

# Artifacts in Oral Biopsy Specimens

## Histological Analysis and Assessment of Clinical Knowledge in a Brazilian Reference Center

INVESTIGATION





**Artefactos en especímenes de biopsias orales**  
Análisis histológico y evaluación del conocimiento clínico en un centro de referencia brasileño

**Artefatos em Espécimes de Biópsias Oraís**  
Análise Histológica e Avaliação do Conhecimento Clínico em um Centro de Referência Brasileiro

### Abstract

Artifacts in histopathology are artificial structures or tissue alterations produced during biopsy or tissue processing that can modify the sample or lead to diagnostic errors. This study aimed to assess dental clinicians' knowledge of artifacts and to identify the most common artifacts found in histological slides from an oral pathology laboratory, with the goal of reducing their prevalence. An online questionnaire was administered to laboratory users, and 200 histological slides were evaluated by two calibrated examiners. Results were analyzed statistically. Forty-four users of the Oral Pathology Laboratory completed the questionnaire. Most respondents were women (63.6%), over 40 years old (54.5%), graduated more than 20 years ago (52.3%), from public universities (72.7%), and non-specialists (54.5%). A total of 88.6% correctly answered more than 70% of the questionnaire. Non-specialists demonstrated greater knowledge of artifacts, while other comparisons were not significant. The most frequent artifact during biopsy was the crush or compression artifact (30.5%), whereas in the laboratory, tears or splits were the most common (92%). All slides presented at least one processing artifact. Although most users possess basic knowledge of the topic, the prevalence of artifacts remains high. Understanding artifacts is essential for clinicians and pathologists to reduce their prevalence and prevent diagnostic errors.

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## Resumen

Los artefactos en histopatología son estructuras artificiales o alteraciones del tejido que se producen durante la biopsia o el procesamiento del tejido, y que pueden modificar la muestra o inducir a un diagnóstico erróneo. El objetivo de este estudio fue evaluar el conocimiento de los odontólogos sobre los artefactos y analizar los artefactos más comunes presentes en láminas histológicas de un laboratorio de patología oral, con el fin de reducir su ocurrencia. Se aplicó un cuestionario en línea a odontólogos usuarios del laboratorio y se evaluaron 200 láminas histológicas por dos examinadores calibrados. Los resultados fueron analizados estadísticamente. Cuarenta y cuatro usuarios del laboratorio respondieron el cuestionario. La mayoría eran mujeres (63,6%), mayores de 40 años (54,5%), graduados hace más de 20 años (52,3%), egresados de universidades públicas (72,7%) y no especialistas (54,5%). El 88,6% respondió correctamente más del 70% del cuestionario. Se observó que los no especialistas tenían mayor conocimiento sobre los artefactos. El artefacto más común durante la biopsia fue el de compresión (30,5%), mientras que en el laboratorio fueron los desgarros o fisuras (92%). Todas las láminas presentaron al menos un artefacto de procesamiento. Aunque la mayoría de los usuarios tiene conocimientos básicos sobre el tema, la prevalencia de artefactos sigue siendo alta. La comprensión de los artefactos es fundamental para los odontólogos y patólogos a fin de reducir su prevalencia y evitar errores diagnósticos.

**Palabras clave:** Artefactos histológicos; Histología; Patología bucal; Biopsia; Diagnóstico.

## Introduction

Histopathology consists of the microscopic study of tissues obtained from an individual for diagnostic or research purposes. For tissue samples to be efficiently analyzed under the microscope, the specimen must go through several stages: fixation, processing (dehydration, clearing, infiltration), embedding, microtomy, staining, and mounting.<sup>(1)</sup> Each of these stages, from specimen collection to processing, requires careful execution and handling, as artifacts can be introduced at any time.

Artifacts can be defined as artificial structures or tissue alterations present on a slide as a result of external factors,<sup>(2)</sup> which can interfere with the diagnostic process. These alterations can range from minor changes

## Resumo

Os artefatos em histopatologia são estruturas artificiais ou alterações teciduais produzidas durante a biópsia ou o processamento do tecido, que podem modificar a amostra ou induzir a erros diagnósticos. O objetivo deste estudo foi avaliar o conhecimento de cirurgiões-dentistas sobre artefatos e identificar os mais comuns em lâminas histológicas provenientes de um laboratório de patologia oral, visando à redução de sua ocorrência. Aplicou-se um questionário online a profissionais da odontologia e foram avaliadas 200 lâminas histológicas por dois examinadores calibrados. Os resultados foram analisados estatisticamente. Quarenta e quatro usuários do laboratório responderam ao questionário. A maioria era do sexo feminino (63,6%), com mais de 40 anos (54,5%), formados há mais de 20 anos (52,3%), oriundos de universidades públicas (72,7%) e não especialistas (54,5%). Do total, 88,6% responderam corretamente mais de 70% do questionário. Os não especialistas demonstraram maior conhecimento sobre artefatos. O artefato mais frequente durante a biópsia foi o de esmagamento (30,5%), enquanto no laboratório foram os rasgos (92%). Todas as lâminas apresentaram ao menos um artefato de processamento. Apesar do conhecimento básico observado, a alta prevalência de artefatos reforça a importância do tema para evitar erros diagnósticos.

