

Artifacts in Histopathology: A Potential Cause of Misinterpretation.

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ABSTRACT

The intra-operative histological assessment of fresh tissue can provide valuable diagnostic information and guide surgical management. Artifacts in histopathology, could create serious errors and cause misdiagnosis. In some cases, the degree of artifactual damage is so large that may involve the entire specimen, rendering it suboptimal or useless for diagnostic purposes. Biopsy specimens of oral cavity are of small size and fine texture resulting into higher effectiveness of artifacts on them. Some artefacts are easily distinguished from normal or pathological tissue components, and some are difficult to distinguish from such entities. Artefacts may encounter at various stages which include during routine collection of tissues, fixation, processing, cutting and staining of tissues. This review incorporates all artifacts occurs during each stage of histopathological processing of tissues, in an attempt to identify the artifacts, their causative factors and appearance histopathologically so to differentiate them from that of pathological entity.

INTRODUCTION

Artefacts are defined as being any structure or feature that has been produced by the processing of a tissue. Some artefacts are easily distinguishable from normal or diseased tissue components and some are difficult to distinguish from such entities. In histological and cytological terms an artifact can be defined as a structure that is not normally present in the living tissue. The problem is recognizing artifacts as such when they do occur and not confusing them with normal tissue components or pathological changes. In some situations the presence of an artifact can compromise an accurate diagnosis.

Artefacts results in alteration of normal morphologic and cytologic features or even lead to complete uselessness of the tissue. It is important to know and understand about artifacts as by learning to recognize them, we can avoid misdiagnosis [4,2]. These artifacts can be minor, involving only small portion of the specimen and therefore do not interfere with the pathologist's ability to provide an accurate diagnosis. In some cases, however the degree of artifactual damage is excessive or may involve the entire specimen, rendering it suboptimal or useless for diagnostic purposes [4]. Therefore, aim of this publication is to promote an awareness of the various artifacts which may be encountered in histopathology, to provide a guide for their recognition, to explain their causes and to suggest, where possible, the means by which their occurrence can be avoided.

Pre-Fixation Artifacts:

Pre-fixation artifacts are produced in tissues before fixation. They may take the form of deposits such as tattoo pigment, or result from a surgical procedure as with laser knife damage or crush artifact. Contaminants can also be introduced into tissues during surgery or whilst handling prior to, or during specimen dissection. This type of artifact can only be avoided by ensuring that those involved are fully aware of the consequences of allowing a specimen to become contaminated or otherwise damaged.

Artifacts due to surface preparation

The excision margins of fresh surgical specimens are sometimes marked with coloring agents to allow appropriate orientation of the specimen and assessment of these margins microscopically [3]. Biopsy area is also

prepared by using tincture iodine. Reagents commonly used for surface marking include Indian ink, Silver nitrate, Alcian blue or Alcian green. (Fig: 1) Preparation of the area of biopsy with tincture or other colored solutions should be cautiously used and should be clearly mention along with biopsy details, as it can interfere with tissue processing and staining procedures.

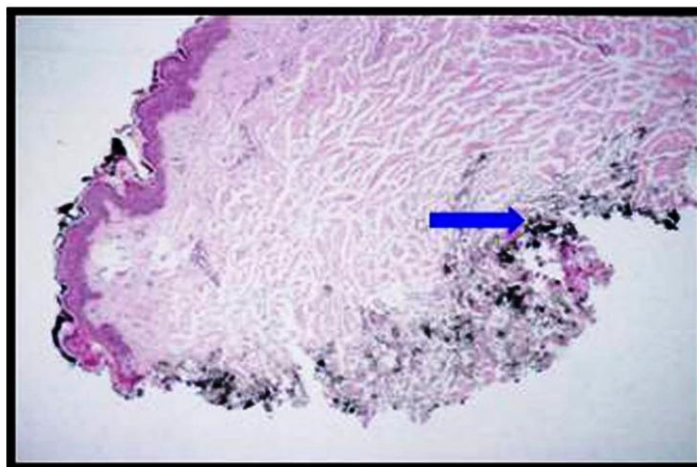


Figure 1: Artifacts due to surface preparation and tattoo pigmentation: margin of a skin biopsy marked with silver nitrate. The reagent stains the soft tissues and has penetrated into the dermis. Intense staining of keratin at the edge of the specimen can also be seen; H&E Stain.

