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REVIEW ARTICLE

# REVIEW ON ARTIFACTS IN HISTOPATHOLOGY AND THEIR REMEDIES

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**ABSTRACT:** Histopathological examination is an essential tool in medical diagnostics, allowing for the assessment of tissue samples to identify pathological conditions. However, artifacts introduced during tissue processing can obscure accurate interpretation, potentially leading to diagnostic errors. These artifacts may result from improper handling during fixation, sectioning, staining, or mounting. This review aims to highlight the types of artifacts encountered in histopathology, their causes, and potential remedies to enhance diagnostic precision.

**Keywords**: Tissues, Tissue processing, Common Artifacts, Remedies.

## INTRODUCTION:

Histopathology plays a vital role in medical diagnosis by enabling microscopic examination of tissues. Through meticulous processes such as sectioning, staining, and fixation, pathologists can detect various pathological conditions. However, the introduction of artifacts during any of these stages can lead to misinterpretation, complicating disease diagnosis. Artifacts are artificial changes that may mimic or obscure tissue morphology, causing potential diagnostic pitfalls [1,2].

Accurate histopathological analysis requires a thorough understanding of the various artifacts that can arise and strategies to minimize their impact. Pathologists must differentiate between true pathological changes and artifacts to provide precise diagnoses [3]. This review categorizes common artifacts based on their occurrence during different stages of histopathological processing and explores strategies to prevent them.

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For pathologists to accurately diagnose various lesions under the microscope, it is essential to prepare tissue sections, often stained, that closely replicate their natural structure. Achieving high-quality tissue sections demands considerable expertise and proficiency in laboratory practices [4]. However, it is not uncommon for pathologists to encounter slides that have been compromised due to improper fixation or mistakes made during tissue processing. These mishandled procedures can lead to changes in the tissue details that affect the accuracy of interpretation [5]. Artifacts are defined as artificial structures or changes in tissue observed on a microscopic slide caused by external factors. Artifacts pose a significant challenge in diagnostics, often complicating or misleading the interpretation process. This makes understanding the sources of these artifacts, as well as methods for identifying and minimizing their impact, crucial for ensuring accurate diagnosis and avoiding potential diagnostic pitfalls [5].

Histopathology is a field centered on the microscopic examination and interpretation of tissue samples. The presence or alteration of foreign substances within tissue details can sometimes lead to confusion and potentially result in incorrect or inconclusive diagnoses [6]. Artefacts can develop at various stages, including before tissue fixation, during specimen securing, grossing, processing, sectioning, staining, or preservation [7]. While some artefacts are easily distinguished from normal or pathological tissue components, others can be challenging to differentiate, making them a significant source of diagnostic challenges [8].

For instance, poor fixation can result in autolysis, which mimics necrotic tissue, leading to misdiagnosis in inflammatory or neoplastic conditions. Similarly, formalin pigment deposition, caused by improper fixation, may be mistaken for hemosiderin, potentially leading to an erroneous conclusion of hemorrhage or iron overload disorder[9]. Another common artifact, chatter or microtome-induced knife marks, can resemble glandular structures, sometimes resulting in the misinterpretation of benign tissue as malignancy. Such diagnostic pitfalls underscore the importance of recognizing and mitigating artifacts to improve the reliability of histopathological evaluations [10].

**Common Artifacts and Their Causes**

Artifacts in histopathology arise at various stages of tissue processing. These include:

1. **Prefixation Artifacts:** These occur before the fixation stage, often due to poor biopsy techniques, mishandling, or exposure to external contaminants. Common prefixation artifacts include forceps-induced crushing, curling due to tissue shrinkage, and splitting caused by excessive handling [11]. Forceps-induced crushing can lead to distorted cellular morphology, mimicking necrosis or inflammatory cell infiltration, which may result in an incorrect diagnosis of tissue injury or an inflammatory condition. Similarly, curling of the tissue can create uneven staining patterns, complicating the evaluation of cellular structures and potentially leading to misinterpretation of architectural distortion in neoplastic or dysplastic lesions. Splitting due to excessive handling may resemble tissue fragmentation seen in necrotic tumors, causing diagnostic confusion [12].
2. **Fixation Artifacts:** Fixation is crucial for preserving tissue morphology, but improper fixation can lead to artifacts. Formalin pigment formation, ice-crystal artifacts, and tissue shrinkage due to prolonged fixation can affect the quality of the sample. The use of unbuffered formalin often results in dark-brown formalin pigment deposits, complicating interpretation [13].
3. **Tissue Processing Artifacts:** Dehydration and clearing errors lead to improper embedding and sectioning. Over-dehydration causes shrinkage artifacts, while incomplete dehydration results in poor paraffin infiltration, making the tissue

