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ARTIFACTS IN ROUTINE HISTOPATHOLOGY- *AN ENIGMA TO PATHOLOGISTS WHILE DIAGNOSIS*

Oral Pathology

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ABSTRACT

Artifacts are very common and bound to occur during microscopic preparation and may be caused in various ways. A histopathological tissue under goes various laboratory steps before it is kept on the diagnostic table for confirmatory diagnosis. This procedure is not only lengthy but also technique sensitive. Hence some unintentional technical errors introduced in the slide preparation may pose and enigma to pathologists. This article reviews the most common artifacts encountered by pathologists in their day to day life with their remedies.

KEYWORDS

Artifacts, Histopathology, Diagnosis

INTRODUCTION

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 confuse them with normal tissue components or pathological changes. In some situations the presence of an artifact can compromise an accurate diagnosis.[1]

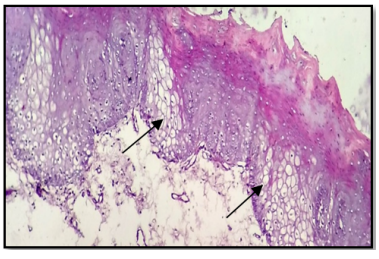
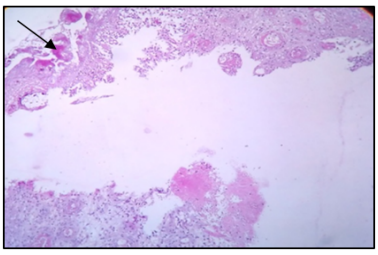
MOST COMMON TYPES OF ARTIFACTS

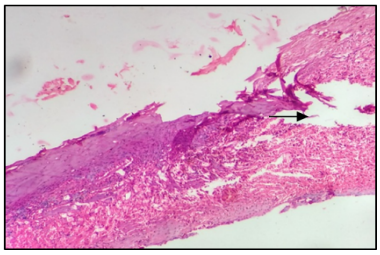
1. Pre-fixation Artifacts
2. Fixation Artifacts
3. Tissue-Processing Artifacts
4. Artifacts of Microtomy and Section Mounting
5. Staining Artifacts
6. Section Preservation Artifacts

1.PRE FIXATION ARTIFACTS

Pre fixation artifacts are produced in tissues before fixation. They may be in the form of intra lesional injections, or result from a surgical procedure as with laser knife damage or crush artifact.[2]

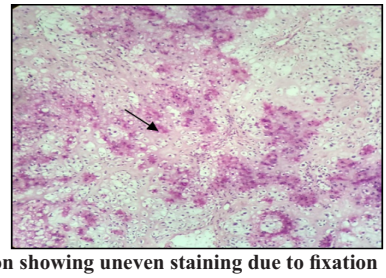
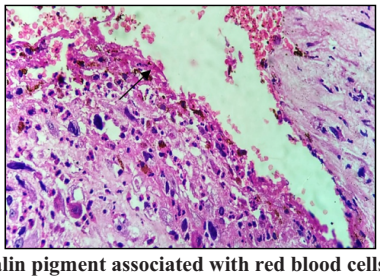
Contaminants can also be introduced into tissues during surgery or whilst handling prior to, or during specimen dissection. This type of artifact can only be avoided by ensuring that those involved are fully aware of the consequences of allowing a specimen to become contaminated or otherwise damaged.[3]

Intra-lesional injection	Intra lesional injection of anesthetic solution into the tissue leads to vacuolization of cells. It can produce hemorrhage with extravasation of RBCs, and separation of connective tissue bands with vacuolization.3 **Remedy-Local anesthetic injection should be given around the periphery of the lesional tissue.**	 **Intralesional injection leading to vacuolization of cells.(Arrow) (H&E)**
Heat damage	Heat damage is often seen along the margin of the biopsies which has been subjected to electrocautery or lasers. It takes the form of strong acidophilic staining in a local area with loss of nuclear and cytoplasmic detail. Connective tissue fibers may also be coagulated due to the effects of heat. **Remedy- Maintenance of the heat produced during the surgery by proper coolant or applying less force on to the tissues.**	 **Intense Acidophilic areas due to excess heat (H&E)**