Tattoo pigment artefact

Various colored insoluble pigments used in producing decorative tattoos are occasionally encountered in sections of skin. There is no tissue reaction to the presence of deposits

Surgical Artifacts

Forceps artifacts or crush/squeeze artifacts

When the teeth of the instrument penetrate the specimen, it results in voids or tears and compression of surrounding tissue. Other possible cause for artifacts during surgical handling are: *Mechanical causes*: instruments commonly used for biopsy procedures like hemostat and mosquito hemostat, suction tips, Adson forceps with or without teeth, atraumatic forceps and Allis clamps. *Chemical causes*: injection of large local anesthetic solution in excess and in to the lesional tissues and application of antiseptic medication like Betadine solution. *Thermal cause*: instruments used for coagulation and cutting electrodes like electrocautery [4,5]. This causes the surface epithelium to be forced through the connective tissue, producing small “pseudocysts” and also causes loss of cytoplasmic and nuclear features [6]. These artifacts can be avoided by handling the specimen gently, avoiding applying unnecessary force and holding the tissue in an area away from the lesional tissue.(Fig: 2A)

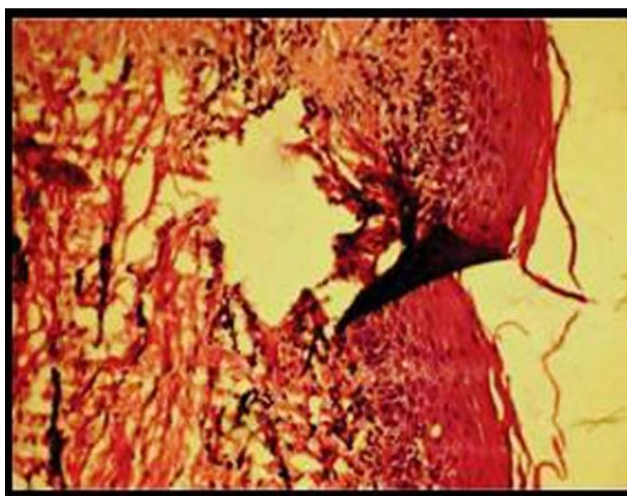


Figure 2A: Surgical Artifacts: A) Forcep artefact: Tearing of tissue due to penetration of forcep; H&E.

Fulgeration artifacts: Heat produced while using laser or electrosurgical procedures may lead to marked alteration in both epithelium and connective tissue [4]. Epithelial cells may appear detached and the nuclei assume a spindle, pallisading configuration, separation of the epithelium from the basement membrane also occurs. The fibrous connective tissue, fat and muscle may show opaque, amorphous appearance. This artifact can be avoided by using knives, use low milliamperage current, use of combination knife and electrical points.

Injection artifact: Injection of large amounts of anesthetic solution into the area to be biopsied can produce hemorrhage with extravasation which masks the normal cellular architecture. Injecting the solution into the lesional tissue will cause vacuolation of epithelium and connective tissue and in addition causes separation of connective tissue. To avoid this use block technique instead of infiltration technique and inject the solution well away from the lesional tissue. Large quantities of local anaesthetic solution should not be injected [4,5].

Sutural artifact: Suture material is an occasional inclusion in histological specimens. It may consist of isolated fragments or complete fibre-bundles cut in transverse, oblique or longitudinal planes [5,7]. Detail of the fibre structure can sometimes be seen upon careful examination of H&E-stained sections and these silk sutures exhibit a strong birefringence under polarized light and this can be useful in their identification (*Fig: 2B*). The presence of a suture in a histological specimen may not be of any pathological significance. It can damage the microtome knife leading to tears and knife lines in sections. Visible sutures should be removed wherever possible.



Figure 2B: Surgical Artifacts: B) Sutural artefact: Bifringent suture material from stitch granuloma under Polarized light.

Artifacts due to contamination

Certain artifacts can be arised due to contamination which mainly includes, Starch artifact due to contamination of specimen with starch powder used as lubricant in surgical glove. These starch granules are refractile, glassy, plyzonal, PAS positive bodies generally 5-20mm in diameter. They have spore like structures with dark central area which could be misinterpreted as a pyknotic nucleus or as one undergoing mitosis (*Fig: 3A*), Specimen-specimen contamination artifact probably occurs during dissection where tissue from a previous specimen is transferred via the instruments used (such as scalpel blades) or from fragments which remain on the dissecting board surface. Reusable processing components (such as tissue cassette lids), if not thoroughly cleaned, can also carry fragments of previous specimens (*Fig: 3B*). Thorough rinsing of the board and instruments between specimens or covering the dissection board with separate paper sheets will avoid this problem. Foreign body contamination artifact often makes the interpretation of the specimen quite difficult. These artifacts are encountered as a contaminant arising from paper, cotton gauze or a cork board used during specimen preparation. They are usually found on the surface of the specimen but can be implanted mechanically during dissection [1,3] (*Fig: 3C*).

