

The Impact of Histological Tissue Processing On Diagnostic Accuracy In Histopathology: A Narrative Review

Rhemadhyaksa Tegar Arrondiwa¹, Khairun Nisa¹, Pratiwi Soesilawati², Zahiyah Wulan Supriyono¹, Valerian Rayhan Wijaya¹

¹Faculty Of Dental Medicine Airlangga University, Surabaya, Indonesia

²Department of Oral Biology, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia

Corresponding author: Rhemadhyaksa Tegar Arrondiwa

Email: rhemadhyaksa.tegar.arrondiwa-2025@fkg.unair.ac.id

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Abstract

Background/Aim: Histopathological diagnosis depends on high-quality tissue processing to preserve tissue morphology and ensure diagnostic accuracy. Inadequate processing may induce artifacts and compromise diagnostic interpretation. This narrative review aimed to summarize current evidence on the impact of histological tissue processing on tissue quality, artifact formation, and diagnostic accuracy.

Methods: A narrative review was conducted using a structured systematic literature search guided by the PRISMA 2020 statement. The review question was formulated using the PEO framework. English-language studies published between 2021 and 2026 were retrieved from PubMed, Scopus, and ScienceDirect using predefined inclusion and exclusion criteria, and the eligible studies were synthesized narratively.

Results: FFPE remains the gold standard for preserving tissue morphology and structural integrity. However, processing-related artifacts and formalin-induced molecular alterations may reduce diagnostic accuracy. Emerging approaches, including alternative processing reagents, microwave-assisted and automated processing, and artificial intelligence-assisted quality control, show promise for improving specimen quality and diagnostic reliability.

Conclusion: Optimizing and standardizing histological tissue processing is essential to improve diagnostic accuracy and support advances in molecular pathology, digital pathology, and precision medicine.

Keywords: Histology; Paraffin Embedding; Formaldehyde; Artifacts; Diagnostic Accuracy; Digital Pathology.

I. INTRODUCTION

1.1 Histopathology as the Gold Standard for Disease Diagnosis

Histopathology is widely recognized as the gold standard for disease diagnosis, serving as a critical component of clinical practice and biomedical research across multiple medical disciplines.¹ The diagnostic process typically begins with biopsy acquisition, followed by microscopic examination of tissue architecture and cellular morphology to establish definitive diagnoses, particularly in oncology and other pathological conditions.² Among various specimen preparation approaches, formalin-fixed paraffin-embedded (FFPE) tissue remains the most widely adopted and is considered the gold standard preparation method due to its high image quality, long-term stability, and excellent preservation of tissue morphology.^{3,4}

1.2 Importance of Histological Tissue Processing

The diagnostic value of histopathological examination depends substantially on specimen

quality, which is largely determined by histological tissue processing.⁵ Histological tissue processing represents a critical pre-analytical stage that bridges tissue collection and microscopic examination. Its primary purpose is to preserve tissue architecture and cellular morphology while preventing autolysis, putrefaction, and structural degradation following tissue excision.⁶ To achieve these objectives, tissue specimens undergo a series of coordinated procedures, including fixation, dehydration, clearing, infiltration, and embedding, which collectively prepare tissues for sectioning and microscopic evaluation.⁵ Proper tissue processing ensures the preservation of cellular and architectural details, optimal staining characteristics, and sufficient structural integrity for histological, immunohistochemical, and molecular analyses.^{1, 5} Conversely, deficiencies during processing may result in morphological distortion, staining inconsistencies, and compromised diagnostic interpretation.⁶

1.3 Relationship Between Tissue Processing and Diagnostic Accuracy

A growing body of evidence suggests that tissue processing quality directly influences diagnostic accuracy by affecting the reproducibility and reliability of histological and molecular analyses.⁶ Suboptimal processing can compromise tissue integrity and introduce diagnostic uncertainty, whereas appropriately processed specimens facilitate accurate morphological assessment and biomarker evaluation.⁷

Furthermore, processing-related artifacts such as tissue folds, shrinkage, incomplete staining, and artificial deposits may obscure tissue morphology or mimic pathological conditions, potentially contributing to false-positive or false-negative interpretations.^{7, 8} These artifacts may adversely affect both conventional histopathological assessment and emerging artificial intelligence-assisted diagnostic systems.^{7, 9} Consequently, tissue processing is increasingly recognized as a key determinant of diagnostic reliability in modern histopathology.

1.4 Research Gap and Rationale of the Review

Numerous studies have investigated various aspects of histological tissue processing, including fixation protocols, tissue preservation techniques, processing-related artifacts, and emerging processing technologies.^{3, 6, 7} In parallel, advances in immunohistochemistry, molecular pathology, digital pathology, and artificial intelligence have increased the demand for high-quality tissue specimens and standardized pre-analytical procedures.^{9, 10} Despite these developments, existing evidence remains fragmented, with most studies focusing on individual processing stages, specific artifacts, or particular technological innovations rather than evaluating their collective impact on diagnostic accuracy.

Furthermore, the relationship between tissue processing quality, artifact formation, morphological preservation, and downstream diagnostic performance has not been comprehensively synthesized across conventional histopathology, immunohistochemical applications, and molecular analyses.^{6, 7} This lack of integration limits a broader understanding of how processing-related variables influence diagnostic reliability and clinical decision-making. Therefore, an updated review is needed to consolidate current evidence, identify persistent challenges, and evaluate emerging technologies that may improve diagnostic accuracy in modern histopathology.