Crush or Squeeze artifact	In the fresh state some tissues are highly susceptible to damage from crushing or squeezing by forceps or other surgical instruments. This artifact is typically seen at the periphery of the specimens as a small, localized areas appearing like pseudocyst.[1,2] **Remedy- It is recommended not to apply excessive force with tissue holding forceps while holding the tissues after incision to avoid such artifacts.**	 **Tissues showing tearing and pseudocyst formation; (Arrow) H&E**

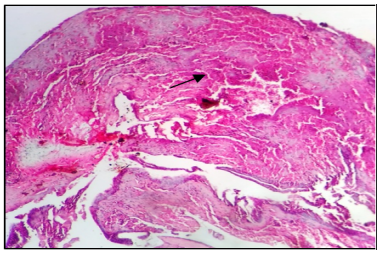
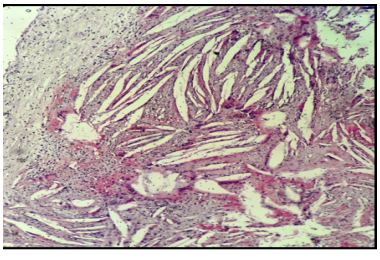
2.FIXATION ARTIFACTS

The process of fixation can produce artifacts in tissues 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.

Zonal Fixation	It occurs when the fixative penetrates slowly producing various degrees of fixation at different levels within the specimen. Common causes for this are insufficient time in fixative, attempting to fix a specimen which is too large, or by using a reagent with ***a poor penetration rate.***[1,2,4,5] **Remedy- Keeping the tissue for sufficient time in fixative solution, if the tissue is too large it should be grossed into bits to improve penetration of the fixatives.**	 **Section showing uneven staining due to fixation effects.**
Formalin Pigment	This pigment appears as a brown, with, or in the vicinity of, red blood cells. It is most often seen in tissues which have had prolonged fixation. It forms when acid formalin reacts with hemoglobin to form acid formaldehyde hematin.[1] **Remedy- It is hence recommended not to overfix the tissues in formalin and also use fresh 10% NBF for fixation procedures.**	 **Formalin pigment associated with red blood cells.**

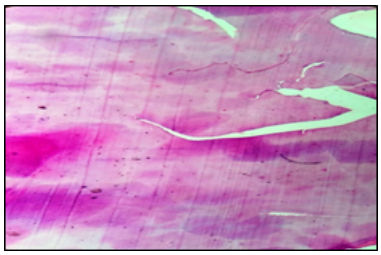
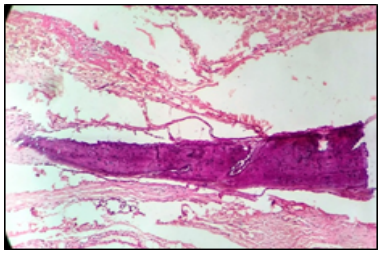
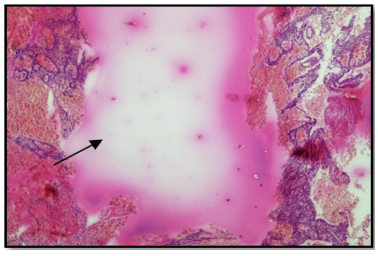
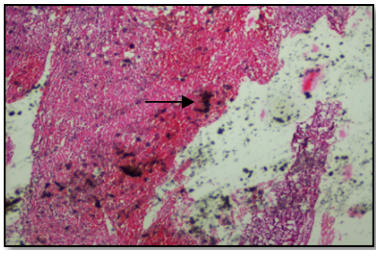
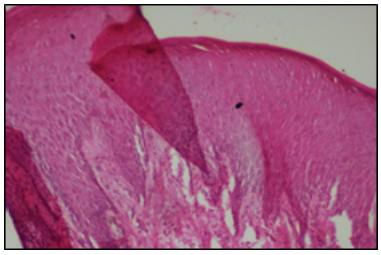
3.TISSUE-PROCESSING ARTIFACTS

Artifacts that occur during processing to paraffin wax may be the result of inadequate or incomplete fixation or some processing fault. It is inevitable that some shrinkage and distortion will occur during processing.

