

Exploratory Comparison of 10% NBF and Methanol Fixation in Adenomyosis Uterine Tissue

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Nasywa^{1*}, Gina Andyka Hutasoit², Tri Setyawati³, Vera Diana Towidjojo⁴, Emi Sunarya⁵

¹Medical Study Program, Faculty of Medicine, Tadulako University, Palu, Indonesia

²Department of Anatomical Pathology, Faculty of Medicine, Tadulako University, Palu, Indonesia

³Department of Biochemistry, Faculty of Medicine, Tadulako University, Palu, Indonesia

⁴Department of Parasitology, Faculty of Medicine, Tadulako University, Palu, Indonesia

⁵Faculty of Medicine, Tadulako University, Palu, Indonesia

Email Corresponding:

nasywa.nas09@gmail.com

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Abstract

Background: Fixation is critical for routine histology, but alternative alcohol fixatives may produce different staining characteristics from 10% neutral buffered formalin. **Objectives:** This exploratory laboratory study compared hematoxylin and eosin staining quality in adenomyosis uterine tissue from two hysterectomy donors. **Methods:** Forty histology slides were evaluated, with 20 slides in the 10% neutral buffered formalin group and 20 in the methanol group. Nuclear, cytoplasmic, and cell boundary staining were scored as adequate or good by light microscopy at 10x and 40x magnification. **Results:** Good scores were more frequent with formalin than methanol for nuclei (60.0% vs 25.0%), cytoplasm (65.0% vs 20.0%), and cell boundaries (70.0% vs 25.0%). Mann Whitney output showed asymptotic p values of 0.027, 0.004, and 0.005, respectively; the reported exact value for nuclear staining was 0.060 and was not corrected for ties. **Conclusions:** Because only two biological donors contributed the slides, findings are interpreted descriptively as a pilot comparison. Under the tested laboratory conditions, formalin showed more favorable slide level staining scores, while confirmation in a larger donor series with standardized allocation and masked scoring is required.

Keywords: Adenomyosis; Fixation; Histology; Methanol; Neutral buffered formalin.

Introduction

Histological fixation is a preanalytical step intended to preserve tissue architecture and cellular detail before processing, sectioning, and staining. In routine surgical pathology, 10% neutral buffered formalin is widely used because formaldehyde produces protein cross linking that stabilizes tissue morphology. Fixation quality can influence nuclear detail, cytoplasmic appearance, cell boundaries, antigenicity, and the performance of downstream molecular assays^{1,2}.

Methanol is chemically distinct from formaldehyde. Methanol is an alcohol that

primarily produces coagulative fixation through protein precipitation, whereas formaldehyde is an aldehyde that forms cross links between macromolecules^{3,4}. This distinction is important because stronger stain intensity does not automatically indicate better overall preservation, and the effects of alcohol fixation can differ across tissue types, concentrations, processing protocols, and diagnostic targets^{5,6}.

Adenomyosis is characterized by endometrial glands and stroma within the myometrium, and interpretable hematoxylin and eosin sections require recognizable epithelial and stromal structures. Standardized

tissue handling is therefore important for morphological interpretation. Studies in diagnostic pathology have compared formalin with alcohol based or other nonformalin fixatives and reported variable findings across nuclear contour, cytoplasm, cell boundaries, staining uniformity, molecular targets, and processing time^{7,8}.

Indonesian histopathology studies published in *Healthy Tadulako Journal* illustrate the importance of reproducible morphological assessment in endometrial lesions, meningioma, astrocytoma, breast carcinoma, prostate lesions, and colorectal carcinoma^{9,10,11,12,13,14}. These studies do not compare formalin with methanol, but they demonstrate why interpretable histological features and standardized preparation are essential for clinicopathological assessment.

The present study was conducted in a basic histology and anatomical pathology laboratory and explored whether 10% neutral buffered formalin and methanol produced different slide level staining appearances in adenomyosis uterine tissue. The source study included 40 slides originating from only two hysterectomy donors; therefore, the biological sample size is two donors rather than 40 independent patients. This distinction is central to interpretation because multiple sections from the same donor may be correlated.

The objective was to describe and compare nuclear, cytoplasmic, and cell boundary staining scores between the two fixation groups under the documented laboratory conditions. The study was treated as an exploratory pilot comparison, with emphasis on transparent descriptive results and cautious interpretation of inferential statistics.

Materials and Methods

Study Design

This exploratory laboratory study used a quasi experimental posttest comparison of two

fixation conditions: 10% neutral buffered formalin and methanol. The study was conducted in August 2025 at the Histology and Anatomical Pathology Laboratory, Faculty of Medicine, Tadulako University. The biological source consisted of adenomyosis uterine tissue obtained from two patients who underwent hysterectomy at Undata Regional General Hospital, Palu. The analytic material comprised 40 histology slides, with 20 slides evaluated in each fixation group.