1.5 Objectives of the Review

The primary objective of this review is to comprehensively analyze the impact of histological tissue processing on diagnostic accuracy in histopathology. Specifically, this review aims to:

1. Examine current evidence regarding the effects of tissue processing procedures and processing-related variables on tissue morphology, staining quality, antigen preservation, and molecular integrity.^{3, 6}
2. Identify common processing-induced artifacts and evaluate their implications for histopathological interpretation and diagnostic reliability.^{8, 11}
3. Explore emerging technologies and future perspectives in histological tissue processing, including automated processing systems, alternative processing approaches, and artificial intelligence-assisted quality assessment tools.^{9, 10}

II. METHODS

2.1 Review Design

This study was conducted as a narrative review using a structured literature search strategy. A systematic approach was applied during literature identification, screening, and study selection to improve transparency and minimize selection bias. The review process consisted of literature searching, study screening, eligibility assessment, data extraction, and thematic synthesis. The selected studies were synthesized narratively to provide a comprehensive overview of the impact of histological tissue processing on diagnostic accuracy in histopathology.

2.2 Research Question and PEO Framework

Research Question: What is the impact of histological tissue processing procedures and processing-related factors on tissue quality and diagnostic accuracy in histopathology?

Population (P): Histopathological tissue specimens, biopsy samples, surgical pathology specimens, formalin-fixed paraffin-embedded (FFPE) tissues, and fresh frozen tissues used for diagnostic evaluation.

Exposure (E): Histological tissue processing procedures and processing-related factors, including fixation, dehydration, clearing, paraffin infiltration, embedding, alternative fixatives, alternative clearing agents, microwave-assisted tissue processing, automated tissue processing, and processing-related artifacts.

Outcome (O): Tissue quality, morphological preservation, staining quality, artifact formation, histopathological interpretation, diagnostic accuracy, immunohistochemical performance, and molecular integrity.

2.3 Literature Search Strategy

A structured literature search was conducted using PubMed, Scopus, and ScienceDirect databases to identify studies relevant to histological tissue processing and diagnostic accuracy in

histopathology. The search was limited to articles published between 2021 and 2026 and written in English. Search terms were developed based on the review objectives and PEO framework, encompassing concepts related to histological tissue processing, tissue quality, processing-related artifacts, diagnostic accuracy, and emerging technologies in histopathology. Boolean operators (AND, OR) were used to combine keywords according to database-specific requirements. The complete search strategy is presented in Table I (see Tables section).

Table I. Search Strategy Used in the Literature Search

Concept	Keywords/Search Terms
Histological tissue processing	"histological tissue processing", "tissue processing", "histopathological processing", FFPE
Tissue quality	"tissue quality", morphology, "morphological preservation", staining quality
Processing-related artifacts	artifact*, "processing artifact", "histological artifact"
Diagnostic accuracy	"diagnostic accuracy", diagnosis, interpretation, diagnostic reliability
Emerging technologies	"microwave-assisted tissue processing", "automated tissue processing", "alternative fixative", "digital pathology", "artificial intelligence"

Search terms were adapted to the syntax requirements of each database. Additional searches were performed using combinations of keywords related to fixation, dehydration, clearing, embedding, tissue preservation, and molecular pathology when necessary.

Eligibility Criteria

Inclusion Criteria

1. English-language full-text articles published between 2021 and 2026.
2. Studies discussing histological tissue processing, tissue quality, processing-related artifacts, diagnostic accuracy, immunohistochemistry, molecular pathology, digital pathology, or emerging tissue-processing technologies.
3. Original research articles, comparative studies, experimental studies, methodological studies, protocol papers, review articles, and relevant case reports.

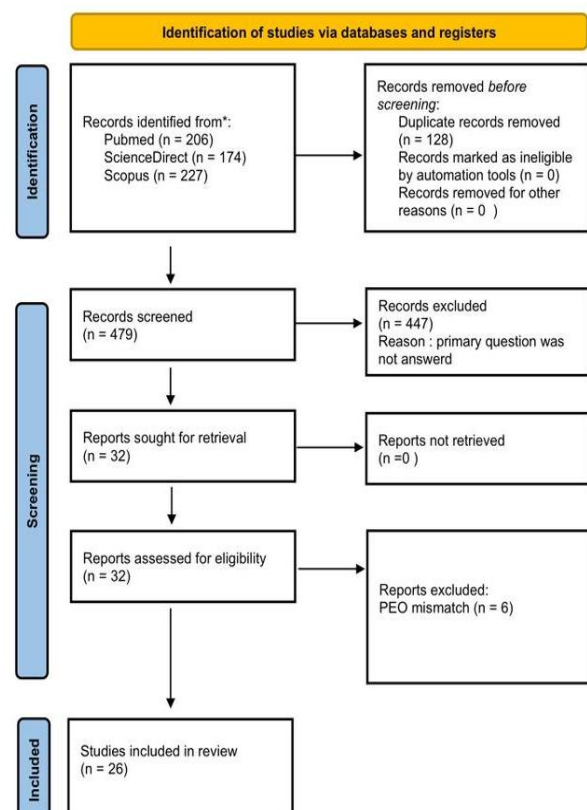
4. Studies reporting outcomes related to tissue morphology, staining quality, artifact formation, diagnostic interpretation, immunohistochemical performance, molecular integrity, or diagnostic accuracy.

Exclusion Criteria

1. Editorials, commentaries, letters to the editor, and conference abstracts without full text.
2. Duplicate publications.
3. Non-English publications.
4. Studies unrelated to histological tissue processing or its diagnostic implications.
5. Case reports without relevance to tissue processing, tissue quality, artifact formation, or diagnostic interpretation.

2.4 Study Selection

Retrieved records were imported into Rayyan AI for duplicate removal, title and abstract screening, and full-text eligibility assessment. Studies were screened according to the predefined inclusion and exclusion criteria. The study selection process was documented using a PRISMA flow diagram (Picture 1).



2.5 Ethics Statement

Not applicable. This manuscript is a narrative literature review that did not involve human participants, human tissue samples, human data, or animal subjects; therefore, ethics committee approval and informed consent were not required.