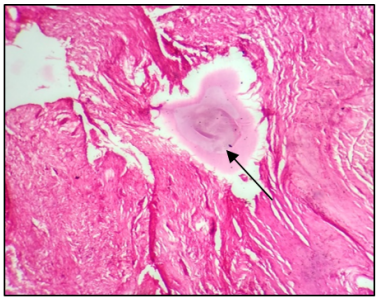
Poor Processing	Extensive loss of architectural detail may reflect inadequate fixation, but can also be caused by faults in tissue processing. Too short a processing cycle, use of exhausted reagents.[1,2] **Remedy- Keep the tissue in freshly prepared solution for appropriate time in adequate quantity or 20 times the size of tissue.**	 **Excessive tissue disruption within the loose connective tissue stroma.**
Loss of Soluble Substances	Cholesterol is seen as tapering needle-like crystals. A more common example of the loss of soluble substances is seen when neutral lipid is dissolved from adipose cells leaving regular ovoid spaces surrounded by a rim of cytoplasm.[1]	 **Tapering needle like crystal spaces observed after lipids being lost during tissue processing.**

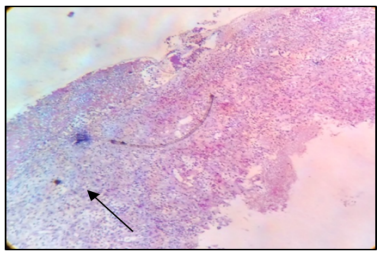
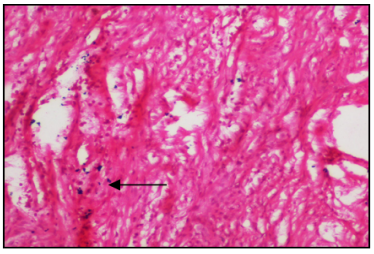
4.ARTIFACTS OF MICROTOMY AND SECTION MOUNTING

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.

Knife Lines (Venetian Blind Effect)	Fine parallel cracks in sections is usually caused by tiny vibrations in the knife edge as it passes through hard, brittle blocks. It most often occurs when disposable blades are not properly supported in the knife holder. Excessive hardness and brittleness in blocks caused by over-processing, or excessively rough or rapid cutting may also produce this effect.[1] **Remedy- before microtomy the tissue block should be kept under refrigeration for 1 hour and we should also check the sharpness of the microtome blade and its stability.**	 **Thin vertical knife lines seen along the entire section.**
Floater Effect	Floater artifact caused by specimen-to-specimen contamination during dissection where tissue from a previous specimen is transferred via the instruments used (such as scalpel blades) or through contaminated water bath during processing. Tissue cassettes, grossing table, water of the floatation bath if not thoroughly cleaned, can also carry fragments of previous specimens.[1] **Remedy- Fresh scalpel blade need to be used during grossing and floatation water bath must be devoid of previous tissue sections.**	 **A section of bone seen amidst of the muscle tissue.**
Tidemark due to adhesive	The pale amorphous, eosinophilic stained deposits are caused by the pooling and subsequent evaporation of flotation fluid containing albumin adhesive. The pools are more likely to occur in irregular, poor quality sections where protein-based adhesives are used and sections are not drained of excess adhesive before drying.[1,3,5,6] **Remedy- exact amount of adhesive to be used in an uniform stroke on the glass slide by a clean paintbrush and excess adhesive to be drained off before drying the slide.**	 **Tide mark due to adhesive (albumin), H&E**
Contamination of Mounted sections	Mounted, unstained sections left uncovered, can become contaminated with airborne fibers, hair from the brush used to transfer sections from knife edge to flotation bath and dirt .[1,2,3,5,6] **Remedy- glass slide on which the tissue is loaded should be clean and it should be mounted soon after the staining is complete.**	 **Dirt contaminating the section (H&E)**
Folds in a section	Tissue sections likely to show folds after flotation. Folding is caused by tissue shrinking during processing followed by variable expansion on flotation. These faults are readily identified macroscopically. **Remedy-Careful microtomy and flotation techniques minimize this problem.**	 **Folds seen in the Stratified Squamous Epithelium. (H&E)**

