Artifacts in histology: A 1-year retrospective study

ABSTRACT

Introduction: Histology is a science of the analysis of tissue architecture; however, the presence of artifacts in microscopic sections may result in misdiagnosis. Despite documented common occurrences, studies on the patterns of artifacts in Nigeria are however scant. The rarity of descriptions in this clumsy but important component of histology stimulated our interest in demonstrating the various patterns of artifacts in a laboratory. This 1-year retrospective study was conducted in Federal Medical Centre, Asaba, Delta State. **Materials and Methods:** Tissue sections were viewed and with the aid of a microscope to check for the various patterns of artifacts. These artifacts were seen as artificial structures or tissue alternations on the prepared slide. Histological images were captured using eyepiece Scopetek DCM 500, 5.0 Megapixel connected USB 2.0 computer. Data were obtained by standard microscopic techniques in which the various patterns of tissue alterations were described. Permission for this study was obtained from the hospital Ethics Committee (ethical number FMC/ASB/T/A81/198). **Results:** This review of patterns of artifacts showed that during the 1-year period, of the 388 slides reviewed, 94.59% had the presence of artifacts. The results also revealed that fold artifacts were the most prevalent patterns constituting about 33.00% of the total tissue sections observed, followed by artifacts attributed to microtomy which accounted for 18.47% and formalin pigment artifacts, 14.78%. The least was heat and hemorrhagic artifacts which contributed to about 0.25%. **Conclusion:** In conclusion, fold artifacts were the most prevalent patterns observed in this study due to the thin sections which easily stretch around other structures having different constituencies if the tissue is not carefully lifted from the water bath.

Key words: Artifacts, fold, histology, misdiagnosis, retrospective

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INTRODUCTION

The alteration of tissue detail emanating from the occurrence of foreign implants during the preparation and processing of tissue or from the tools of histological analysis (microscope) has been known to result in misdiagnosis in histopathology. These entities called artifacts may alter the original picture or result in alteration of normal tissue cytomorphological architecture.[1]

It has previously been noted that some artifacts are easily distinguishable from the original component of the tissue, whereas others may be more difficult to identify.[2] Tissue alterations of these magnitudes have been classified as minor or major, depending on the degree to which the original tissue is sufficient for accurate diagnosis.[3]

Several factors have been recorded to account for artifacts, and these include clinical application of chemicals, local injection of anesthetics, surgical suctioning, excessive heat, freezing, surgical mishandling of specimens, inadequate tissue fixation, improper fixation medium, faulty tissue processing, embedding sponges, and improper staining among others.[4]

Access this article online	
Quick Response Code:	Website: www.bioanthrojournal.org
	DOI: 10.4103/2315-7992.210253

How to cite this article: Igho OE, Aimakhume A. Artifacts in histology: A 1-year retrospective study. Ann Bioanthropol 2017;5:34-9.

Although artifacts have been demonstrated in most histological and histopathological tissue sections, there is a dearth of data on the proportion of artifacts and the relative frequency of various patterns of artifacts despite the occurrence of these artifacts in several histopathology centers. Scanty literature has been developed to ascertain the frequency owing to the cost and time effect of the occurrence of artifact has necessitated the present study. Therefore, this study was aimed at evaluating the patterns of artifacts in a histopathology laboratory. It is expected that this study will provide a baseline analysis of the patterns of artifacts, the frequency of artifacts both of which could likely influence appropriate funding by the management of various histopathology laboratories.

MATERIALS AND METHODS

Study design

This study was a 1-year retrospective study, which involved records of all histological tissue sections processed at the Department of Pathology, Federal Medical Centre, Asaba, between January 1, 2014, and December 31, 2014.

Inclusion and exclusion criteria

All formalin is fixed and paraffin-embedded histological tissue slides received in the Department of Pathology, Federal Medical Centre, Asaba, Delta State, between January 1, 2014, and December 31, 2014 were included in the study. Histological tissue slides that were broken and slides that were improperly labeled were excluded from the study.

Ethical clearance

Departmental ethical clearance was obtained from the Department of Pathology, Federal Medial Centre, Asaba. Institutional clearance was also obtained from the Ethical Committee Federal Medial Centre, Asaba, with ethical number FMC/ASB/T/A81/198.