Fixation Artifacts

Although fixation is necessary to avoid diffusion of soluble tissue components and decomposition, it by itself constitutes a major cause of artifact. If the procedure is not carried out under optimal conditions, if fixative does not have proper access to the tissues, or because of the nature and quality of the particular reagent used, artifact can occur [8,9]. The most commonly used fixative is 10% formalin. The concentration of the formalin, contamination and prolong fixation time leads to difficulty in sectioning of the specimen.

Pigment Artifact: Such artifacts arise due to formalin , mercuric chloride and picric acid used in various fixative agent which causes Brown-Black granular and yellow stains distributed randomly throughout the tissues (Fig: 4).

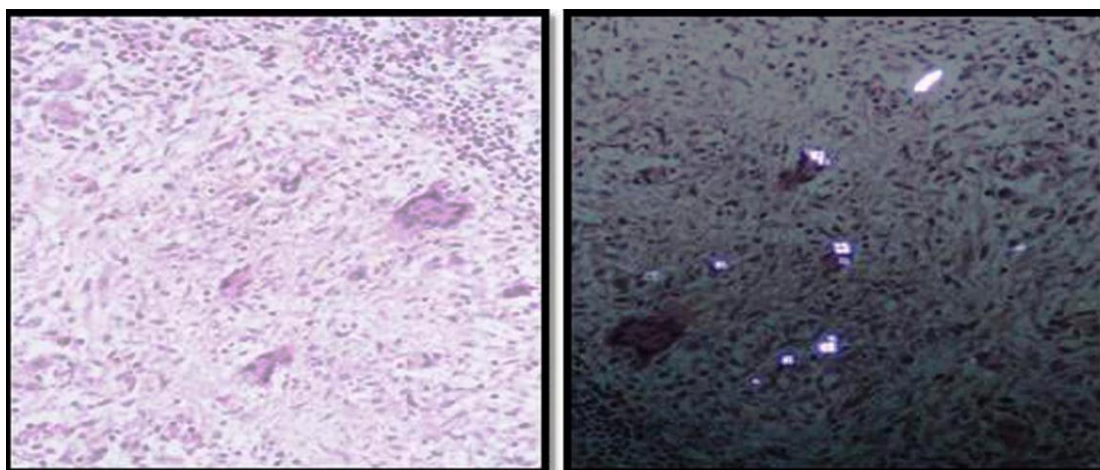


Figure 3 A-Artifacts due to contamination. A) Starch granules: Starch granules in a typical starch granuloma stained with H&E and Polarized light.

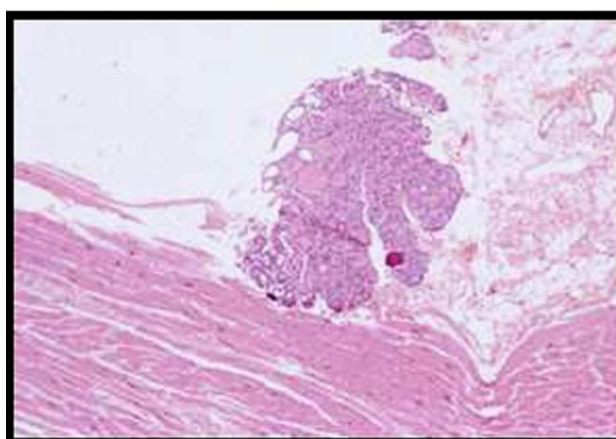


Figure 3 B-Artifacts due to contamination. B) Specimen-specimen contamination: A section of cardiac muscle with a piece of extraneous thyroid tissue present against one surface; H&E.

