

COMMON ARTIFACTS AND REMEDIES IN HISTOPATHOLOGY (A REVIEW)

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Abstract

The essence of preparing a biological specimen for microscopic studies is to get adequate and accurate medical information that is a true representation of the specimen, be it for research or diagnostic purposes. An artifact can be defined as unrelated, self-colored artificial feature found in tissue sections. This has been an age long cause of misinterpretation leading to misdiagnosis to microscopists. The processing of specimen for medical information is subject to a procedure 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 artifacts and attempt to overcome them is the single biggest challenge in the histopathology Laboratory. This article focused on identifying artifacts and their potential cause so that misinterpretation and difficulty in diagnosis can be overcome, and help microscopist to come into definite diagnosis.

Keywords: Artifacts, Histopathologist, Microscopist, Diagnosis

INTRODUCTION

Artifact can be defined as unrelated, self-colored artificial features found in tissue sections (Avwioro, 2014). They occur in tissue sections before fixation, during fixation, grossing of specimen, tissue processing, sectioning, staining and preservation of tissue section. Some artifacts are easily distinguishable from normal or diseased tissue components while some are difficult to distinguish from such entities. In some situations the presence of an artifact can compromise an accurate diagnosis. Artifacts may result in alteration of normal morphologic and cytological features or even lead to complete uselessness of the tissue. It is important to know and understand about artifacts, as learning to recognize them, can prevent misdiagnosis. Ficarra et al., (1987), Margarine et al (1985). These artifacts can be minor, involving only small portion of the specimen and therefore do not interfere with the pathologists' 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 Ficarra et al., (1987). Therefore, this article is to promote an awareness of the various common 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.

TYPES OF ARTIFACT

Pre-Fixation Artifact

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.

Examples of pre-fixation artifact include:

i. 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 Krishnanand et al., (2010). Biopsy area is also prepared by using tincture iodine. Reagents commonly used for surface marking include Indian ink, Silver nitrate, Alcian blue and Alcian green.

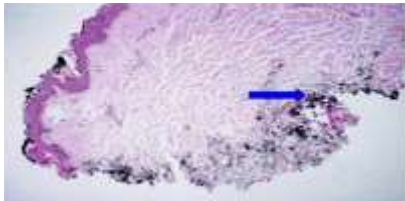


Plate 1: Artifacts due to surface preparation and tattoo pigmentation: margin of a skin biopsy marked with silver nitrate. The reagent stains the soft tissues and has penetrated into the dermis

REMEDY

Preparation of the area of biopsy with tincture or other colored solutions should be cautiously used and should be clearly mentioned along with biopsy details, as it can interfere with tissue processing and staining procedures.

ii. Tattoo pigment artifact

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.

iii. 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 causes of artifacts during surgical handling are:

Mechanical causes: by 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 into the lesional tissues and application of antiseptic medication like Betadine solution.

Thermal cause: instruments used for coagulation and cutting electrodes like

electrocautery[Meghana and Ahmedmujib (2007), (Seoane, 2004). This causes the surface epithelium to be forced through the connective tissue, producing small “pseudocysts” and also causes loss of cytoplasmic and nuclear features (Camacho et al., 2008).



Plate 2: Surgical Artifacts: A) Forceps Artifact: Tearing of tissue due to penetration of forceps

REMEDY

These artifacts can be prevented by handling the specimen gently, avoiding applying unnecessary force and holding the tissue in an area away from the lesional tissue.

iv. Fulgeration artifacts

Heat produced while using laser or electrosurgical procedures may lead to marked alteration in both epithelium and connective tissue Meghana and Ahmedmujib, (2007). 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.

REMEDY

This artifact can be prevented by using knives, use low milliamperage current, use of combination knife and electrical points.

v. Injection artifact

Injection of large amounts of anesthetic solution into the area to be biopsied can produce hemorrhage with extravasations 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 (Meghana and Ahmedmujib, 2007; Seoane, 2004).

REMEDY

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.

vi. 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. (Seoane, 2004), Ramirez et al., (2007) 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. The presence of a suture in a histological specimen may not be of any pathological significance but it can damage the microtome knife leading to tears and knife lines in sections.



Plate 3: Sutural artifact: Bifringent suture material from stitch granuloma under Polarized light.

REMEDY

Visible sutures should be removed wherever possible

vii. Artifacts due to contamination

Certain artifacts can be due to contamination:

- (a) Starch artifact due to contamination of specimen with starch powder used as lubricant in surgical glove. These starch granules are retractile, glassy, poly, 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,



Plate 4 (Artifacts due to contamination). Starch granules: Starch granules in a typical starch granuloma stained with H&E and Polarized light

- (b) 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.