Sample

Fresh endometrial uterine tissue from hysterectomy specimens with a pathological diagnosis of adenomyosis and without gross necrosis was included. The source manuscript documents two donors and 40 histology slides. Because donor specific allocation of tissue pieces, blocks, slides, and microscopic fields was not recorded in the supplied manuscript, the 40 slides were not treated as 40 independent biological observations. Table 1 distinguishes the documented donor level and slide level information to prevent pseudoreplication in interpretation.

Data Collection Technique

The source protocol compared 10% neutral buffered formalin with methanol fixation and subsequently evaluated hematoxylin and eosin stained sections by light microscopy. Morphological scoring covered nuclear staining, cytoplasmic staining, and cell boundary visibility. Each domain used a two category ordinal score: 1 for adequate and 2 for good. For this revision, the outcome is described specifically as perceived staining quality rather than overall morphological preservation or diagnostic accuracy, because the recorded instrument did not directly measure fixative penetration, nucleic acid integrity, lipid retention, antigen preservation, or diagnostic performance.

The original manuscript states that evaluation was performed with a light microscope at 10x and 40x magnification and that scoring was discussed with a pathology specialist. Other potentially influential preanalytical details, including cold ischemia time, tissue piece dimensions, fixative volume

relative to tissue volume, methanol purity, fixation temperature and duration, paraffin processing batch, section thickness, field selection rule, and assessor masking, were not documented in the supplied source. These variables were therefore not reconstructed retrospectively or presented as observed facts.

Table 1. Documented experimental hierarchy and fixation groups

Level	Documented count	10% NBF arm	Methanol arm	Interpretive role
Biological donor	2 patients	Not separately reported by donor	Not separately reported by donor	True biological source
Histology slide	40 slides	20 slides	20 slides	Slide level morphological observations
Microscopic magnification	10x and 40x	Used	Used	Visual assessment
Morphology domains	3 domains	Nucleus, cytoplasm, cell boundary	Nucleus, cytoplasm, cell boundary	Ordinal staining score

Source: Primary data, 2025

Data Analysis Technique

The primary descriptive analysis reported n and percentage of adequate and good scores for each staining domain by fixation group. The original analysis used the Mann Whitney U test at a two sided significance level of 0.05. Because the score has only two categories, ties are extensive. The source output reported both asymptotic and exact significance values, and the exact output was explicitly marked as not corrected for ties. Accordingly, both results are reported transparently rather than selecting the value that favors a hypothesis. The analysis is considered exploratory because the slide observations are clustered within only two biological donors and the available data do not permit a reliable donor level mixed model or cluster robust population inference.

Ethical Consideration

The study received approval from the Ethics Committee of the Faculty of Medicine, Tadulako University, approval number 7621/UN28.10/KL/2025. The source manuscript states that participants provided informed consent before participation and that

collected data were kept confidential in accordance with health research ethics principles. Tissue and slide reporting in this manuscript is deidentified.

Result

The study material originated from two hysterectomy patients with adenomyosis. A total of 40 histology slides were evaluated, consisting of 20 slides in the methanol group and 20 slides in the 10% neutral buffered formalin group. The source manuscript did not provide donor specific demographic characteristics or a donor by fixative allocation table; therefore, only the verified tissue diagnosis and slide counts are reported.

Table 2 presents the distribution of staining quality scores. In the methanol group, adequate scores predominated for nuclei, cytoplasm, and cell boundaries. In the formalin group, good scores predominated for all three domains. Good nuclear staining occurred in 12 of 20 formalin slides (60.0%) and 5 of 20 methanol slides (25.0%). Good cytoplasmic staining occurred in 13 of 20 formalin slides (65.0%) and 4 of 20 methanol slides (20.0%). Good cell

boundary staining occurred in 14 of 20 formalin slides (70.0%) and 5 of 20 methanol slides (25.0%).

The Mann Whitney output is summarized in Table 3. For nuclear staining, the asymptotic p value was 0.027, while the reported exact p value was 0.060 and was not corrected for ties. This discrepancy does not support a categorical

statement that nuclear staining was definitively significant. Cytoplasmic and cell boundary scores had asymptotic p values of 0.004 and 0.005, with reported exact values of 0.014 for both. These p values remain exploratory because slides from only two donors may be correlated and donor allocation was not available for reconstruction.