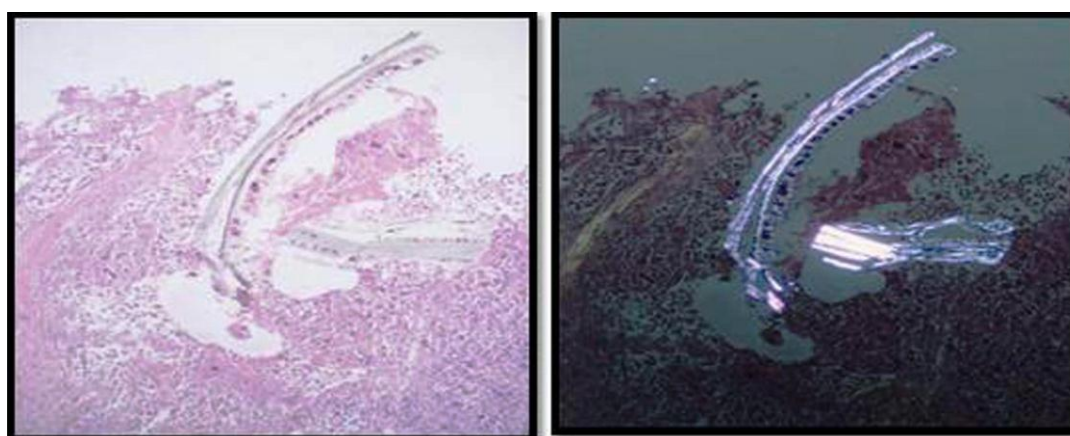


Figure 3 C-Artifacts due to contamination. C) Foreign body contamination: Cellulose in an H&E-stained section and under Polarized light.

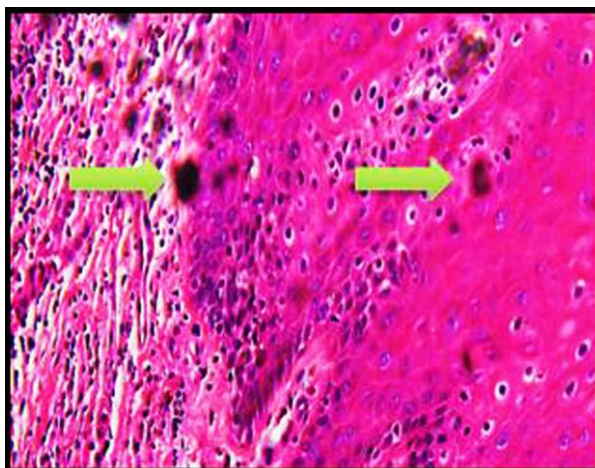


Figure 4: Fixation Artifact. Pigment artefact: Formalin pigments deposition as brown stains; H&E Stain.

Shrinkage artifacts: During fixation, tissues change in volume. This is due to inhibition of cellular respiration and changes in membrane permeability. As a result tissues that are attached in life may be pulled away from each other, leaving empty spaces [8], this is a very common artifact. Some of the non protein precipitants cause swelling of tissue following fixation in formalin.

Streaming artifacts: This is an important type of artifact due to diffusion of unfixed material to give false localization by coming to rest in same place other than original location well known example of this is glycogen. Fixation of tissue for glycogen study should be prompt as there is an initial sharp loss of glycogen in postmortem solution and it should be carried out at 4 degrees in 80% alcohol.

Diffusion artifacts: Materials may sometimes diffuse out of the tissue. Apart from large molecules, small molecules like inorganic ions and biogenic amines can be lost from tissue. For example; when cut end of adrenal gland tissue are placed in iodate for iodate reaction, late cholamines can be seen leaving the tissue as a red cloud of aminochromes.

Artifacts due to microwave fixation: Optimum temperature for microwave fixation in 45-55 C°. Under heating results in poor sectioning quality whereas overheating above 65 C° produces vacuolation, overstained cytoplasm and pyknotic nuclei.

Artifacts during freeze-drying - ice crystal artifacts: During fixation using freeze drying method, the tissue must be plunged into isopentane cooled to -160 C° to -180 C° with liquid nitrogen immediately. Low temperature is important because unless the whole tissue is frozen, large ice crystals are formed causing disruption artifacts¹⁰. This artifact cause total distortion of the tissue and diagnostic difficulty.

Artifacts due to prolonged fixation: Prolong fixation causes secondary shrinkage and hardening which leads to separation of portion of tissues giving appearance of empty spaces.

Processing Artifacts

Tissue processing is designed to remove all extractable water from tissue, replacing it with a support medium that provides sufficient rigidity to enable sectioning of the tissue without damage or distortion and artifacts are produced at each step if proper care and procedures are not followed.

Artifacts during post fixation treatment: Tissue that are fixed in chrome if not washed for 24 hours in running tap water it could lead to the formation of chromeoxide pigment.