Methods

Already processed histological sections were used, and these tissue sections were obtained from Pathology Department. These tissue sections were viewed and with the aid of a microscope (Leica DM500) to check for the various patterns of artifacts. These artifacts were seen as artificial structures or tissue alternations on the prepared slide.[1] Histological images were captured using eyepiece Scopetek DCM 500, 5.0 Megapixel connected USB 2.0 computer.

The data obtained from this study were presented in tabular form [Table 1].

RESULTS

The present review of the patterns of artifacts in a histopathology laboratory during the 1-year period showed that of the 388 slides reviewed, 367 were characterized by artifacts. In the slides investigated, 406 artifacts were recorded with some slides displaying one or more different types of artifacts.

Table 1: Results

Patterns	*f*	Percentage of prefixative artifacts	Percentage of total artifacts
Prefixative artifacts			
Heat*	1	1.45	0.25
Crush[ł,§]	28	40.58	6.9
Split[¶,†]	36	52.17	8.86
Contaminant**	3	4.35	0.74
Hemorrhagic***	1	1.45	0.25
Total	69	100.00	17.0
Fixative artifacts	***f***	**Percentage of fixative artifacts**	**Percentage of total artifacts**
Formalin pigment[††,§§]	60	100	14.78
Tissue processing artifacts	***f***	**Percentage of tissue processing artifacts**	**Percentage of total artifacts**
Microtomy[¶¶,ll]	75	35.89	18.47
Fold[†††,‡]	134	64.11	33.00
Total	209	100.00	51.47
Staining and mounting artifacts	***f***	**Percentage of staining and mounting artifacts**	**Percentage of total artifacts**
Residual wax[‡‡,§§§]	19	27.94	4.68
Stain deposit[¶¶¶,****]	29	42.65	7.14
Contaminant[‡‡‡]	3	4.41	0.74
Air bubble entrapment[††††,§§§§]	17	25.00	4.19
Total	68	100.00	16.75
Grand total	406		100.00

Key: *Occurred once in combination with other patterns; łOccurred twice singly; §Occurred 26 times in combination with other patterns; ¶Occurred 8 times singly; †Occurred 28 times in combination with other patterns; **Occurred thrice in combination with other patterns; ***Occurred once in combination with other patterns; ††Occurred 8 times singly; §§Occurred 52 times in combination with other patterns; ¶¶Occurred 14 times singly; llOccurred 61 times in combination with other patterns; †††Occurred 33 times singly; ‡Occurred 101 times in combination with other patterns; ‡‡Occurred 4 times singly; §§§Occurred 15 times in combination with other patterns; ¶¶¶Occurred 6 times singly; ****Occurred 23 times in combination with other patterns; ‡‡‡Occurred thrice in combination with other patterns; ††††Occurred twice singly; §§§§Occurred 15 times in combination with other patterns

Prefixative artifacts

There were 36 cases of split artifact contributing to 52.17% in this group, [Figure 1] 28 cases (40.58%) were crush artifact, whereas only three cases (4.35%) were artifacts attributed to contaminant and only one case (1.45%) of hemorrhagic and heat artifact each was observed in this study.

Fixative artifacts

The only artifact observed at this stage was formalin pigment artifact which had 60 cases (100%) [Figure 2].

Tissue processing artifacts

The only pattern of artifact observed which was due to lifting of tissue from the water bath was fold artifact which was observed in 134 cases (64.11%) [Figure 3] which was the highest observed artifact due to tissue processing. Seventy-five cases (35.89%) were due to faulty microtomy, [Figures 4 and 5] some of these artifacts which were observed included knife line artifacts, scratch line artifact, and crumbling artifacts.

Staining and mounting artifacts

Twenty-nine cases (42.65%) observed were due to stain deposited on the prepared histological section, these patterns were the highest in this stage. Nineteen cases (27.94%) observed showed artifact due to residual wax, [Figure 6] 17 cases (25.00%) observed were due to air bubble entrapment during mounting [Figure 7]. Finally, three cases (4.41%) observed were due to contaminant [Figure 8].