All discussed data were derived from previously published, publicly available studies.

III. RESULTS

3.1 Data Extraction

Data were extracted from all eligible studies using a standardized extraction form. The extracted information included author, year of publication, study design, tissue processing method or topic, study objective, principal findings, and diagnostic implications. The full literature extraction table is presented as Table II.

The included studies were synthesized narratively and grouped into four major thematic categories:

1. Histological Tissue Processing and Tissue Quality
2. Processing-Related Artifacts and Diagnostic Challenges
3. Impact of Tissue Processing on Diagnostic Accuracy
4. Emerging Advances in Histological Tissue Processing

The thematic categorization of all included studies is presented in Table III (see Tables section).

3.2 Data Synthesis

Table II. Literature Extraction Table

No.	Author (Year)	Study Design	Objective/Aim	Tissue Processing Method/Technology	Key Findings	Relevance to Diagnostic Accuracy
1	Bright et al. (2024)	Comparative Study	Evaluate virgin coconut oil as a safer alternative to xylene for clearing.	Coconut oil (60°C) vs. xylene control.	Preserved nuclear-cytoplasmic contrast and sectioning rigidity well, but caused more macroscopic tissue shrinkage than xylene.	Maintains H&E staining stability and cellular clarity, ensuring an undisrupted diagnostic foundation.
2	Gundlapalle et al. (2022)	Comparative Study	Evaluate thermal artifacts from different mucosal biopsy excision tools.	Scalpel vs. diode laser excision.	Diode lasers caused severe artifactual necrosis and hyperchromatism; scalpels left clear, intact margins.	Thermal artifacts can mask tumor cells or mimic disease, confounding margin assessment.
3	Falahkheir et al. (2022)	Experimental (AI)	Bridge the quality gap between artifact-prone fresh frozen (FF) and FFPE slides.	Cycle-GAN AI model transforming FF images to "virtual FFPE".	AI successfully synthesized high-resolution FFPE-like images from FF, improving inter-pathologist diagnostic agreement.	Eliminates visual freezing artifacts, enabling intraoperative diagnoses with certainty equivalent to archival specimens.
4	Cavalcanti et al. (2022)	Experimental Study	Validate a low-cost, carcinogen-free tissue preservation method.	D-Limonene clearing and acrylic polymer dry preservation.	Rehydrated specimens maintained morphology and DNA/RNA quality for 20 years without formalin-like shrinkage.	Guarantees long-term specimen reliability and molecular stability for future retrospective diagnostics.

No.	Author (Year)	Study Design	Objective/Aim	Tissue Processing Method/Technology	Key Findings	Relevance to Diagnostic Accuracy
5	Aziz & Zeman - Pocrnich (2022)	Review	Outline best practices for optimizing tissue processing fluid exchange.	Review of dehydration, clearing, and infiltration variables.	Processing times must adapt to tissue size/fat. Over-exposure to solvents causes brittleness; under-exposure causes mushiness.	Precise processing standardization prevents slide degradation and ensures diagnostic readability.
6	Hagbabin et al. (2022)	Experimental Study	Develop a universal, formaldehyde/xylene-free cold microwave protocol.	Variable power cold microwave (VCT) with Diazolidinyl Urea.	Eliminated toxic reagents and processed heterogeneous tissues in 97 mins with excellent morphological preservation.	Shrinkage-free, homogeneously heated processing prevents cytological distortion for accurate evaluation.
7	Kanwal et al. (2024)	Experimental (AI)	Create an AI framework to detect artifacts and quantify prediction uncertainty.	Deep Kernel Learning (DKL) with Gaussian Process on WSIs.	Highly precise in segmenting folds/blur, providing a predictive margin of error for ambiguous slide regions.	Prevents diagnostic AI models from making overconfident misdiagnoses on mechanically defective tissues.
8	Flint et al. (2022)	Case Report	Resolve incomplete staining artifacts during Mohs surgery frozen sections.	Modified pre-stain washing to separate acetone and hematoxylin.	Acetone residues caused eosinophilic stains obscuring cells. A 15-second water rinse eliminated this artifact.	Eliminating false stains ensures accurate evaluation of surgical margins intraoperatively.
9	Weng et al. (2024)	Experimental (AI)	Validate an AI tool (GrandQC) for automated digital slide quality control.	Deep-learning GrandQC module for WSI artifact segmentation.	Successfully identified and quarantined artifacts (blur, folds, ink), preventing misinterpretation by downstream AI.	Ensures image quality, safeguarding the reliability of Computer-Aided Diagnosis (CAD) systems.
10	Wang et al. (2025)	Experimental (AI)	Develop "ArtiDiffuser" to digitally restore artifacts and synthesize training data.	Counterfactual Denoising Diffusion Probabilistic Model (DDPM).	Precisely removed artifacts (bubbles, folds, ink) to reveal true pathology, improving downstream tumor detection.	Salvaging damaged slides computationally ensures clear structures for accurate human and AI disease grading.
11	Olave et al. (2026)	Case Report	Document chemical artifacts mimicking intestinal disease.	Observational analysis of metal push-pin exposure during processing.	Fixative reacted with metal pins, depositing iron crystals in tissue that perfectly mimicked iron pill mucosal injury.	Highlights how hardware-induced "disease mimics" can cause severe false-positive diagnoses.