 

difficult to cut. Clearing agent residues, such as excess xylene, may cause brittleness [14]. Incomplete dehydration is particularly problematic as it leads to inadequate paraffin embedding, causing spongy or poorly supported tissue sections that are prone to tearing or collapsing during microtomy [15]. This may result in artifacts that resemble autolysis or tissue breakdown, potentially leading to a misdiagnosis of necrosis or degenerative changes. Additionally, clearing agent residues, such as excess xylene, may cause tissue brittleness, making sectioning more challenging and affecting overall histological quality [16].

4. **Microtomy Artifacts:** Errors during sectioning, such as tearing, compression, and chatter, often result from blunt blades or incorrect cutting angles. Chatter artifacts create alternating thick and thin areas in tissue sections, while compression artifacts distort tissue structures, leading to misinterpretation [17].
5. **Staining Artifacts:** Improper staining techniques introduce artifacts such as incomplete staining due to residual wax, excessive acid application affecting eosin staining, and hematoxylin crystallization leading to pigment deposits. Overstaining and under-staining result from poor reagent preparation and handling [18].
6. **Mounting Artifacts:** The final step in histopathology involves mounting the stained sample onto a slide for examination. Errors such as air bubbles, dry mounting, and excessive mounting media affect the clarity of microscopic observations. Air bubbles trapped under the coverslip create refractive issues, while excess mounting medium can cause a foggy appearance [19].

**Remedies for Common Artifacts**

Each stage of histopathology presents unique challenges that require specific interventions to prevent artifacts. Integrating these remedies into routine workflows and laboratory training programs can enhance diagnostic accuracy by minimizing errors and ensuring consistency. Standardization of protocols across laboratories, including the use of automated tissue processors and adherence to best practices, can further improve the quality and reproducibility of histopathological samples [20]. The following measures can be implemented:

1. **Prefixation Remedies:** Careful handling of biopsy specimens using appropriate forceps minimizes mechanical damage. Immediate fixation in appropriate media prevents dehydration and curling of tissues [1]. Implementing standardized specimen collection protocols and training personnel in proper biopsy handling can reduce inconsistencies and improve tissue integrity.
2. **Fixation Remedies:** Using buffered formalin prevents formalin pigment deposition. Ice-crystal artifacts can be avoided by employing rapid freezing techniques, and shrinkage artifacts can be reduced by using compound fixatives. Standardizing fixation times and ensuring uniform fixation conditions across laboratories help maintain sample quality and reduce variability in histological interpretation [20].
3. **Tissue Processing Remedies:** Gradual dehydration in increasing alcohol concentrations prevents excessive shrinkage. Proper clearing and embedding techniques ensure uniform paraffin infiltration. Utilizing automated tissue processors ensures consistent dehydration times and paraffin embedding, minimizing human error and variability. Regular quality control checks on processing reagents and protocols can further enhance reliability [21].
4. **Microtomy Remedies:** Using sharp blades and adjusting the clearance angle reduces tearing and chatter artifacts. Cooling wax blocks before sectioning prevents compression artifacts [14]. Training histotechnologists in standardized cutting techniques and maintaining equipment

 

properly can significantly reduce sectioning errors. Additionally, implementing quality control measures, such as routine assessment of blade sharpness and microtome calibration, helps ensure optimal sectioning outcomes.