DISCUSSION

This study revealed that over the 1-year period, 406 artifacts were observed in 388 tissue sections in Pathology Department of Federal Medical Centre, Asaba, Delta State. Most tissue sections had the presence of more than one pattern of artifacts which occurred in different locations, and 21 tissue sections were devoid of artifacts. Several studies have described artifacts as any artificial structure or tissue alteration on a prepared tissue section - the result of an extraneous factor, which can result in alteration of normal tissue morphologic and cytological features which might have occurred as a result of the way tissue were handled, right from biopsy, which was surgically obtained till the entire histopathological procedure of fixation, processing, embedding, sectioning, and staining are performed. These procedures themselves have been shown to be subject to both human and material error. Artifacts can be classified into prefixative artifacts, fixative artifacts, tissue processing and sectioning artifacts, and staining and mounting artifacts.[1,5,6] Fold artifact accounted for the majority of cases, heat and hemorrhagic artifacts constituted the least observed artifact.

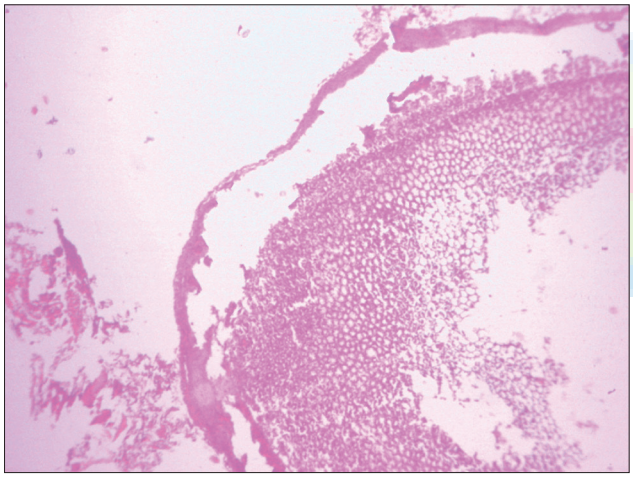

Figure 1: Split artifact in a section of the intestine, ×40

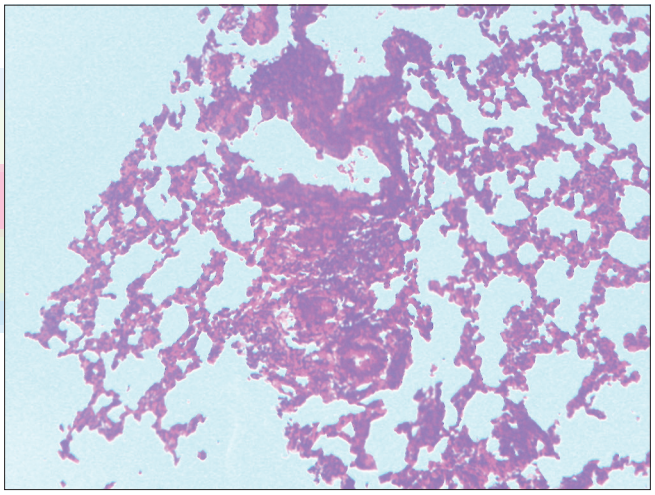

Figure 2: Formalin pigment in a section of the lungs, ×100

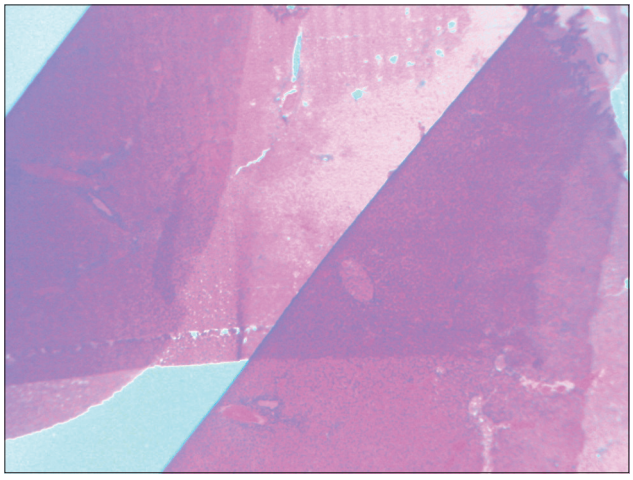

Figure 3: Fold artifact in a section of the liver, ×40

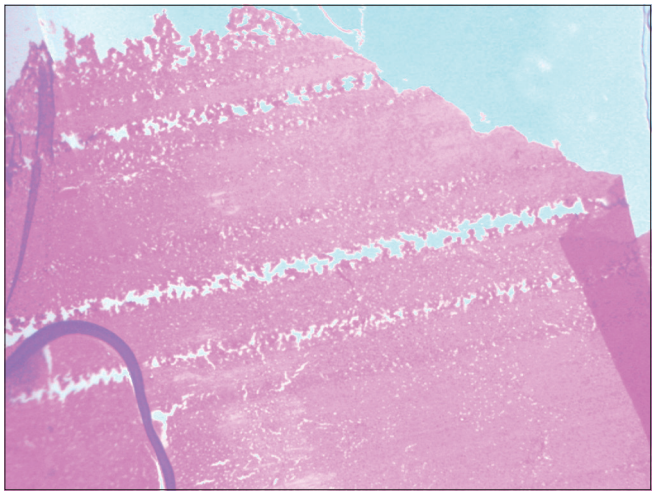

Figure 4: Scratch lines in a section of the liver, ×40

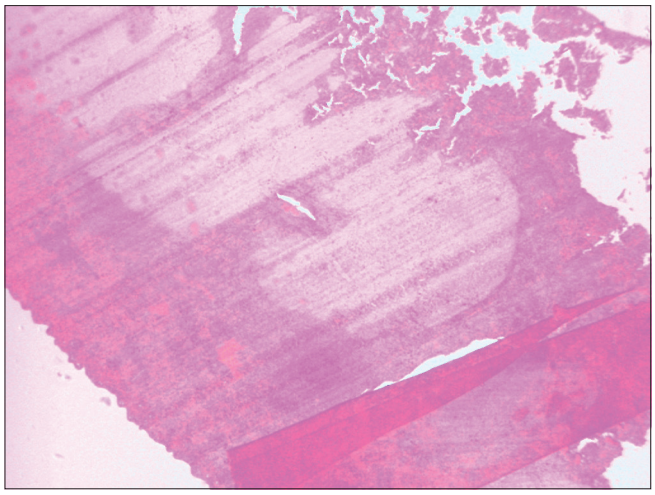

Figure 5: Knife lines and fold artifact in a section of the spleen, ×40

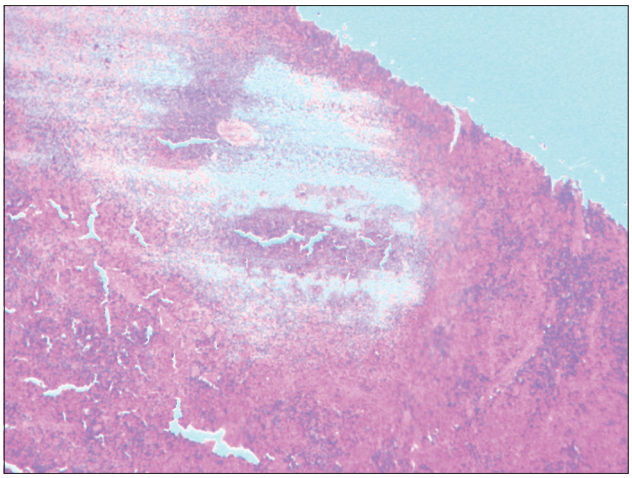

Figure 6: Residual wax in a section of the spleen, ×100

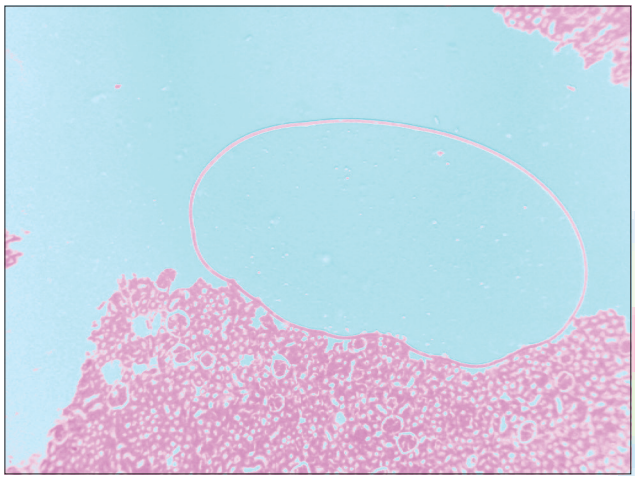

Figure 7: Air bubble entrapment in a section of the kidney, ×40

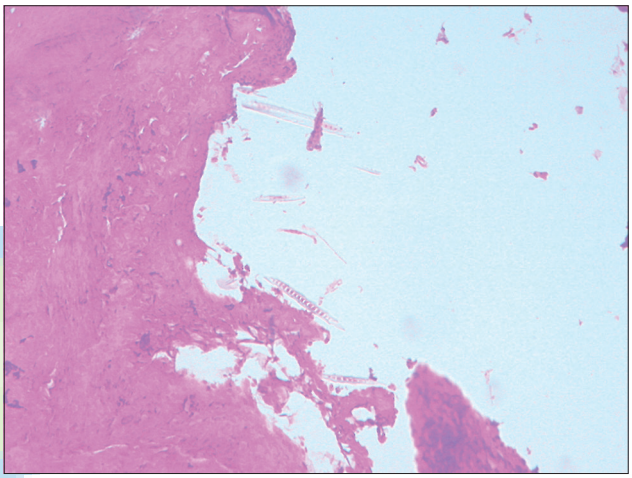