No.	Author (Year)	Study Design	Objective/Aim	Tissue Processing Method/Technology	Key Findings	Relevance to Diagnostic Accuracy
12	Tay et al. (2025)	Experimental Study	Optimize fixation and IHC protocols for mouse nervous tissue.	10% NBF fixation (24h) with Proteinase K antigen retrieval.	Extended 10% NBF preserved morphology well; Proteinase K restored antigenicity without background noise.	Preserving epitopes prevents non-specific noise, ensuring absolute accuracy in cell quantification.
13	Rahman et al. (2022)	Comparative Study	Compare an alcohol-based fixative (EMA) against formalin for molecular preservation.	EMA fixative vs. 10% NBF control.	EMA prevented cross-linking, yielding stronger immunofluorescence and higher purity DNA/RNA than NBF.	Avoiding molecular masking minimizes false-negative risks in IHC biomarker testing.
14	De Martino et al. (2022)	Experimental Study	Examine the impact of ISO-standardized cold transport on DNA/RNA yield.	ISO cold-chain transport tested across 4 fixatives.	Cold transport halted RNase activity, preserving long genomic sequences, though 1-year storage still degraded RNA.	Temperature control preserves macromolecule integrity, essential for credible molecular diagnostics.
15	Villanacci et al. (2023)	Review	Explore the impact of 3D spatial orientation of biopsies on diagnosis.	Vertical alignment using cellulose acetate filters.	Misalignment causes tangential slicing, creating illusionary crowded nuclei and "twin" villi.	Proper embedding prevents visual overlap artifacts, avoiding false-positive overdiagnosis (e.g., Celiac disease).
16	Borges et al. (2023)	Comparative Study	Determine the best short-term fixative for ovarian follicle cells.	Short-Time Ethanol (ST-EtOH) vs. Carnoy's vs. Paraformaldehyde.	Carnoy's best preserved follicular structures; prolonged ST-EtOH caused cytoplasm retraction.	Prevents tissue shrinkage, allowing valid pathological assessment without structural distortion.
17	Hacihasanoglu et al. (2024)	Retrospective	Assess the impact of slide defects on inter-pathologist agreement for PD-L1.	Audit of inter-observer agreement for PD-L1 scoring.	Mechanical "crush/squeeze" artifacts were the primary cause of low pathologist agreement.	Artifacts damage biomarker anatomy, reducing the reliability of targeted therapy scoring.
18	Hamed et al. (2026)	Retrospective	Identify surgical heat artifacts mimicking appendiceal malignancies.	Conventional vs. energy-assisted thermocoagulation resection.	Thermal devices caused vacuolar changes that perfectly mimicked goblet cell adenocarcinoma.	Prevents misdiagnosing procedure-induced thermal vacuolization as true tumor invasion at margins.

No.	Author (Year)	Study Design	Objective/Aim	Tissue Processing Method/Technology	Key Findings	Relevance to Diagnostic Accuracy
19	Winter et al. (2026)	Comparative Study	Compare histomorphological accuracy of FFS vs. FFPE.	Fresh Frozen Sections (FFS) vs. FFPE.	FFPE significantly outperformed FFS in structural integrity and reliable quantitative cell counts.	Clearer FFPE architecture improves the validity of diagnostic cell quantification compared to FFS.
20	Ghebeh et al. (2021)	Retrospective	Analyze PD-L1 scoring differences between FFPE and fresh frozen tissues.	PD-L1 assessment in FFPE vs. matched fresh frozen tissues.	Formalin cross-linking in FFPE reduced PD-L1 signal intensity, dropping sensitivity by up to 67% vs. frozen tissue.	Epitope masking from formalin causes false-negatives, risking denied targeted immunotherapy.
21	Basting et al. (2021)	Experimental Study	Calibrate rapid IHC preparation for decalcified bone (TMJ).	Formic acid decalcification + pressure cooker antigen retrieval.	Pressure cooking with citrate buffer effectively restored antigenicity (vimentin) while preserving joint structure.	Preserves bone marker signals and morphology, ensuring accurate bone disease detection.
22	Yadav et al. (2025)	Experimental Study	Formulate a technique for single-cell chromatin profiling from FFPE.	scFFPE-ATAC assay with a reverse cross-linking protocol.	Reverse cross-linking freed DNA without injury, yielding epigenetic profiles highly concordant with fresh tissues.	Enables precision molecular mapping and cancer subtyping directly from standard archived FFPE blocks.
23	Oliveira et al. (2021)	Comparative Study	Compare DNA methylation profiling quality in FFPE vs. fresh frozen tissues.	DNA methylation array of FFPE vs. matched frozen tumors.	FFPE maintained highly stable and equivalent DNA methylation patterns compared to fresh frozen specimens.	Validates FFPE archives as a reliable source for long-term epigenetic diagnostic testing.
24	Ong et al. (2023)	Comparative Study	Reduce processing time without losing morphological quality.	Domestic microwave-assisted processing vs. conventional method.	Microwave heating reduced processing to 62 minutes while maintaining 80% morphological quality.	Compressing pre-analytical time safely expedites diagnostic results without compromising quality.
25	Thangamani et al. (2025)	Review	Review the capabilities and hazards of eco-friendly alternative fixatives.	Evaluation of natural fixatives (honey, jaggery, vinegar).	Natural fixatives prevent early autolysis well but are highly prone to fungal contamination and degradation.	Eco-friendly options are viable, but fungal artifacts must be monitored to prevent false diagnostics.

No.	Author (Year)	Study Design	Objective/Aim	Tissue Processing Method/Technology	Key Findings	Relevance to Diagnostic Accuracy
26	Wiedenmann et al. (2024)	Experimental (AI)	Develop an AI model immune to multiplex immunofluorescence (mxIF) artifacts.	CycleGAN to restore chemical stripping artifacts.	Repeated washing distorted H&E profiles. CycleGAN recalibrated these colors, restoring segmentation accuracy.	Computational artifact correction prevents CAD algorithms from misclassifying boundaries.