Table 2. Slide level staining quality scores by fixation group

Outcome	Fixative	Adequate n (%)	Good n (%)	N	Mean rank
Nuclear staining	Methanol	15 (75.0)	5 (25.0)	20	17.0
Nuclear staining	10% NBF	8 (40.0)	12 (60.0)	20	24.0
Cytoplasmic staining	Methanol	16 (80.0)	4 (20.0)	20	16.0
Cytoplasmic staining	10% NBF	7 (35.0)	13 (65.0)	20	25.0
Cell boundary staining	Methanol	15 (75.0)	5 (25.0)	20	16.0
Cell boundary staining	10% NBF	6 (30.0)	14 (70.0)	20	25.0

Source: Primary data, 2025

Table 3. Mann Whitney output for staining quality scores

Outcome	Mann Whitney U	Wilcoxon W	Z	Asymptotic p	Reported exact p	Interpretation
Nuclear staining	130.0	340.0	-2.211	0.027	0.060	Discordant p values; descriptive interpretation prioritized
Cytoplasmic staining	110.0	320.0	-2.842	0.004	0.014	Exploratory slide level difference
Cell boundary staining	110.0	320.0	-2.814	0.005	0.014	Exploratory slide level difference

Note: Primary interpretation is descriptive because of donor clustering.

Representative micrographs from the source manuscript are shown in Figures 1 and 2. The panels illustrate hematoxylin and eosin stained adenomyosis tissue under the two fixation conditions and show the epithelial and

stromal regions used for morphological comparison. The images are interpreted descriptively; staining intensity alone is not treated as proof of global tissue preservation or diagnostic accuracy.

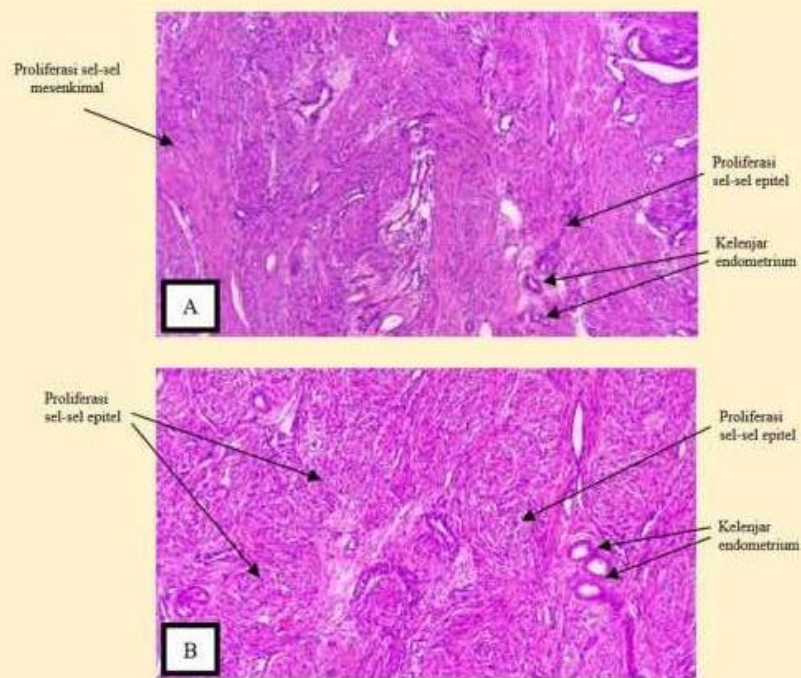


Figure 1. Representative hematoxylin and eosin (H&E) stained histological sections of adenomyosis tissue at 10× magnification, comparing (A) 10% neutral buffered formalin (NBF) fixation and (B) methanol fixation, showing endometrial glands, epithelial cell proliferation, and surrounding mesenchymal cell proliferation.

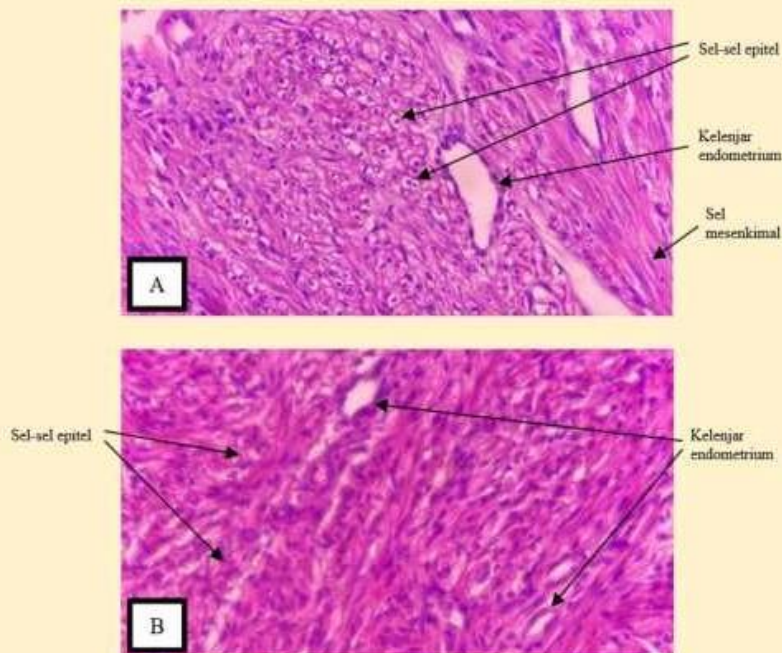


Figure 2. Representative hematoxylin and eosin (H&E) stained histological sections of adenomyosis tissue at 40× magnification, comparing (A) 10% neutral buffered formalin (NBF) fixation and (B) methanol fixation, showing endometrial glands, epithelial cells, and surrounding stromal tissue.