Figure 8: Contaminant artifact in a section of the bone marrow, ×100

Prefixative artifacts have been shown to be introduced into the tissue before fixation.[4] They have been reported to be caused by factors related to the surgical procedures, during handling using forceps.[7,8] Heat artifacts were observed along the margin of surgical biopsies and were seen microscopically as strong acidophilic staining with loss of nuclear and cytoplasmic details, the distribution and features were also documented in another study.[4] In the index study, heat artifacts were the least observed pattern of artifact because this was introduced into the tissue when fixed with heat and majority of the tissues used for this study were fixed with a chemical fixative. To prevent the occurrence of this artifact, other methods of fixation are advised.

When toothed forceps were used, and their teeth were able to penetrate the tissue, it resulted in voids or tears and compression of the surrounding tissues.[3,9] Microscopically, the tissue architecture was rearranged and the chromatin of the nucleus was squeezed out. In the index study, it was observed that crush artifact was one of the most prevalent prefixative artifacts, similar frequencies of this pattern of artifact were reported in another study.[5] However, in this study, fewer number of crush artifacts were observed at the base as well as in the specimen proper and when compared with other studies,[3] this could be attributed to the facts that blunt forceps were used instead of toothed forceps during biopsies for larger tissues and the tissues were handled at the base of the specimen where fat and muscle were usually present. Regarding the presence of split artifact which was secondary to the surgical technique employed, this pattern of artifact was the highest observed prefixative artifact in this study, and the reason for the high occurrence of these patterns was because most of the tissues