**Palavras-chave:** Artefatos histológicos; Histologia; Patologia oral; Biópsia; Diagnóstico.

that affect only part of the specimen without compromising the final diagnosis to making it completely unsuitable for analysis. In some cases, artifacts may form structures that resemble real pathological findings, which can lead to an incorrect diagnosis that does not reflect the true state of the tissue or the characteristics of the pathology.<sup>(1,3)</sup>

The most common artifacts related to biopsy originate from the instruments used to obtain the tissue (scalpel, punch, scissors, forceps), which can cause tearing, compression artifacts, or thermal damage when an electrosurgical scalpel is used. In addition, local anesthesia injection may produce artifacts such as bleeding or artificial spaces, which could mislead the pathologist.<sup>(4)</sup> During fixation, artifacts are related to the type, quality,

and duration of the fixative, which can cause hardening, retraction, pigment formation, and chemical alterations. Good fixation practices—such as using the appropriate fixative, in sufficient volume, and for the correct amount of time—are essential to prevent these problems.<sup>(5)</sup> After fixation, responsibility generally falls on the laboratory technician or pathologist to carry out the following stages: decalcification (if necessary), processing, embedding, microtomy, staining, and mounting.<sup>(1)</sup>

Although the goal of histological processing is to obtain a preparation that represents as faithfully as possible the original structure of the tissue, this is not always achieved, as the presence of artifacts is extremely common. Therefore, it is essential that dentists, laboratory technicians, and pathologists are familiar with artifacts, understand their causes and microscopic appearance, and know how to prevent them. Our objective was to evaluate dentists' knowledge of artifacts and to analyze the most frequent artifacts found on histological slides in an oral pathology laboratory in order to reduce their occurrence.

## Materials and Methods

This research was approved by the Institutional Ethics Committee (CAAE 57368822.6.0000.0077). This cross-sectional study was conducted with users of the Oral Pathology Laboratory of the Institute of Science and Technology, São Paulo State University (UNESP), who have used the service since 2017 ( $n = 235$ ), the year in which the online system for submitting requests and issuing reports was implemented.

### QUESTIONNAIRE

A self-administered digital questionnaire (Google Forms) was used to assess participants' knowledge of histopathological artifacts and their biopsy practices. The questionnaire included 24 closed-ended, Likert-type questions organized into three sections: (1) one initial question about whether the professional performed biopsies in their clinical practice; (2) fifteen questions regarding the knowledge and prevention of histopathological artifacts, including the choice of instruments, timing of specimen fixation, influence of surgical technique and anesthesia on histological analysis, use of methods such as electrosurgery and laser, types and volumes of fixatives used, and methods of container identification; and (3) eight questions about the participant's sociodemographic and professional profile.

The questionnaire underwent a preliminary validation process that included a pilot test with ten labora-

tory users to assess the clarity and consistency of the questions, as well as review by two specialists in the field, who confirmed the adequacy, relevance, and scope of the content. After the necessary adjustments were made, the final version was distributed by e-mail to the entire target population. Two weeks after the first mailing, it was re-sent to those who had not responded, and one month later, the results were tabulated.

Upon completion of the questionnaire, participants received by e-mail an e-book containing recommendations on good practices for performing biopsies to minimize the prevalence of artifacts. Subsequently, the e-book was updated to include the information obtained from this study.

For the analysis of the total number of correct answers, questions related to personal experiences, individual preferences, and the use of toluidine blue were excluded, leaving 11 items. Responses were considered correct when they acknowledged: the influence of surgical technique and tissue alterations on histological analysis; the importance of properly handling thin specimens; that the use of forceps or direct suction can interfere with interpretation; and that fixation should be performed within 10 minutes after biopsy, using 10% buffered formalin in a volume at least ten times greater than that of the specimen, with proper identification of the container.

The responses were analyzed descriptively (absolute and relative frequencies) and by means of statistical tests: Chi-square for qualitative variables and Student's t-test to correlate the number of correct answers with sex, educational institution, and specialist status (Sphinx®, Sphinx Brasil, Canoas, RS, Brazil).

### LITERATURE REVIEW

An exploratory literature review was conducted to identify and describe the main histopathological artifacts reported in biopsy specimens, with the aim of guiding the analysis of the histological slides included in this study. For this purpose, several databases (PubMed, Scopus, and SciELO) were searched using terms such as *histological artifacts*, *artifacts in histopathology*, and *artifacts in oral biopsy specimens*, as well as gray literature located through Google, including manuals, books, and teaching materials. In addition, the reference lists of selected papers were reviewed to locate additional relevant information. The findings obtained were organized and compiled into tables that include the mode of artifact production and their microscopic description.

## HISTOLOGICAL SLIDES

A total of 200 histological cases submitted to the Oral Pathology Laboratory were selected. Half were processed by each of the two laboratory technicians and stained with hematoxylin and eosin. One out of every five cases was chosen, following the order of receipt of the specimens, always selecting the first slide prepared from each case.

The slides were jointly evaluated by two previously calibrated examiners. The artifacts identified in each histological section, whether produced during surgery or in the laboratory, were recorded using a dichotomous classification (present or absent).

## Results

### QUESTIONNAIRE

Responses were obtained from 52 participants (22.1% of laboratory users), of whom 15.4% did not perform biopsies, possibly representing users who had submitted material only once. The remaining 44 constituted the final sample for analyzing knowledge of histopathological artifacts and practices related to biopsy collection and processing, in addition to sociodemographic and professional data.

The sample was mainly composed of women over 40 years of age, who had graduated more than 20 years ago, and most were graduates of public universities. Regarding specific knowledge and clinical practices, most respondents had heard of artifacts, correctly identified the site of anesthesia, and recognized the influence of surgical technique on histological analysis. All fixed the specimens in 10% formalin within 10 minutes after surgical removal. Detailed results are presented in **Table 1**.