Across the evaluated slides, formalin had a larger proportion of good scores than methanol for nuclear staining, cytoplasmic staining, and cell boundary visibility. The strongest descriptive contrasts were observed for cytoplasm and cell boundaries. The nuclear comparison was statistically ambiguous because the source output reported asymptotic $p=0.027$ but exact $p=0.060$. Given the two donor design, the findings are best regarded as pilot slide level observations that require confirmation in independent donors.

Discussion

This exploratory study found more frequent good hematoxylin and eosin staining scores in slides assigned to 10% neutral buffered formalin than in slides assigned to methanol. The observed pattern involved nuclear, cytoplasmic, and cell boundary appearance, but the biological sample consisted of only two adenomyosis donors. Consequently, the result describes performance under the tested laboratory conditions and does not establish general superiority of one fixative for uterine tissue.

The findings are biologically plausible because formaldehyde and alcohols preserve tissue through different mechanisms. Neutral buffered formalin forms cross links that stabilize proteins, whereas methanol produces coagulative fixation and can alter tissue hydration and protein configuration^{1,3,4}. Comparative pathology studies have shown that formalin may provide favorable nuclear contour and cytoplasmic detail in some settings, while alcohol based fixatives may provide satisfactory cell boundaries or selected molecular advantages^{7,15}. Such variation indicates that fixation performance depends on the outcome being measured rather than on a single universal definition of quality.

The current scoring system measured perceived staining quality on an ordinal two category scale. It did not directly assess nucleic acid preservation, antigenicity, lipid retention, tissue shrinkage, fixation penetration, or diagnostic concordance. Therefore, possible chemical explanations should not be interpreted as mechanisms demonstrated by this experiment. Preanalytical variables such as fixation duration, specimen thickness, fixative volume, processing schedule, staining batch, and field selection can affect tissue appearance and could contribute to the observed contrasts^{2,16,17}.

The nuclear result illustrates why statistical reporting requires caution. The asymptotic Mann Whitney p value was 0.027, whereas the exact value reported by the source output was 0.060 and was not corrected for ties. With a binary score, ties are common, and neither value should be selected solely because it supports a preferred conclusion. More importantly, multiple slides from the same donor are not equivalent to multiple independent patients. With only two donors, standard cluster adjustment cannot provide reliable population level uncertainty.

Histopathological interpretation depends on clear and reproducible structural features. Studies in Healthy Tadulako Journal examining endometrial lesions, meningioma, astrocytoma, breast carcinoma, prostate lesions, and colorectal carcinoma emphasize that morphological and immunohistochemical interpretation relies on standardized tissue preparation and interpretable architecture⁹⁻¹⁴. These studies provide diagnostic context rather than evidence about fixative superiority.

For routine laboratory translation, future comparisons should include a larger number of independent hysterectomy donors, split each donor specimen between fixation arms,

standardize cold ischemia time, tissue dimensions, fixative volume, concentration, duration, temperature, processing and staining batches, and use masked assessors with predefined field selection.

Multiple assessors or repeated readings should be used to estimate agreement when subjective morphology scores are primary outcomes^{18,19}. A donor matched design would allow within donor contrasts and reduce confounding by biological variability.

The strengths of this study include direct side by side evaluation of two commonly accessible fixation approaches on adenomyosis tissue and transparent reporting of the observed slide distributions. Its limitations are the two donor biological sample, uncertain independence of slide observations, lack of donor specific allocation records, binary subjective scoring, limited documentation of preanalytical variables, and single laboratory setting. These limitations define the work as a pilot study and guide the design of a more definitive validation study.

Conclusion

Under the laboratory conditions evaluated in this two donor pilot study, slides fixed with 10% neutral buffered formalin more frequently received good scores for nuclear staining, cytoplasmic staining, and cell boundary visibility than slides fixed with methanol. The nuclear statistical result was not robust across the two significance outputs, and the limited number of biological donors prevents population level inference. These findings support further donor matched evaluation with standardized fixation, processing, masked scoring, and a larger independent sample before any routine protocol recommendation is made.

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Conflict of Interest Statement

The author(s) declare no commercial, financial, or personal conflicts of interest related to this research. All authors approved the final manuscript and consented to its publication in *Healthy Tadulako Journal*.

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