Table III. Thematic Categorization of Included Studies

Theme	Included Studies
Histological Tissue Processing and Tissue Quality	1, 5, 12, 13, 14, 16, 19, 21
Processing-Related Artifacts and Diagnostic Challenges	2, 8, 11, 17, 18
Impact of Tissue Processing on Diagnostic Accuracy	15, 20, 23
Emerging Advances in Histological Tissue Processing	3, 4, 6, 7, 9, 10, 22, 24, 25, 26

3.3 Histological Tissue Processing and Tissue Quality

Principles of Histological Tissue Processing

Tissue processing is a fundamental physical and chemical procedure that preserves tissue morphology and prepares specimens for microscopic evaluation.^{5, 6} By maintaining tissue architecture, minimizing structural damage during microtomy, and preventing autolysis and putrefaction, it ensures specimen quality for accurate histopathological interpretation.^{1, 6} Consequently, tissue processing plays a critical role in supporting reliable histopathological diagnosis, the gold standard for disease diagnosis and clinical decision-making.³

Conventional Tissue Processing Workflow

- **Fixation:** Fixation is the first and most critical step, preserving tissue morphology and preventing autolysis. 10% neutral-buffered formalin (NBF) remains the standard fixative because it stabilizes proteins through covalent cross-linking, although this may mask epitopes and reduce nucleic acid recovery.^{5, 12, 13}
- **Dehydration:** Tissue water is removed using ascending alcohol concentrations before paraffin infiltration. Inadequate dehydration impairs clearing and infiltration, whereas excessive dehydration causes tissue shrinkage and morphological distortion.⁵
- **Clearing:** Alcohol is replaced with a clearing agent compatible with both alcohol and

paraffin. Although xylene remains the standard agent, safer alternatives such as D-limonene and plant-based oils have been developed because of its toxicity.^{1, 4}

- **Paraffin Infiltration and Embedding:** Tissues are infiltrated and embedded in molten paraffin to provide support for microtomy. Wax melting temperature influences tissue quality, with higher temperatures increasing thermal artifacts and nucleic acid degradation, whereas lower temperatures better preserve immunohistochemical and molecular integrity.⁵

Factors Affecting Tissue Quality

Processed tissue quality is influenced by technical and biological factors, particularly processing duration and temperature, which should be adjusted according to tissue size, thickness, and composition to ensure adequate reagent penetration.^{5, 12} Reagent quality, concentration, and freshness also affect tissue preservation and staining, while contaminated or exhausted reagents may compromise specimen quality. Standardized processing and transport protocols further reduce enzymatic degradation and improve the preservation of tissue morphology and molecular components.¹⁴ Collectively, these factors determine specimen quality and the reliability of histopathological, immunohistochemical, and molecular analyses.

3.4 Processing-Related Artifacts and Diagnostic Challenges

Mechanisms of Artifact Formation

Artifacts are artificially introduced tissue alterations that possess no diagnostic relevance and can obscure normal morphology.⁷

- **Pre-analytical factors:** Issues arising before processing, such as delayed fixation leading to autolysis, or the use of exogenous hardware like metal push-pins to orient luminal gastrointestinal specimens on grossing boards.¹¹
- **Processing-related factors:** Variables such as improper dehydration, extreme heating during paraffin infiltration, or exhausted clearing agents cause severe chemical and thermal distortions.⁵
- **Technical handling errors:** Mistakes during embedding, such as incorrect spatial orientation, or during microtomy and mounting, including dull blades or trapped air under coverslips, introduce physical and visual artifacts.¹⁵

Common Histological Artifacts

- **Tissue Shrinkage:** Often caused by the aggressive dehydrating properties of alcohols or the fixative itself (like formalin), resulting in the contraction of cells and artificial widening of intercellular spaces.¹⁶
- **Tissue Folds:** Occur due to improper placement or stretching of the tissue section during the mounting process, causing the tissue to overlap itself and appear artificially thick and dark.⁷
- **Cracks and Mechanical Distortion:** Blunt trauma from forceps, needle crushing during core biopsies, or microtome streak artifacts from hardened paraffin create "crush/squeeze" artifacts that severely deform cellular anatomy.¹⁷
- **Incomplete Infiltration:** Arises from inadequate dehydration or clearing, leaving tissue soft, sunken, or prone to fragmentation

or structural disruption when placed in a water bath.⁵

- **Staining Artifacts:** Chemical interactions, such as residual acetone reacting with hematoxylin during frozen section processing, can precipitate amorphous dye deposits that obscure cellular details.⁸
- **Thermal Artifacts:** Energy-assisted surgical devices (diode lasers or electrocautery) boil intracellular water, inducing tissue necrosis, nuclear elongation, hyperchromatism, and optically clear vacuolar spaces at the resection margins.^{2, 18}
- **Foreign Material and Deposition Artifacts:** Chemical reactions between fixatives and external materials, such as formalin interacting with dissection push-pins, can leach heavy metals (iron crystals) deep into the tissue.¹¹

Diagnostic Implications of Histological Artifacts

The presence of artifacts heavily confounds microscopic interpretation, leading to diagnostic uncertainty. Artifacts can result in false-positive diagnoses by creating "disease mimics".¹¹ For example, iron leached from push-pins is virtually indistinguishable from iron pill-induced mucosal injury, and thermal vacuolization in appendiceal margins can closely mimic the infiltrative glands of goblet cell adenocarcinoma.¹⁸ Tangential slicing from improper orientation can create "twin villi," mimicking celiac disease.¹⁵ False-negative diagnoses also occur when artifacts mask true pathology, such as when incomplete staining artifacts obscure malignant cells during Mohs Micrographic Surgery.⁸ Furthermore, artifacts profoundly degrade the performance of Artificial Intelligence (AI) and digital pathology systems, often causing computational models to fail silently, misclassify tumor boundaries, or generate false-positive cancer detections.^{7, 9} The common histological artifacts, their causes, and diagnostic implications are summarized in Table IV (see Tables section).