The correct answer rate for the 11 selected questions ranged from 63.6% (11.4% of the sample) to 100% (29.5% of the sample), with an average of 85.7%. A total of 88.6% of participants answered correctly at least 72.2% of the questionnaire (8 or more questions) (**Figure 1**). Participants were divided into two groups: those with higher scores (90.9% or 100%, corresponding to 10 or 11 correct answers; 47.7% of the sample) and those with lower scores (63.6%, corresponding to 7 correct answers; 11.4% of the sample). When analyzing the number of correct answers together with epidemiological data, it was observed that participants who scored 90.9% or higher were predominantly non-specialists (61.9%), female (58.9%), under 32 years of age

(42.9%), graduates between 2013 and 2021 (47.6%), and graduates of public institutions (71.4%). In contrast, those who scored below 63.6% were also mostly women (69.4%), but had graduated before 2003 (100%), came from public universities (60%), were specialists (80%), and were between 40 and 49 years old (60%).

Statistical analysis of the number of correct answers in relation to sex, year of graduation, educational institution, specialty, and age revealed differences only between specialists and non-specialists, with significantly higher scores among non-specialists ( $t = 2.1$ ;  $p = 0.04$ ).

### LITERATURE REVIEW

As a result of the exploratory literature review, the main histopathological artifacts reported during tissue processing, both in clinical and laboratory settings, were identified and described. These findings are summarized in **Tables 2** and **3**, which list the artifacts associated with the surgical procedure and those arising during histotechnical processing, along with their mechanisms of occurrence and microscopic features.

### HISTOLOGICAL SLIDES

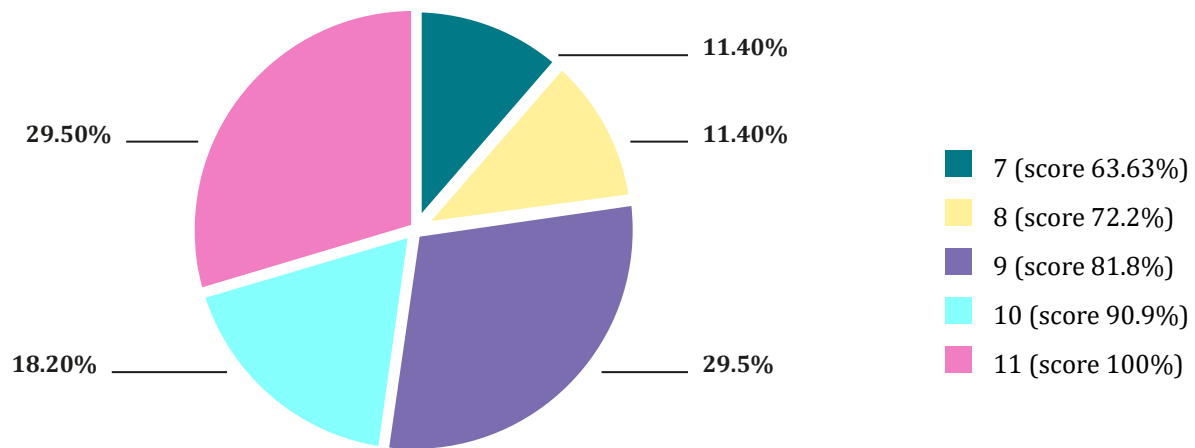
After analyzing 200 slides, crush artifacts were identified as the most frequent clinician-induced artifact, present in 30.5% ( $n = 61$  slides), followed by curling, observed in 14.5% ( $n = 29$ ) of the analyzed slides. No artifacts related to inadequate fixation or anesthetic injection into the specimen were observed (**Table 3**).

Regarding laboratory-induced artifacts, at least one artifact was found in all slides. The most frequent were tears or splits, present in 92% ( $n = 184$  slides), contamination in 86.5% ( $n = 173$  slides), and inadequate contrast in 71.5% ( $n = 143$  slides) (**Table 4**). The number of artifacts per slide ranged from 1 to 9, with a mean of 5.1 artifacts per slide.

**Figures 2** and **3** show representative examples of artifacts induced by the clinician and those produced during laboratory processing, respectively. It is illustrated how these alterations can hinder histological interpretation.

**Table 1.** Participant characteristics and questionnaire results (n = 44).

Variable	Category	n (%)
<b>SAMPLE PROFILE</b>		
Age	> 40 years	24 (54.5)
	Range: 24–73 years	–
Sex	Female	28 (63.6)
Year of graduation	> 20 years	23 (52.3)
Graduation institution	Public	32 (72.7)
Registered specialty	Yes	20 (45.5)
	Periodontics	7 (35.0 of specialists)
	Oral medicine	6 (30.0)
	Oral surgery	2 (10.0)
<b>KNOWLEDGE AND PREVENTION OF HISTOPATHOLOGICAL ARTIFACTS</b>		
Previous knowledge of artifacts	Yes	35 (79.5)
Correct identification of anesthesia site	Yes	32 (72.7)
Instruments used in biopsies	Conventional scalpel	44 (100.0)
	Punch	15 (34.1)
	Scissors	14 (31.8)
	Electrosurgical scalpel	7 (15.9)
Influence of surgical technique	Acknowledges impact	44 (100.0)
Precautions with thin specimens	Yes	32 (72.7)
	Spread on paper	10 (22.7 of total / 31.3 of those taking precautions)
Direct suction on the lesion	Acknowledges impact	35 (79.5)
Handling of specimen with forceps	Acknowledges impact	37 (84.1)
Use of dyes to mark biopsy site	Does not use	37 (84.1)
Information on the use of dyes to mark the biopsy site	Indicated in the exam request form	6 (85.7 among those who use dyes)
	Did not indicate on exam request form	15 (34.1)
Fixation in 10% formalin within ≤ 10 min	Yes	44 (100.0)
Fixative volume ≥ 10:1	Yes	32 (72.7)
Container labeling	Yes	43 (97.7)



**Figure 1.** Distribution of participants (n = 44) according to the number of correct answers out of 11 questions. Each color represents the percentage of respondents who achieved a given number of correct answers, with the corresponding overall percentage of correct responses indicated in parentheses.