REMEDY

Thorough rinsing of the board and instruments between specimens or covering the dissection board with separate paper sheets will prevent this problem.

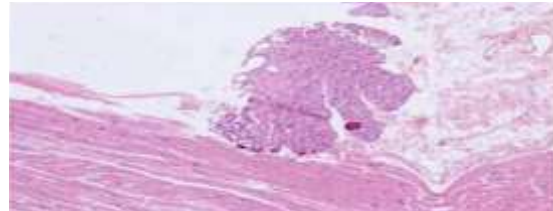


Plate 5-.Artifacts due to contamination. Specimen-specimen contamination: A section of cardiac muscle with a piece of extraneous thyroid tissue present against one surface; H&E.

- (c) 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 (Ficarra et al., 1987; Krishnanand et al., 2001)



Plate 6-.Artifacts due to contamination. Foreign body contamination: Cellulose in an H&E-stained section and under Polarized light.

REMEDY

Artifact due to contaminations can be prevented by careful manipulation of specimen at every step of preparation and cleaning dissecting board after treating each specimen.

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. (McInnes, 2005;Weir, 1976). 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. Fixation artifacts arise due to formalin, mercuric chloride and picric acid used in various fixative agents which causes Brown-Black granular and yellow stains distributed randomly throughout the tissues. (Samar et al., 2014)

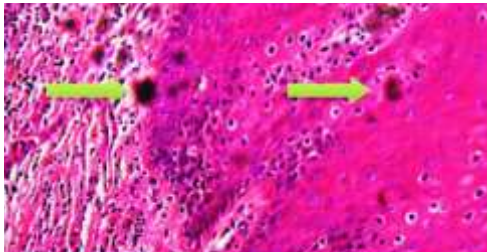


Plate 7. Fixation Artifact. Pigment artifact: Deposits of formalin pigments are brown here in H&E Stain

REMEDY

Treat sections with saturated alcoholic picric acid solution or prevent it by fixing in buffered formalin

i. Shrinkage artifacts: During fixation, tissues change in size. 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 (McInnes E 2005), this is a very common artifact. Some of the non protein precipitants cause swelling of tissue following fixation in formalin.

REMEDY

Shrinkage artifact can be prevented by using compound fixatives compensating for disadvantages of individual fixatives as seen in Bouin's fluid.

ii. 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 its original location. A well known example of this is glycogen.

REMEDY

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

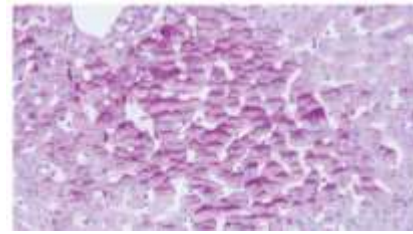


Plate. 8 streaming of glycogen, Seen in hepatic tissue

iii. 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. (Samar et al., 2014)

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

Remedy: Maintain optimal temperature during this procedure

v. Artifacts during freeze-drying - ice crystal artifacts: During fixation using freeze drying method, the tissue must be plunged into isopentane cooled to -160 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 artifacts (Thomson A 2007). This artifact causes total distortion of the tissue and diagnostic difficulty.

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

Vii Artifacts during post fixation treatment: Tissue that are fixed in chrome if not washed for 24 hours in running tap water could produce chromeoxide pigment

TISSUE 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. Examples of tissue processing artifact include the following.

i. 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 to increase in possibility of cell distortion (Margarine et al., 1985) (McInnes E 2005)

2. Over dehydration makes the tissue hard, brittle and shrunken causing difficulty while cutting and also interfere with staining properties of section (Grizzle et al., 2001)

3. Under or Incomplete dehydration results in improper infiltration of paraffin and block made is difficult to section thus leading to distorted or fragmented tissue sections which causes artifactual features.

ii. Artifacts During Clearing:

Artifacts may arise due to over and under clearing of tissue causing excessive hardening, and thus obstruct paraffin impregnation of tissue making it difficult to cut during sectioning. Krishnanand et al., (2010), Margarine et al., (1985)

iii. Artifacts During Impregnation:

The function of wax impregnation is to remove clearing agent (wax solvent) from the tissue and for it to be completely permeated by the paraffin wax which is subsequently allowed to harden to produce a block from which sections are cut. The artifact produced during this procedure is Crystallization: The thicker the tissue, the more clearing agent it absorbs and hence it requires multiple change of molten wax to be completely impregnated. Contamination of molten wax by clearing agent causes incomplete impregnation leading to crumbling and crystallization during sectioning (Samar et al., 2014)

iv. Artifacts During Embedding:

Artifacts due to improper orientation are frequently encountered during embedding procedures which will lead to damage to

microtome, tearing of section thereby making microscopic study of tissue difficult.

v. Artifacts Due to Poor Processing:

Extensive loss of architectural details and clarity within loose connective tissue may reflect inadequate fixation. These can 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. Krishnanand et al., (2010), Grizzle et al., (2001)

ARTIFACT DUE TO MICROTOMY AND SECTION MOUNTING

i. 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.



ii. Artifacts Related to Tissue Floating on Water Bath:

Mainly three types of artifacts can result from floating:

(a) Air Bubble Entrapment: trapped water bubble under section, 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.

(b) Increase temperature of water bath results into expansion of tissue beyond its limit and “Parched Earth (crackes)” effect is noted.

(c) Floaters Artifacts are pieces of tissues that appear on slide that do not belong there, they are mainly extraneous to the floated section and these results mainly from unclean floating bath.

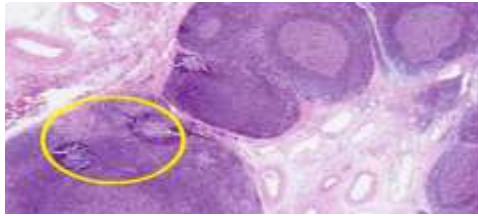


Plate 10 water bubble trapped in section
Artifacts in water bath: Air bubble entrapment; Section of lymph node showing circular darkly stained areas with pale centers and surrounding radial cracks. These faults indicate collapsed bubble artifact; H&E Stain.

iii. Artifacts related to oven/hot plate:

The temperature setting of the oven/hot plate 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. Cells appear to be completely devoid of nuclear details.

iv. Artifacts During Lifting of Tissue Sections:

Artifacts such as Tissue Folds can be produced when tissue adheres to the under surface of the blade, seen most commonly with fatty tissues and mainly seen due to dull blade. 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. This is mainly seen due to dull blade, these tearing can be avoided by using clean and replaced blade.



Plate 11: Artifacts during lifting of tissues: Tissue folds; H&E Stain.

v. Artifacts due to section adhesive and mounting:

Thick coat of section adhesive will take the stain and background stain may be detected resulting in irregular, poor quality sections. Mounted, unstained section left uncovered can be contaminated with various materials such as

microorganisms, airborne fibres, cellulose fibres and dirt particles.

STAINING ARTIFACTS

i. Artifacts due to residual wax:

Residual wax in a section will prevent penetration of both aqueous and alcoholic dye solutions leaving areas 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 (Krishnanand et al., 2010; Wynnchuk, 1990)

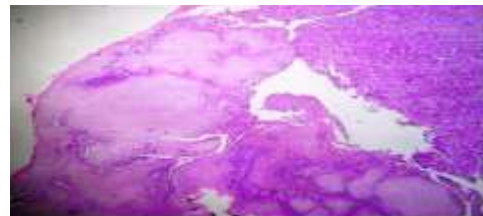


Plate 12 Artifact due to residual wax

REMEDY

Prolonged xylene treatment and re-staining will overcome this problem

ii. Artifacts due to contaminated staining solution

Staining solutions can be contaminated by microorganisms, dust/ foreign particle and could serve as a potential cause of misdiagnosis.

Artifacts due to stain deposits:

Undissolved and precipitated stain will lead to deposits on sections.

v. 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. Grizzle et al., (2001)



Plate .13 Artifacts due to incomplete staining

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

Remedy

Ensure that microscopes are well kept and regularly maintained

Section Preservation Artifacts

This include majorly drying artifact and bleaching artifact



Plate. 14 bleaching present due to exposure of stained section to light.

SUMMARY

The processing of specimen for adequate medical information is subject to a procedural protocol that results in a tissue fit for diagnosis and interpretation. The procedures themselves are subject to human, reagents and material errors which may result in 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 artifacts and attempt to overcome them is the single biggest challenge in the histopathology Laboratory. This review article focused on identifying artifacts, their potential cause and remedies so that misinterpretation and difficulty in diagnosis can be overcome and help microscopist to come into definite diagnosis.

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