Table IV. Common Histological Artifacts and Their Diagnostic Implications

Artifact Type	Cause	Histological Appearance	Potential Diagnostic Consequence (Reference)
Deposition Artifact	Reaction between formalin and metallic dissection push-pins	Crystalline and diffuse granular iron deposits in mucosa	False-positive: Misdiagnosed as iron-pill-induced mucosal injury or gastric siderosis (Olave et al., 2026)
Thermal Artifact	Laser or energy-assisted surgical resection	Vacuolar degeneration, clear spaces, hyperchromatism, and necrosis	False-positive: Misinterpreted as goblet cell adenocarcinoma invasion at margins (Hamed et al., 2026; Gundlapalle et al., 2022)

Artifact Type	Cause	Histological Appearance	Potential Diagnostic Consequence (Reference)
Incomplete Staining	Interaction between acetone dehydration and hematoxylin	Amorphous, eosinophilic blotches crossing cellular boundaries	False-negative: Obscures malignant cellular features during Mohs surgery (Flint et al., 2022)
Crush / Squeeze	Mechanical trauma from needles or forceps during biopsy	Severely flattened, elongated, and distorted nuclei/cells	Diagnostic uncertainty: High inter-pathologist discordance for PD-L1 biomarker scoring (Hacihanoglu et al., 2024)
Tangential Slicing	Improper 3D spatial orientation during paraffin embedding	Overlapping cells, artificial crypt distortion, "twin villi"	False-positive: Misdiagnosed as celiac disease due to illusion of high lymphocyte density (Villanacci et al., 2023)

3.5 Impact of Tissue Processing on Diagnostic Accuracy

Morphological Assessment

The method of tissue preservation dictates the clarity of cellular and tissue architecture. Formalin-fixed paraffin-embedded (FFPE) sections provide vastly superior structural integrity, cellular delineation, and physical stability compared to fresh frozen sections (FFS).¹⁹ Studies demonstrate that FFPE sections consistently yield significantly higher and more reliable quantitative counts of diagnostic features, such as lymphocytes and blood vessels, minimizing measurement errors that occur in artifact-prone frozen tissues.¹⁹

Tumor Diagnosis and Histological Grading

Accurate tumor identification and grading rely on the preservation of precise cellular variegation and margin intactness. Processing artifacts directly compromise grading accuracy and oncologic margin assessment. For instance, incomplete staining deposits or thermal laser artifacts at the edges of resected tissue severely obscure distinguishing features between benign and malignant cells, increasing the risk of misdiagnosis and subsequent surgical recurrence.^{2,8}

Immunohistochemistry

Tissue processing variables heavily influence the reliability of biomarker assessment. Formalin fixation induces extensive protein cross-linking that structurally alters protein folding, effectively masking target epitopes and reducing antibody binding affinity.¹² Consequently, FFPE tissues frequently demonstrate a weaker detection signal for critical biomarkers like PD-L1 compared to fresh frozen tissues, occasionally yielding false-negative

results.²⁰ While heat-induced antigen retrieval methods can partially unmask these proteins, alternative fixatives (like alcohol-based solutions) avoid this cross-linking altogether, providing

superior antigenic stability and stronger immunofluorescence signals without requiring retrieval steps.^{13, 21}

Molecular Pathology

The integrity of DNA and RNA is highly sensitive to pre-analytical processing. Formalin fixation is detrimental to nucleic acids, causing rapid fragmentation and degradation over time, which impedes advanced molecular testing.¹⁴ Conversely, alcohol-based fixatives operate by controlling dehydration rather than cross-linking, allowing for the extraction of significantly higher yields and purer quality of genomic DNA and RNA.¹³ Despite formalin's limitations, emerging technologies like scFFPE-ATAC now enable robust single-cell epigenomic profiling directly from archived FFPE samples.^{22, 23} Furthermore, employing rapid, cold temperature protocols (ISO standard cold transport) minimizes RNase enzymatic activity, protecting the molecular profiles essential for modern precision medicine.¹⁴

3.6 Emerging Advances in Histological Tissue Processing

Automated Tissue Processing

Modern high-throughput laboratories utilize automated fluid transfer (enclosed) processors, which utilize computerized retorts to automatically cycle tissues through fixation, dehydration, clearing, and infiltration.⁵ The primary advantage of these systems is the application of vacuum/pressure, heat, and fluid agitation to enhance reagent penetration while minimizing human handling errors and toxic fume exposure. However, the limitation remains their extensive processing time, typically requiring overnight runs of 12 to 18 hours, delaying the final diagnostic turnaround.²⁴

Microwave-Assisted Tissue Processing

Microwave-assisted processing exploits microwave irradiation to excite polar water molecules within

the tissue, generating instantaneous and uniform heat that drastically accelerates reagent diffusion. Current evidence proves that microwave protocols can shrink the overall processing time from 18 hours to just 60–97 minutes while producing morphological quality equivalent or superior to conventional automated processing.²⁴ Novel systems, such as Variable power cold microwave tissue processing (VCT), offer the added advantage of processing fatty and non-fatty tissues simultaneously in a single universal protocol without using toxic formaldehyde or xylene.⁶

Xylene-Free and Alternative Processing Approaches

Driven by occupational health hazards and environmental safety concerns regarding formaldehyde (a known carcinogen) and xylene (highly toxic to human organ systems and aquatic life), alternative reagents are gaining traction.²⁵

- **Alternative Clearing Agents:** Virgin coconut oil has proven to be a highly effective, bio-friendly substitute for xylene, maintaining crisp nuclear-cytoplasmic contrast and sufficient microtomy rigidity, albeit with slightly higher macroscopic tissue shrinkage.¹
- **Alternative Fixatives:** Alcohol-based fixatives (EMA: ethanol, methanol, and acetic acid) provide faster penetration and superior molecular/antigen preservation compared to formalin.¹³ Additionally, environmentally sustainable natural fixatives — such as honey, jaggery, sugar syrup, and aloe vera — have demonstrated short-term morphological preservation comparable to formalin.²⁵ However, these natural alternatives face significant limitations, including vulnerability to mold formation, attraction of insects, and limited shelf life.⁴

Artificial Intelligence and Digital Pathology Applications

The digital transformation of pathology has spurred the development of powerful AI algorithms designed to tackle processing artifacts.