Artifacts during dehydration: Artifacts can be encountered during dehydration due to **1.** Improper gradient of dehydration, if the concentration gradient between the fluid inside and outside the tissue is excessive, diffusion current cross the cell membranes during fluid exchange thus resulting the increase in possibility of cell distortion [2,8] **2.** Over dehydration makes the tissue hard, brittle and shrunken causing difficulty while cutting and also interfere with staining properties of section [11] **3.** Under and Incomplete dehydration results in improper infiltration of paraffin and block made is difficult to section thus distorted with fragmented tissue sections will lead to artifactual changes.

Artifacts during clearing : Artifacts may arise due to over and under clearing of tissue specimens to be over harden and will obstruct paraffin to impregnate properly in paraffin wax thus making it difficult to cut during sectioning [2,3].

Artifacts during impregnation: The function of wax impregnation is to remove clearing agent (wax solvent) from the tissue and for them to be completely permeated by the paraffin wax which is subsequently allowed to harden to produce a block from which sections may be cut. The artifact produced during this procedure is Crystallization: Thicker the tissue the more clearing agent it will carry and hence it requires more change of wax to be removed it. Even if a small amount of clearing agents contaminates the wax it will lead to crumbling and crystallization of tissue during cutting.

Artifacts during embedding: Artifacts due to improper orientation are frequently encountered during faulty embedding procedures which will lead to damage to microtome or render it difficult to study tissue microscopically.

Artifacts due to poor processing: Extensive loss of architectural detail and clarity within loose connective tissue may reflect inadequate fixation. Can also be caused by faults in tissue processing such as too short processing cycle, inappropriate choice of reagents, use of exhausted reagents or error in replacing solvents on the processing machine [3,11].

Cutting Artifacts

Artifacts related to Microtomy: Various forms of mechanical damage produced during section cutting and flotation, together with a range of contaminants from a variety of sources, are commonly encountered in sections. **Table 1** showing various artifacts and there possible cause during micrtome cutting. (Fig: 5)



Figure 5: Artifacts during microtomy : Splitting of section at right angle and Knife lines.

Artifacts related to tissue floating water bath: Mainly three types of artifacts seen in tissue floating water bath 1). Air Bubble Entrapment care should be taken to prevent water bubbles being trapped under the section, these entrapment mainly occurs due to poor floatation technique where sections are dropped rather than pull gently across the water surface or due to bubbles already present in water bath dislodged by the slide and rise up under the section. These air bubbles trapped in a section after flotation and mounting can collapse on drying ,leaving zones which cracks and fails to adhere properly to the slide (Fig: 6). 2. Increase temperature of water bath results into expansion of tissue beyond its limit and “Parched Earth (crackes)” effect is noted. 3.Floaters Artifacts are pieces of tissues that appear on slide that do not belong there, they have floated during processing or due to dirty uncleaned water bath.

Table 1: Showing various artifacts and there possible cause during microtome cutting.

Artifacts	Potential cause	Precaution
Thick and thin section	Loose blade/block or Insufficient clearing angle	To tighten block and blade, To increase clearance angle
Chatter Artifacts	Vibrations of knife edge, knife/block loose in holder, excessive steep clearance angle	Replacing or using new blade, reducing angle, re-embedding in fresh paraffin
Splitting of sections at right angle	Nick in blade, presence of hard particle or tissue, hard particle contaminant	Using different parts of blade, surface decalcification of hard tissue, removal of foreign particle with sharp points scalpel
Knife lines	Damaged knife edge	New knife to be used
Venetian blind effects	Hard and brittle blocks, blades not properly supported in knife holder	Cutting thinner sections or softening the blocks before cutting
Excessive compression	Due to dull blade, paraffin wax is soft for the tissue	Replacing blade, cooling block surface and recut
Incomplete sections	Incomplete impregnation of tissue, incorrectly embedded tissue, superficial cut sections	Re-processing and embedding of tissue with proper orientation and cutting deeper sections

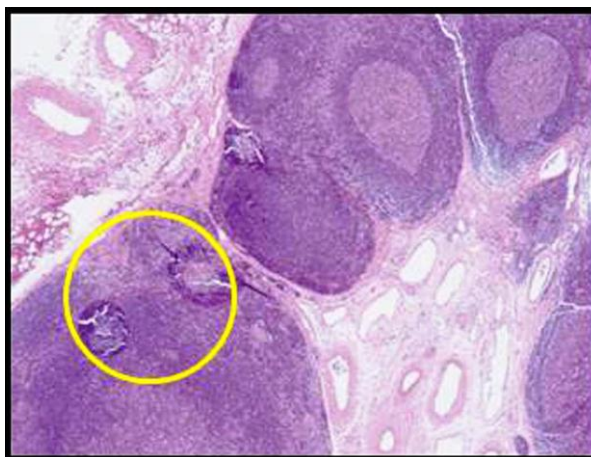


Figure 6: Artifacts in water bath: Air bubble entrapment; Section of lymph node showing circular darkly stained areas with pale centres and surrounding radial cracks. These faults indicate collapsed bubble artifact; H&E Stain.