**Table 2.** Description of artifacts produced during biopsy by the clinician, along with their microscopic characteristics.<sup>(6-8)</sup>

Artifact	How it occurs	Microscopic description
Anesthetic injection	When the anesthetic is injected directly into the lesion, tissues may exhibit edema and hemorrhage unrelated to the existing pathology.	Hemorrhage, separation of connective tissue bands, and vacuolization of epithelial cells.
Crush artifact	Improper handling of the specimen can cause compression or crushing, altering its three-dimensional structure.	Crushing (basophilia), architectural distortion, and marks (cavities, pseudocysts) ( <b>Figures 1A, 1B</b> ).
Thermal damage	Heat generated by the electrosurgical scalpel or laser burns the tissue at the lesion margin, hindering adequate histological analysis.	Marginal basophilia or eosinophilia, tissue coagulation, amorphous appearance, and reticulated epithelium ( <b>Figures 1C, 1D, 1E, 1F</b> ).
Suction artifact	Direct suction on the lesion produces vacuolated structures in the tissue.	Vacuolated structures resembling fat or blood vessels ( <b>Figure 1G</b> ).
Insufficient specimen for diagnosis	Occurs when the excised specimen is too small and does not adequately represent the clinical lesion.	Insufficient or unrepresentative tissue.
Curling	Occurs in thin specimens, such as plaques, that tend to curl during fixation, losing their original shape.	The specimen curls or bends instead of maintaining its original configuration.
Suture thread	Caused when a loop is tied around the specimen as an auxiliary technique during biopsy.	Central cavity in the tissue visible in all sections ( <b>Figure 1F</b> ).
Inadequate fixation	Occurs when the specimen is not fixed immediately after removal, leading to cellular autolysis and impaired tissue morphology.	Autolysis and bacterial proliferation due to delayed fixation; morphological alterations depending on fixative type and concentration ( <b>Figures 1I, 1J</b> ).

**Table 3.** Description of how artifacts are produced in the laboratory, along with their microscopic characteristics.<sup>(6-8)</sup>

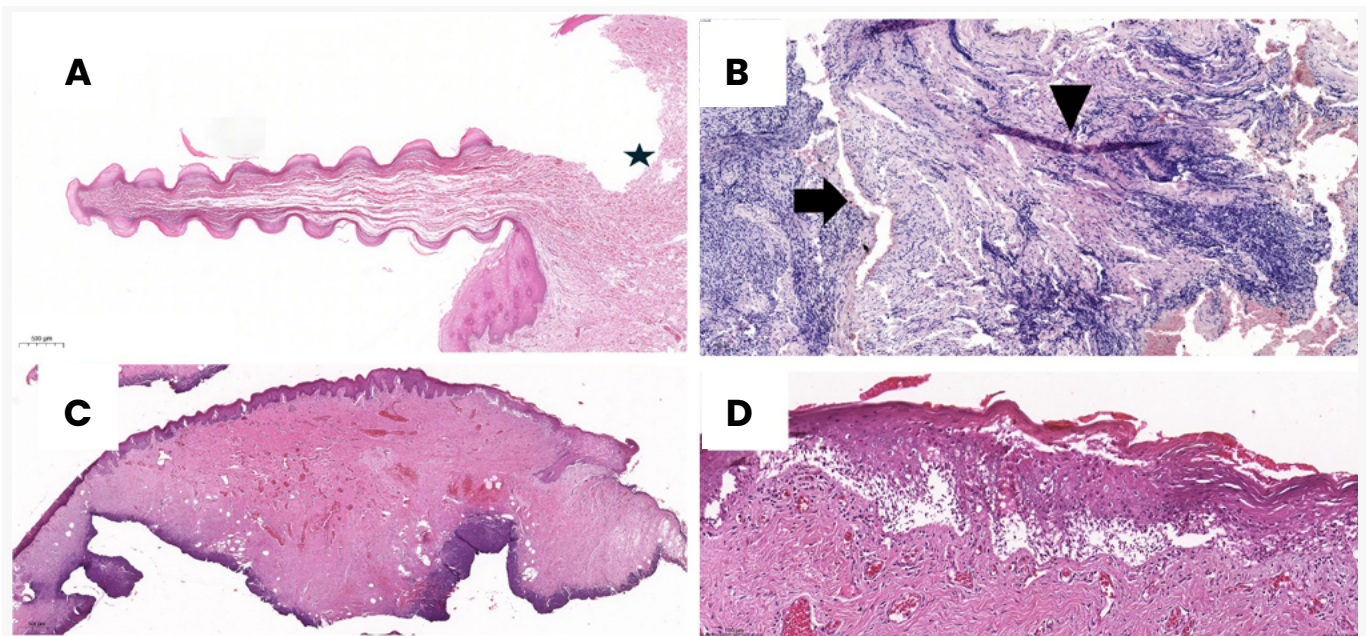
Artifact	How it occurs	Microscopic description
Pigments	Caused by unfiltered dyes, exogenous pigments, or formaldehyde pigment (a product of acid formalin in contact with hemoglobin).	Granular or pigmented deposits, often brown or black, on tissue structures.
Incomplete decalcification	The tissue is not exposed to the decalcifying agent for enough time.	Cracks, fissures, and distorted morphology; specimen may appear intensely eosinophilic.
Over-decalcification	The tissue remains in acid longer than necessary.	Loss of nuclear detail, cytoplasmic discoloration, and destruction of soft tissue structures.
Tangential cuts	The specimen is misoriented during embedding, resulting in an incorrect cutting plane.	Section parallel to the tissue surface, with incomplete epithelial architecture and short or absent epithelial ridges ( <b>Figure 3B</b> ).
Blunt blades	Caused by defects or damage to the microtome blade due to a dull or nicked edge; may also result from hard tissue or foreign bodies within the block.	Linear, parallel, and equidistant tears aligned with the direction of the cut; repetitive pattern across multiple sections ( <b>Figures 2F, 2G, 3A</b> ).
Tears/splits	Usually caused by inadequate fixation, insufficient dehydration, or tissues with heterogeneous consistency; may also result from excessive pressure during cutting.	Irregular, serrated fissures following natural weak points or structural interfaces; non-parallel and non-uniform ( <b>Figures 2A, 2B</b> ).
Sections too thick	The thickness of the tissue section is excessive.	Hyperintense staining, overlapping cellular details, and multiple planes of focus visible.
Folds	The tissue folds during cutting or when placed in the flotation bath.	Overlapping bands with more intense staining than the surrounding tissue, with loss of continuity ( <b>Figures 2B, 3A</b> ).
Paraffin residue	Incomplete deparaffinization before staining.	Unstained or unevenly stained areas.
Inadequate contrast	Caused by degraded or low-quality dyes, incorrect staining times, improper hydration or dehydration steps, inadequate pH, or poor differentiation.	Poor structural differentiation; section appears uniformly basophilic or eosinophilic.
Weak staining	Use of old, low-quality, or degraded dyes; may also occur due to prolonged exposure of the specimen to light.	Uniformly pale staining; poor visualization of nuclear and cytoplasmic details.
Excessive staining	Occurs when the section is over-stained or excessively thick.	Intensely stained sections that may obscure fine histological details.
Uneven H&E staining	Caused by poor deparaffinization, incomplete hydration, or uneven dye distribution.	Irregular staining with alternating light and dark areas; inconsistent visualization of tissue components.
Contamination (same focal plane)	Tissue fragments, debris, or foreign material introduced during embedding, microtomy, or in the flotation bath.	Foreign particles (talc, cotton fibers, graphite, insects) in sharp focus, which can be mistaken for real structures ( <b>Figures 2G, 3C</b> ).
Contamination (different focal plane)	Particles introduced during mounting or coverslip placement.	Out-of-focus foreign material (dust, debris) above the section; generally less interfering for diagnosis ( <b>Figure 3D</b> ).
Air bubbles	They form on the stained section during coverslip placement.	Bubbles (tiny spheres) on the surface of the section.