- **Quality Control and Artifact Detection:** Deep Convolutional Neural Networks (DCNNs) and comprehensive tools like GrandQC provide high-precision, pixel-wise segmentation of multi-class artifacts (folds, blur, pen markings, air bubbles) across Whole Slide Images, automatically excluding clinical noise from diagnostic pipelines.⁹ Furthermore, Deep Kernel Learning (DKL) models have been introduced to provide predictive variance and uncertainty quantification, preventing AI systems from making overconfident misdiagnoses on mechanically damaged tissues.⁷

- **Artifact Restoration and Image Enhancement:** Generative AI models are actively being utilized to salvage damaged slides computationally. ArtiDiffuser, a counterfactual diffusion model, successfully purifies structures obscured by physical artifacts without altering the original stain style.¹⁰ Similarly, Cycle-Consistent Generative Adversarial Networks (CycleGAN) have proven highly effective in digitally recalibrating color profiles distorted by chemical stripping during multiplex immunofluorescence,²⁶ and translating artifact-prone fresh frozen images into archival-quality "virtual FFPE" images.³ These advancements offer massive future opportunities to rescue substandard diagnostic slides and synthesize diverse data augmentations to train highly robust computational pathology systems.

IV. DISCUSSION

4.1 Summary of Main Findings

The present review demonstrates that histological tissue processing is an important pre-analytical determinant that directly influences morphological preservation, molecular integrity, and overall diagnostic accuracy.⁵ Proper tissue processing, particularly conventional formalin-fixed paraffin-embedded (FFPE) techniques, generally provides superior structural preservation, cellular delineation, and section stability compared with fresh frozen sections (FFS).¹⁹ However, processing-related artifacts including incomplete staining, thermal vacuolization, and mechanical crushing may obscure cellular architecture and mimic pathological conditions, thereby contributing to false-positive or false-negative interpretations.^{8, 17, 18}

In immunohistochemical and molecular applications, conventional formalin fixation may reduce antigen accessibility and compromise nucleic acid integrity through extensive protein cross-linking, whereas alternative alcohol-based fixatives and standardized cold-transport protocols have demonstrated improved preservation of DNA, RNA, and selected biomarker signals.^{13, 14} Furthermore, emerging technologies including microwave-assisted and automated tissue processing, alternative processing reagents, and artificial intelligence-assisted quality control and image restoration offer promising strategies to address processing-related and artifact-related challenges, and shorten diagnostic turnaround times while maintaining diagnostic reliability.^{6, 9, 10}

4.2 The Relationship Between Tissue Processing and Diagnostic Accuracy

Tissue processing strongly influences diagnostic accuracy because the quality of histopathological interpretation and computational image analysis

depends heavily on the visual and molecular characteristics of the prepared tissue specimen.^{3,9} A close inverse relationship exists between tissue quality, artifact formation, and diagnostic reliability. For example, mechanical crush and squeeze artifacts in needle core biopsies may substantially increase diagnostic uncertainty and reduce inter-observer agreement during biomarker assessment, including PD-L1 scoring.¹⁷ Similarly, exogenous processing artifacts, such as iron deposition resulting from dissection push-pins, may create convincing morphological mimics of iron pill-induced mucosal injury and contribute to false-positive diagnoses.¹¹ Preservation of morphology, antigenicity, and molecular integrity remains essential for modern oncologic pathology. Comparative studies indicate that FFPE tissues generally provide superior cytoarchitectural preservation and facilitate more reliable quantitative assessment of histological features compared with fresh frozen sections.¹⁹ Nevertheless, molecular preservation remains a challenge, as FFPE processing may reduce biomarker detectability and produce weaker immunohistochemical signals compared with matched frozen tissues.²⁰ Recent technological developments seek to address this limitation. For instance, scFFPE-ATAC has demonstrated that archived FFPE samples can still yield high-quality epigenomic information when optimized molecular workflows are applied.²²

4.3 Current Challenges in Histological Tissue Processing

Variability in Processing Protocols

Differences in fixation duration, dehydration procedures, clearing agents, and laboratory workflows remain major sources of variability in tissue quality.⁵ Under-fixation may fail to adequately preserve tissue structure and prevent enzymatic degradation, whereas over-fixation can increase protein cross-linking and negatively affect immunohistochemical staining performance.¹² Such variability contributes to inconsistencies in diagnostic interpretation and may reduce reproducibility across institutions.

Processing-Related Artifacts

Artifact formation continues to represent a persistent challenge throughout the tissue processing workflow. Thermal artifacts, staining artifacts, tissue folds, crush artifacts, and deposition artifacts may distort normal morphology and closely resemble pathological conditions.^{8,18} Consequently, these artifacts may increase diagnostic uncertainty and contribute to both false-positive and false-negative interpretations.