Artifacts related to Oven/Hot Plate: The temperature setting should be approximately that of the melting point of paraffin. This type of artifact arises due to increase in temperature beyond melting point of paraffin. If the oven is too hot there may be distortion to the cells. Causing dark pyknotic nuclei or nuclear bubbling. Giving appearance of “Heating Artifact” Cells appears to be completely devoid of nuclear details.

Artifacts during lifting of tissue sections: Artifacts such as Tissue Folds produce when tissue adheres to the under surface of the blade, seen most commonly with fatty tissues and mainly seen due to dull blade (*Fig: 7*). They can be avoided by transferring the sections to a new water bath or by passing light of Bunsen burner over the section and also by adding small amount of detergent may be helpful. Tissue Tearing is produced when tissue adheres to the undersurface of the blade mainly seen due to dull blade ,these tearing can be avoided by using clean and replaced blade.



Figure 7: Artifacts during lifting of tissues: Tissue folds; H&E Stain.

Artifacts due to section adhesive and mounting: Thick coat of section adhesive will take the stain and background stain may be detected resulting into irregular, poor quality sections. Mounted, unstained section left uncovered can be contaminated with various materials such as microorganism, airborne fibres, cellulose fibres and dirt particles.

Staining Artifacts

Artifacts due to residual wax: Residual wax in a section will prevent penetration of both aqueous and alcoholic dye solutions leaving area totally devoid of stain, traces of residual wax have a subtle effect on nuclear staining producing small patches in sections where nuclei appear muddy and lack detail ^[3,12] (*Fig: 8*). Prolonged xylene treatment and re-staining will overcome this problem.

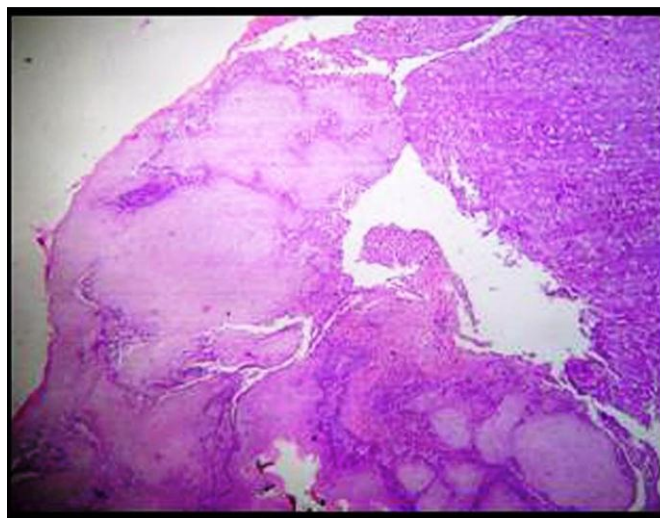


Figure 8: Staining Artifacts. Residual Wax; wax deposits deproving the cellular details; H & E Stain.

Artifacts due to contaminated staining solution: Contamination by microorganisms/foreign particle/expired solution seen a deposition on the section [3].

Artifacts due to stain deposits: Undissolved and precipitated stain will lead to deposition on the sections.

Artifacts due to incomplete or unstained areas: Inadequately filled staining dish or accumulation of staining solution at the top of slide will cause such artifacts [14].

Mounting Artifacts

During mounting air bubble entrapment, residual water and excessive use of mounting media will bring artifactual changes. These changes can be avoided if proper mounting technique is incorporated.

Microscopy Artifacts

Dust particles impurities that can be internal or external present on slide will bring artifactual changes. Fatty films are observed due to uncleaned lenses or greasy deposits on eyepieces due to eyelashes will results in foggy appearance.

SUMMARY

The processing of an oral biopsy specimen is subject to a procedural protocol that results in a tissue fit for diagnosis and interpretation. The procedures themselves are subject to human and material errors and the result is an artifact that in the least may interfere with adequate diagnosis or at the most render the tissue so distorted as to be undiagnosable. The need to recognize these artefacts and attempt to overcome them is the single biggest challenge in the oral pathological laboratory. The present review focus on identifying artifacts and their potential cause so that misinterpretation and difficulty in diagnosis can be overcome and help pathologist to come into definite diagnosis.

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