**Table 4.** Number and percentage of surgeon- and laboratory-induced artifacts identified on histological slides.

Artifact	Percentage (n)
<b>ARTIFACTS CAUSED BY THE CLINICIAN</b>	
Crushing/compression	30.5 % (61)
Curling	14.5 % (29)
Coagulation by electrosurgical scalpel	5.5 % (11)
Suction artifact	4.5 % (9)
Insufficient specimen for diagnosis	2.5 % (5)
Suture thread	2.0 % (4)

#### ARTIFACTS CAUSED IN THE LABORATORY

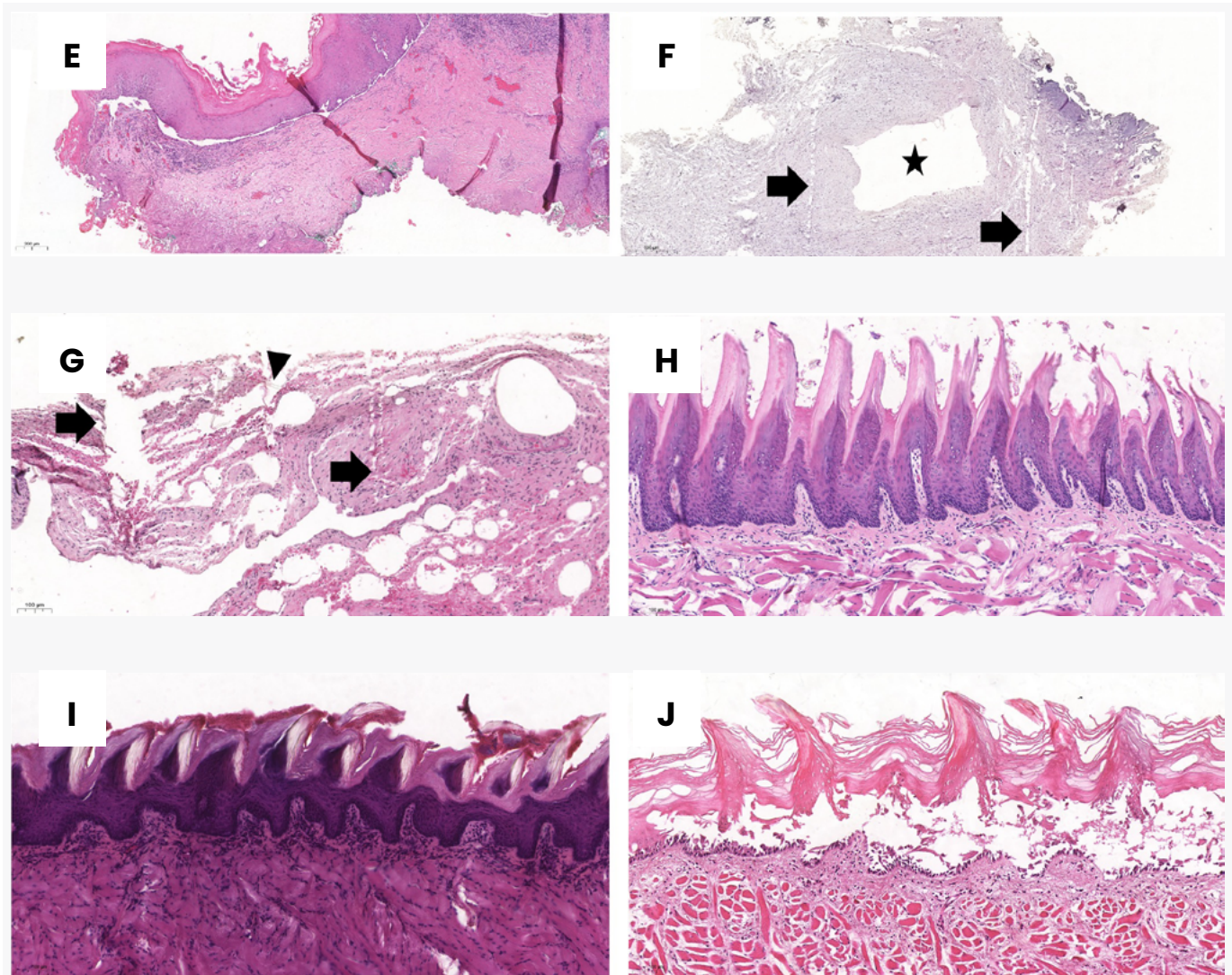
Tears/splits	92.0 % (184)
Contamination (different focal plane)	86.5 % (173)
Inadequate contrast	71.5 % (143)
Pigments	50.5 % (101)
Folds	48.0 % (96)
Contamination (same focal plane)	41.5 % (83)
Blunt blades	29.0 % (58)
Weak staining	16.5 % (33)
Uneven H&E staining	11.0 % (22)
Tangential cuts	7.0 % (14)
Excessive staining	3.0 % (6)
Incomplete decalcification	0.5 % (1)
Over-decalcification	0.5 % (1)