Balancing Morphological and Molecular Preservation

A major challenge in contemporary pathology is achieving optimal preservation of both tissue morphology and molecular integrity.¹⁴ While formalin fixation remains highly effective for preserving tissue architecture, it may reduce antigen accessibility and induce nucleic acid fragmentation.¹³ Conversely, alcohol-based fixatives may provide improved molecular preservation but can introduce morphological alterations such as tissue shrinkage and hardening if not carefully optimized.^{16,13}

Resource and Infrastructure Limitations

Many conventional tissue processing protocols still require prolonged processing cycles, often extending overnight, which may delay diagnostic turnaround times.²⁴ In addition, optimal preservation of tissue morphology and molecular integrity frequently depends on standardized transport systems, controlled laboratory environments, and specialized processing equipment. These requirements may be difficult to implement in resource-limited settings due to financial constraints, infrastructure limitations, and shortages of trained personnel.^{6,14}

4.4 Future Perspectives

Alternative and Sustainable Processing Reagents

Growing concerns regarding the toxicity of formaldehyde and xylene have stimulated the development of safer and environmentally sustainable alternatives. Bio-based clearing agents such as coconut oil and naturally derived fixatives including honey, jaggery, and aloe vera have demonstrated promising tissue preservation capabilities while reducing occupational and environmental hazards.^{1,4,25} Future research should focus on improving the long-term stability, reproducibility, and molecular compatibility of these alternatives.

Standardization of Pre-Analytical Variables

Increasing emphasis is being placed on the standardization of tissue handling and processing protocols. Variations in fixation duration, transportation conditions, reagent quality, and processing schedules remain important sources of inter-laboratory variability. The implementation of standardized protocols and quality assurance systems may improve reproducibility, preserve molecular integrity, and enhance diagnostic consistency across institutions.¹⁴

Rapid and Automated Tissue Processing

Advances in automation and microwave-assisted tissue processing offer opportunities to substantially reduce processing time while maintaining tissue quality. Technologies such as variable power cold microwave processing have demonstrated the ability

to shorten turnaround times from many hours to less than two hours while reducing dependence on hazardous chemicals.^{6, 24} Further large-scale validation studies are required to confirm their performance across different tissue types and routine clinical workloads.

Integration with Molecular Pathology, Digital Pathology, and Artificial Intelligence

As precision medicine continues to evolve, tissue processing protocols must increasingly support histomorphological assessment, immunohistochemistry, molecular diagnostics, and computational pathology. Technologies such as scFFPE-ATAC have demonstrated the feasibility of extracting high-quality molecular information from routinely processed FFPE tissues.²² Simultaneously, AI-based systems for artifact detection, quality control, and image restoration may assist pathologists in minimizing the impact of processing-related artifacts and improving diagnostic reliability.^{7, 9, 10} Nevertheless, extensive multi-institutional validation remains necessary before these technologies can be routinely implemented in clinical practice.

4.5 Limitations of the Review

Several limitations should be considered when interpreting the findings of this review. First, the available literature on histological tissue processing encompasses multiple areas of investigation, including tissue preservation, artifact formation, immunohistochemistry, molecular pathology, digital pathology, and artificial intelligence applications, resulting in the use of diverse outcome measures and evaluation methods.^{7, 8, 17} Consequently, direct quantitative comparison across studies was limited, and a narrative synthesis was considered the most appropriate approach for integrating the available evidence. In addition, several emerging technologies and alternative processing approaches remain under active investigation and require further validation across different tissue types, laboratory settings, and clinical workflows.^{9, 10, 25}

Nevertheless, this review integrates evidence from multiple domains of modern pathology and provides a comprehensive overview of the current knowledge regarding the influence of histological tissue processing on tissue quality, artifact formation, molecular preservation, and diagnostic accuracy, while also identifying important opportunities for future research and technological development.

4.6 Clinical and Scientific Implications

Implications for Pathologists

The findings of this review highlight the importance of recognizing processing-related artifacts and disease mimics during histopathological evaluation. Awareness of artifacts such as iron deposition

caused by dissection push-pins or thermal changes induced by energy-based surgical devices may help reduce diagnostic uncertainty and prevent potential false-positive interpretations that could affect patient management.^{11, 18}

Implications for Laboratory Practice

The reviewed evidence suggests that optimization and standardization of tissue processing protocols can improve specimen quality, diagnostic consistency, and laboratory efficiency. Emerging technologies such as automated and microwave-assisted tissue processing systems may offer opportunities to reduce turnaround times, enhance workflow efficiency, and potentially decrease exposure to hazardous processing reagents while maintaining acceptable histological quality.^{6, 24}

Implications for Histotechnology

As histopathology increasingly incorporates molecular diagnostics and digital pathology, histotechnologists play a critical role in ensuring high-quality specimen preparation. The implementation of standardized pre-analytical procedures, including appropriate fixation, controlled tissue handling, accurate specimen orientation, and quality assurance measures, may contribute to improved morphological preservation and more reliable downstream molecular and computational analyses.^{12, 14}

Implications for Future Research

Future research should focus on the large-scale validation of emerging tissue processing technologies across diverse laboratory settings and tissue types. Particular attention should be directed toward evaluating the long-term stability and molecular compatibility of sustainable fixatives, improving rapid processing methodologies, and assessing the clinical applicability of AI-based artifact detection and image restoration systems.^{10, 25} In addition, further refinement of molecular technologies capable of utilizing routinely processed FFPE specimens may enhance the integration of histopathology with precision medicine and advanced biomarker discovery.²²

V. CONCLUSION

Histological tissue processing is a critical determinant of tissue quality and diagnostic accuracy in histopathology. Appropriate processing preserves tissue morphology, molecular integrity, and staining quality, whereas processing-related artifacts may compromise diagnostic interpretation and reliability. Emerging advances, including alternative processing reagents, automated and microwave-assisted processing, and artificial intelligence-assisted quality control, offer promising opportunities to improve specimen quality and

laboratory efficiency. Continued optimization and standardization of tissue processing protocols are essential to support accurate histopathological diagnosis and the growing demands of molecular pathology, digital pathology, and precision medicine.

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Conflict of Interest

The authors declare no conflict of interest.

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