

Review Article**Artefacts in tissue processing: A review**

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ABSTRACT

An artificial feature in tissue sections that is self-colored and unrelated is called an artefact. This has been an age long cause of misinterpretation that leads to misdiagnosis. It can result in alteration of normal morphologic and cytologic features that may occur as a result of the way the tissue has been handled, right from the time the biopsy, which is surgically obtained till the entire histopathological procedures of fixation, processing, embedding, sectioning and staining are performed on it. This article reviews the common artefacts encountered during tissue processing.

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1. Introduction

An artefact can be defined as an artificial structure or tissue alteration on a prepared microscopic slide as a result of an extraneous factor.¹ These artefacts cause typical morphologic and cytologic traits to change, or they may even cause the tissue to become completely unusable.² These have been shown to occur at various stages: During surgical removal, fixation, processing, embedding or staining of tissue sections.³ Understanding and being aware of artifacts is important since it helps to identify them can help avoid misdiagnosis.⁴ Therefore, this article is to promote an awareness of the various common artifacts that may be found during tissue processing, to offer guidance for identifying them, to elucidate their causes and whenever practical, to recommend ways in which their occurrence can be avoided.

1.1. Causes of artefacts¹

1. Clinical application of chemicals
2. Local injection of anesthetics
3. Surgical suctioning
4. Excessive heat
5. Freezing
6. Surgical mishandling of specimen
7. Inadequate tissue fixation
8. Improper fixation medium
9. Faulty tissue processing
10. Embedded sponges
11. Improper staining

1.2. Classification of artefacts^{4,5}

1. During surgery
2. Injection artefacts
3. Forceps artefacts
4. Fulguration artefacts
5. During fixation and transport
6. Fixation artefacts

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7. Freezing artefacts
8. Artefacts during transportation
9. Tissue processing artefacts
10. Other artefacts

1.3. Processing artefacts

A procedural routine is followed during the processing of an oral biopsy specimen in order to produce a tissue fit for diagnosis and interpretation. The processes itself are prone to mistakes by people and materials, producing an artifact that might, at the very least, make a diagnosis more difficult to make or, at the most, cause the tissue to become so warped that it cannot be made. Following fixation, the tissue must be gradually dehydrated, beginning with 50% alcohol.^{5–7}

1.4. Artefacts during dehydration

1.4.1. Artefacts can occur during dehydration due to

1. Improper gradient of dehydration: Diffusion current crosses cell membranes during fluid exchange if there is a high concentration gradient between the fluid inside and outside the tissue, increasing the risk of cell deformation.^{6,8}
2. Excessive dehydration causes the tissue to become hard, brittle, and shrunken, making cutting difficult and also interfering with the staining.⁹
3. Under or Incomplete dehydration results in inappropriate penetration of paraffin and block created is difficult to section therefore resulting in distorted or fragmented tissue sections which generates artefactual features.

1.4.2. Artefacts during clearing

Artefacts may form owing to over and under cleaning of tissue producing excessive hardness, and hence hinder paraffin impregnation of tissue making it harder to cut during sectioning.^{6,10}

1.4.3. Artefacts during impregnation

The purpose of wax impregnation is to rid the tissue of the clearing agent (wax solvent), allowing paraffin wax to fully penetrate it. The wax is then allowed to solidify to form a block from which sections are cut. Crystallization is the artifact that is formed during this process. Since thicker tissue absorbs more cleaning agent, it takes more molten wax to completely impregnate; therefore, repeated changes are needed. When a clearing agent contaminates melted wax, it results in insufficient impregnation, which during sectioning produces cracking and crystallization.¹¹

1.4.4. Artefacts during embedding

During embedding processes, it is common to encounter artifacts resulting from poor orientation. These artefacts can cause damage to the microtome and tear the sections,

making it difficult to study tissue under a microscope.¹²

1.4.5. Artefacts due to poor processing

Inadequate fixation may be the cause of a significant loss of architectural elements and clarity in loose connective tissue. These may result from mistakes made during the tissue processing cycle, such as using chemicals that are depleted, choosing the wrong reagents, or not switching solvents in a timely enough manner.^{9,10}

1.4.6. Sponge artefacts

These are seen in tissues placed in cassettes sandwiched between sponges. The tissue has angulated holes around its periphery, which are frequently triangular in shape and have smooth edges. Occasionally, a small intermediate channel bridging the separate faults connects paired angulated holes. The stromal matrix and tissue consistency are related to the extent of sponge penetration. For instance, sponge brushes tend to penetrate softer, loosely fixed specimens more than they do firmer, well-fixed tissue.^{13,14}

1.5. Prevention: Tissue or lens paper is recommended as a substitute

1.5.1. Loss of soluble substances

Fat solvents are used in the manufacture of paraffin wax, cellulose nitrate, and the majority of synthetic resin embedded sections. Consequently, as adipose tissue is processed, fat will separate from fat cells and manifest as void areas encircled by a cytoplasmic rim, such as lipomas and cholesterol clefts in odontogenic cysts.¹⁵

1.5.2. Orientation artefact

Improper orientation will lead to disorderly arranged histological features in slide. In order for all strata to be visible on the completed slide, the epithelial margins, subcutaneous tissue, and deeper layers must all be oriented such that they are flat to the bottom. To ensure that the sliced section presents a legitimate representation of the tissue submitted, all tissue pieces should be securely embedded into the bottom of the container when more than one specimen is being embedded.¹²

2. Conclusion

During any of the numerous phases a specimen goes through before the pathologist examines its features under a microscope, tissue artifacts may be added to the specimen. Therefore, minimizing artefacts will need meticulous tissue preparation, timely fixation, and appropriate tissue handling. Any level of subpar care could lead to a biopsy that is not diagnostic, which would require repeat surgery for the patient and result in further physical and psychological complications.

3. Source of Funding

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4. Conflict of Interest

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