Excessively thick section	0.5 % (1)
Air bubbles	0.5 % (1)



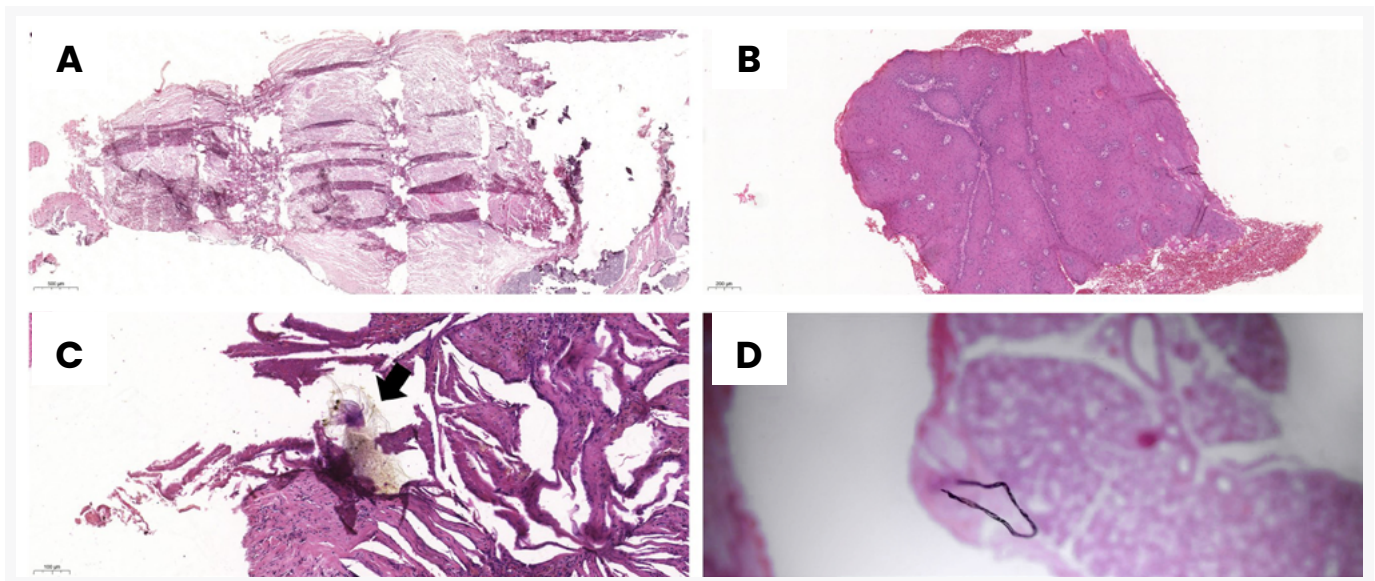
**Figure 2.** Examples of histological artifacts caused by the clinician and during laboratory processing.

- A. Crush artifact caused by compression of the specimen with forceps, showing visible marks from the forceps' teeth. A tear or split (★) is also visible.
- B. Crush artifact with nuclear basophilia. A tear/split (➡) and a fold (▶) are also present.
- C. Thermal damage produced by the electrosurgical scalpel, showing basophilic margins due to tissue coagulation.
- D. Higher magnification of the heat-damaged epithelium from the same case shown in C. These alterations may simulate vesiculobullous lesions.

**Figure 2. (continuation)** Examples of histological artifacts caused by the clinician and during laboratory processing.



