, the need for rapid fixation, such as in certain diagnostic scenarios or to preserve labile cellular components, may favor the use of sponge absorption for smaller samples. Finally, the specific requirements of downstream analyses, such as immunohistochemistry or molecular studies, may dictate the choice of fixative and fixation method to ensure optimal preservation of target antigens or nucleic acids.

Beyond morphological preservation, the *hydrophilic* sponge fixation method positively impacts downstream applications. Antigen retrieval procedures are notably improved due to the consistent fixation achieved, leading to enhanced antibody binding and more reliable immunohistochemical (IHC) staining results. Surprisingly, the stabilization provided by the *hydrophilic* sponge system also facilitates improved recovery of genetic material from the processed specimen. This unexpected benefit opens new possibilities for molecular analysis on tissues that have undergone fixation. The inclusion of formalin gas vapors as part of the fixation process further contributes to the overall stability and preservation of the sample, ensuring long-term integrity and suitability for a range of analytical techniques.

Conclusion:

These findings demonstrated that sponge absorption fixation achieved comparable or even superior tissue penetration and fixation in the initial stages for the 5mm porcine tissue explants. Time-lapse imaging revealed a more rapid and even stabilization of tissue morphology in the sponge-fixed samples compared to immersion fixation, particularly in the peripheral regions. Both methods ultimately resulted in complete fixation of the tissue samples; however, the sponge absorption method exhibited less overall tissue shrinkage and maintained tissue stability more effectively throughout the observation period.

References:

Tingjiao Zhou, Jinbin Yang, Deyong Zhu, Jieyao Zheng, Stephan Handschuh-Wang, *Hydrophilic Sponges for Leaf-Inspired Continuous Pumping of Liquids.* First published: 19 April 2017. https://doi.org/10.1002/advs.201700028.

Atanda, Akinfenwa T, and Solomon Raphael. *“Role of surgeons in determining outcome of histopathology specimens.” Nigerian journal of surgery : official publication of the Nigerian Surgical Research Society* vol. 19,2 (2013): 68-72. doi:10.4103/1117-6806.119242