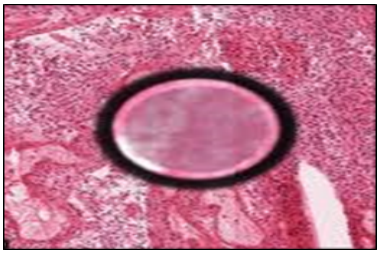
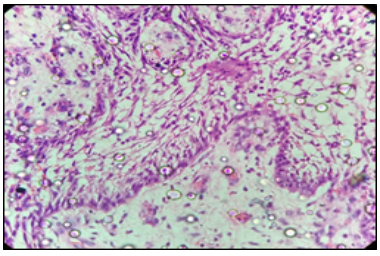
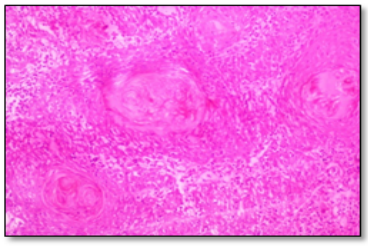
5.STAINING ARTIFACTS

Residual Wax	Residual wax in a section prevents the penetration of staining solutions leaving areas totally devoid of stain. The final clearing of the section before applying cover slips removes all traces of the wax leaving no evidence as to the cause of the patchy staining. **Remedy-Prolonged xylene treatment and re-staining will overcome this problem.** [1]	 **Presence of residual wax . Arrow (H&E)**

Incomplete Staining	Incomplete staining with one dye in a multi-step procedure may result from an inadequate levels of stain in the coplin jars.[1,2] **Remedy- freshly prepared stain with adequate height of the solution to be monitored before staining.**	 **Incomplete staining of tissue (H&E)**
Stain Deposits	This type of artifact may arise from undissolved stain, stain precipitate or any solid component remains in an unfiltered staining solution. **Remedy- the use of sealed staining jars will eliminate most artifacts caused due to precipitates[1,3,5,6] and also remove the oxidized layer of the solution by a filter paper.**	 **Stain deposits in the section. Arrow (H&E)**

6.SECTION PRESERVATION ARTIFACTS

Artifacts which develop in sections during storage may not be evident for months or even years. This can cause major problems when reviews of archival material are required.

Drying Artifact	The common problem of large air bubbles in the mountant blurring the sections. Excessive time taken between removing the slide from xylene and applying the coverslip leads to drying of sections and minute bubbles become trapped over the nucleus when the coverslip is applied.[1,4,5,6] **Remedy- mounting should be done as soon as staining and clearing with xylene is complete.**	 **Air bubble in section (H&E)**
Water in section	Sections not been adequately dried will have water. Sometimes small water droplets may also be seen microscopically which masks microscopic detail.[1] Sections should be cleared, dehydrated and remounted. **Remedy- sections should be adequately dried before mounting.**	 **Water in section (H&E)**
Bleaching of a stain.	Bleaching of stains can result from exposing sections to light for excessive periods. Sections affected in this way can be re-stained. This is caused by exposure of the slide for a short period to the very intense light. **Remedy- Stained sections are best stored in the dark.**	 **Oxidized and bleaching of Hematoxylin with only Eosinophilic area remaining in the section. (H&E)**

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