- E.** Subepithelial separation caused by thermal injury from electrosurgery, also mimicking vesiculobullous pathology.
- F.** Suture thread artifact (★) with a central cavity caused by traction. Blade marks (➡) and thermal damage are also evident.
- G.** Suction artifact with variably sized vacuoles simulating vessels or adipose tissue. Parallel blade marks (➡) and contamination (▶) are also visible.
- H.** Well-preserved tissue fixed in 10% buffered formalin, shown for comparison with the following images.
- I.** Inadequate fixation with undiluted formalin, leading to tissue retraction and hardened consistency; note the compact arrangement of muscle fibers.
- J.** Inadequate fixation by saline immersion, resulting in epithelial autolysis and separation of muscle fibers.



**Figure 3.** Examples of artifacts produced during laboratory processing.

- A.** Folds and blunt blade artifact (parallel tears) caused by the presence of mineralized tissue that was not decalcified, leading to blade damage during sectioning.
- B.** Tangential cut due to incorrect embedding, resulting in poor orientation and incomplete representation of the epithelium.
- C.** Contamination (same focal plane) with the presence of an arthropod (➡) and a concurrent fold.
- D.** Contamination (different focal plane) caused by filamentous debris introduced during coverslip placement.

## Discussion

The accuracy of microscopic tissue analysis depends on proper handling by surgeons, histotechnologists, and assistant pathologists to minimize artifacts, which can pose significant challenges during histopathological examination and potentially compromise diagnosis.<sup>(3)</sup> As these procedures are performed manually, they are susceptible to errors that must be recognized and minimized to avoid interfering with accurate evaluation. In oral pathology, artifacts are particularly relevant compared to other pathology specialties due to the high vascularity and limited access of the oral cavity, which complicates biopsy procedures. These challenges often result in small tissue fragments that are highly susceptible to distortion, even with minimal compression.<sup>(9,10)</sup> Reducing artifact formation can decrease both diagnostic time and costs, as repeated biopsies, deeper cuts, or re-inclusion of fragments are often required to obtain an accurate diagnosis. In this study, knowledge of artifacts was evaluated among users of an oral pathology laboratory, and

their presence was analyzed in histologic slides from the same service, with the aim of highlighting the problem and contributing to its reduction through educational actions.

The profile of our laboratory users was predominantly female (64.6%), over 40 years of age (54.5%), graduated more than 20 years ago (52.3%), from public universities (72.7%), and without a registered specialty (54.5%). Most users demonstrated a basic understanding of the topic, as evidenced by the high percentage of correct answers. However, the significant number of artifacts identified on the slides underscores the critical importance of understanding and implementing strategies to prevent them.

Krishnan et al.<sup>(11)</sup> conducted a similar survey among 70 graduate students and oral and maxillofacial surgeons performing biopsies. Consistent with our findings, more than 90% of participants performed fixation

correctly. However, only 38.5% reported using dye to mark the biopsy site, compared with 85.7% among dye users in our sample. In contrast, 34.1% of our participants did not indicate the use of laser or electrosurgical scalpels when submitting specimens for examination. These findings highlight concerns regarding documentation and communication between surgeons and pathologists. The use of dyes and electrosurgical instruments during biopsy may introduce technical artifacts that simulate structures, complicating or even preventing accurate diagnosis. Electrosurgical instruments can coagulate tissue, generating amorphous areas in connective tissue and inducing epithelial alterations. In small samples, this may render the tissue insufficient for diagnosis.<sup>(3,4)</sup> Therefore, it is essential to include all relevant surgical details in the examination request form addressed to the pathologist.

The high prevalence of artifacts in histologic slides has been reported previously, with frequencies ranging from 90.8% to 100%.<sup>(12-14)</sup> Laboratory-induced artifacts are more frequent than those caused by surgeons,<sup>(14)</sup> with folds and formalin pigments being the most common.<sup>(12-14)</sup> Folds occur during microtome cutting or when placing the specimen on the slide, and their prevention requires technical skill and training.<sup>(3)</sup> In our study, however, tears were the most predominant artifacts. Both are laboratory-induced artifacts and demand particular attention during microtomy, as thin sections increase the risk of both folds and tears.

In the study by Saravani et al.,<sup>(14)</sup> the most common surgeon-induced artifacts were tissue rupture and crushing. Compression or crush artifacts are particularly significant, as they can mimic cystic cavities and make morphological identification of cells difficult or even impossible. Additionally, excessive force can cause infiltration of epithelial cells into the connective tissue, compromising the diagnosis of carcinoma, where epithelial invasion is a decisive criterion for both diagnosis and treatment.<sup>(3)</sup> Therefore, specimens must be handled with care, avoiding toothed forceps and applying controlled force to minimize these artifacts.

In our sample, participants correctly answered questions regarding material fixation and most accurately identified the site of anesthesia application. These findings align with the absence of artifacts related to fixation and anesthetic injection in our slides, suggesting that theoretical knowledge positively influences surgical performance and helps prevent or minimize artifacts. Accordingly, we believe that the e-book developed by our team may contribute to reducing artifact prevalence.

In a previous study, it was shown that general dentists tend to produce more artifacts than oral and

maxillofacial surgeons.<sup>(15)</sup> Interestingly, in our study, specialists had a significantly lower frequency of correct answers on the questionnaire, an unexpected finding. Periodontists were the most represented specialists, followed by stomatologists, oral and maxillofacial surgeons, and other specialties. This result highlights the need to incorporate formal training in biopsy techniques across dental specialties.