5. **Staining Remedies:** Ensuring complete dewaxing, maintaining proper reagent concentrations, and following standardized staining protocols help prevent staining artifacts. Standardizing staining procedures, including automated staining systems, reduces inconsistencies and enhances reproducibility across different laboratories. Regular monitoring of reagent quality and adherence to strict staining protocols further ensures diagnostic reliability [15].
6. **Mounting Remedies:** Using an adequate amount of mounting media and avoiding air bubble entrapment ensures clear visualization of tissue sections. Correct coverslip application prevents drying artifacts and uneven mounting. Automated mounting techniques and adherence to best practices in slide preparation contribute to long-term stability and clarity of histological samples [22].

By incorporating these preventive measures into routine histopathology workflows and promoting standardization across laboratories, systemic improvements in diagnostic accuracy and reproducibility can be achieved. Establishing quality control measures, continuous training programs, and the use of automation where applicable ensures high standards in histopathological processing, ultimately reducing the occurrence of artifacts and enhancing patient outcomes.

## DISCUSSION:

Artifacts in histopathology remain a significant challenge in diagnostic accuracy, often leading to misinterpretation of tissue samples. They can emerge at various stages, including prefixation, fixation, tissue processing, microtomy, staining, and mounting [19]. Each of these artifacts can alter tissue morphology, potentially leading to erroneous diagnoses. For instance, fixation artifacts like formalin pigment deposition can obscure cellular details, while microtomy artifacts such as compression can distort tissue structure [3]. Real-world cases illustrate the clinical impact of histological artifacts. For example, poor fixation can result in autolysis, which mimics necrotic tissue and may lead to an incorrect diagnosis of tumor necrosis in malignancies, potentially altering treatment decisions. Similarly, chatter artifacts caused by improper microtomy techniques can create gland-like structures that may be misinterpreted as adenocarcinoma, leading to unnecessary interventions. Staining artifacts, such as overstaining with hematoxylin, can obscure nuclear details, making it difficult to assess dysplasia accurately. These examples underscore the critical need for identifying and mitigating artifacts to prevent diagnostic errors. Addressing these issues requires implementing standardized laboratory practices, such as using appropriate fixatives, optimizing sectioning techniques, and ensuring proper staining protocols [13]. However, beyond standardization, continuous quality control measures play a vital role in reducing artifacts. Regular calibration of equipment, adherence to standardized processing times, and routine evaluation of reagent quality can significantly minimize errors [23]. Accreditation programs, such as those set by the College of American Pathologists (CAP) or the International Organization for Standardization (ISO), enforce stringent laboratory quality assurance protocols, ensuring that histopathological processes remain consistent and reliable [24]. Furthermore, ongoing training programs for laboratory personnel are essential in reinforcing best practices and keeping up with advancements in histopathological techniques. Routine quality audits, peer reviews, and proficiency testing further help laboratories identify and correct potential sources of artifacts before they impact diagnostic accuracy. Additionally, continuous training and adherence to quality control measures can help minimize errors. Recognizing and differentiating artifacts from pathological changes is crucial in

 

ensuring accurate histopathological evaluations, ultimately contributing to better patient outcomes and treatment decisions [22]. By integrating robust quality control measures and promoting continuous education, laboratories can significantly reduce the prevalence of artifacts, leading to more reliable and precise diagnoses [25].

## CONCLUSION:

Histopathology is an invaluable diagnostic tool, yet the presence of artifacts poses a challenge in achieving accurate interpretations. Artifacts can occur at multiple stages, including prefixation, fixation, tissue processing, microtomy, staining, and mounting. Proper handling techniques, standardized protocols, and quality control measures are essential in minimizing artifacts and ensuring precise histopathological diagnoses. Reducing artifacts directly improves patient outcomes by enhancing diagnostic accuracy, preventing misdiagnosis, and ensuring appropriate treatment decisions. Minimizing artifacts helps pathologists distinguish between true pathological changes and processing-related distortions, reducing the risk of unnecessary treatments or missed diagnoses. This, in turn, leads to timely and effective clinical interventions, ultimately improving prognosis and patient care. Recognizing and addressing these artifacts significantly enhances diagnostic reliability, reinforcing the need for meticulous histopathological practices to support better healthcare outcomes.

Recognizing and addressing these artifacts significantly enhances diagnostic accuracy and patient outcomes, reinforcing the need for meticulous histopathological practices.

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