One limitation of our study is the sample size, which was determined by convenience and represented 22.1% of laboratory users. Furthermore, in the analysis of histological slides, only the work of two experienced technicians was considered when evaluating processing-related artifacts. Since artifacts arising from slide preparation are the most common, their reduction and control are generally easier than those caused by surgical technique. Finally, our laboratory is located within the University, and many users are former students. This proximity may have contributed to the favorable results regarding clinicians' knowledge. Given the University's educational mission, it is customary to report the presence of surgical artifacts in histopathology reports to help reduce their future occurrence. We also consider that any artifact—surgical or laboratory—that hinders or interferes with diagnosis should be explicitly described in the report.

Clinicians and technicians who understand the factors and conditions contributing to artifact formation can significantly reduce their occurrence. Such knowledge facilitates accurate pathological diagnosis, ultimately enabling more effective therapeutic planning for patients.<sup>(4)</sup>

## Conclusions

The findings of this study indicate that, although most professionals possess basic knowledge of histologic artifacts, their prevalence in slides remains high, particularly for artifacts generated during laboratory processing. These results underscore the need to improve clinical and laboratory practices and to implement educational strategies that guide professionals in artifact prevention. Interventions such as developing informative materials can help improve sample quality and enhance diagnostic accuracy in oral pathology.

## REFERENCES

1. Junqueira LCU, Carneiro J. *Histologia Básica: Texto e Atlas*. 14<sup>a</sup> ed. Rio de Janeiro: Guanabara Koogan; 2023.
2. Bernstein ML. Biopsy technique: the pathological considerations. *J Am Dent Assoc*. 1978;96(3):438-443. doi:10.14219/JADA.ARCHIVE.1978.0092
3. Rastogi V, Puri N, Arora S, et al. Artefacts: a diagnostic dilemma - a review. *J Clin Diagn Res*. 2013;7(10):2408-2413. doi:10.7860/JCDR/2013/6170.3541
4. Zargar M. Clinicians' role in the occurrence of oral biopsy artifacts as a potential diagnostic dilemma. *Dent Med Probl*. 2019;56(3):299-306. doi:10.17219/dmp/108597
5. Carson FL. Fixation and processing. In: Brown RW, ed. *Histologic Preparations: Common Problems and Their Solutions*. Northfield, IL: College of American Pathologists; 2009:1-5.
6. Aravindha Babu N, Sridevi Anjuga EP, Masthan KMK, Rajesh E. Artifacts in histopathology – a review. *Indian J Forensic Med Toxicol*. 2020;14(4):1243-1250. doi:10.37506/ijfmt.v14i4.11699
7. Chatterjee S. Artefacts in histopathology. *J Oral Maxillofac Pathol*. 2014;18(5):111-116. doi:10.4103/0973-029X.141346
8. Chatterjee A, Kumar S, Samaddar D, Afridi SK, Kumar M. Artifacts in routine histopathology – an enigma to pathologists. *Int J Med Res Rev*. 2019;7(2):23-26.
9. Ficarra G, McClintock B, Hansen LS. Artefacts created during oral biopsy procedures. *J Cranio-Maxillofac Surg*. 1987;15(C):34-37. doi:10.1016/S1010-5182(87)80012-4
10. Yanduri S, Pandey G, Kumar V, Suma S, Madhura M. Artifacts in oral biopsy specimens: a comparison of scalpel, punch, and laser biopsies. *Indian J Oral Health Res*. 2016;2(2):100. doi:10.4103/2393-8692.196147
11. Krishnan RP, Ramani P, Sherlin HJ, et al. Surgical specimen handover from operation theater to laboratory: a survey. *Ann Maxillofac Surg*. 2018;8(2).
12. Satapute M, Shashikala P, Kavitha GU. Artifacts: a menace in histopathology. *Int J Clin Diagn Pathol*. 2020;3(1):290-292. doi:10.33545/pathol.2020.v3.i1e.185
13. Kargahi N, Keshani F, Khosravian M. Analysis of artifacts in oral and maxillofacial histopathologic sections and related reasons. *Dent Res J (Isfahan)*. 2019;16(6):384-388. doi:10.4103/1735-3327.270788
14. Saravani S, Kadeh H, Shahsavari M, Shahrakipour M, Mosafer B. Evaluation of artifacts in oral and maxillofacial histopathological slides. *J Dentomaxillofac Radiol Pathol Surg*. 2016;5(3):11-16. doi:10.18869/acadpub.3dj.5.3.11
15. Seoane J, Varela-Centelles PI, Ramírez JR, Cameselle-Teijeiro J, Romero MA. Artefacts in oral incisional biopsies in general dental practice: a pathology audit. *Oral Dis*. 2004;10(2):113-117. doi:10.1111/j.1354-523X.2003.00983.x

## SUPPLEMENTARY MATERIAL

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## Ethical approval

This study was approved by the Institutional Ethics Committee (CAAE 57368822.6.0000.0077).

## Data availability

All data supporting the findings of this study are included within the article.

## Conflict of interest statement

The authors declare no conflict of interest.

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## Authorship contribution

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14
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Ana Lia Anbinder	X			X	X	X	X	X	X	X		X	X	X

- |                                 |  |
|---------------------------------|--|
| 1. Project Administration       | 8. Methodology                           |
| 2. Funding Acquisition          | 9. Resources                             |
| 3. Formal Analysis              | 10. Writing - Original Draft Preparation |
| 4. Conceptualization            | 11. Software                             |
| 5. Data Curation                | 12. Supervision                          |
| 6. Writing - Review and Editing | 13. Validation                           |
| 7. Research                     | 14. Visualization                        |

## Acceptance note

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