were benign tumors and inflammatory disorders. This agrees with a study which reported a high incidence of split artifacts with inflammatory disorders, benign tumors, precancerous lesions, and malignancies. It was then observed that this pattern was frequent in biopsies taken from patients with underlying inflammatory processes.[10] A study had reported a high frequency of tissue sections which had splits,[11] and in another study conducted, no split artifact was observed which was explained by the nature of sample studied which were healthy oral mucosa tissues.[5] The use of blunt forceps

is advised, sufficient biopsies should be obtained with care and avoid compression.

Fixative artifacts have been documented to occur during tissue fixation. Formalin pigment artifact was the only pattern noted in the index study and was observed to account for the majority of the cases recorded. This artifact was predominantly seen around blood vessels which contained erythrocytes. Hemoglobin which is a major constituent of erythrocytes is known to react with acid formalin used in the preservation of the specimen and acid formaldehyde hematin, a black to brown opaque pigment is formed, the site and features of this artifact were also reported in another study.[12] The reason for the prevalence of this pattern in the index study could be attributed to the use of acid formalin was used as the fixative for most of the specimen in this center. Hence, the use of other fixatives such as buffered formalin and restricting fixation time can also help reduce the occurrence of these artifacts. Furthermore, the use of picric acid solution before staining has been observed to remove this artifact.

Tissue processing artifacts that occur during this stage maybe as a result of inadequate or incomplete fixation or some processing faults.[4] Microtomy, the means by which tissues are sectioned, so that microscopic examination is possible, some artifacts can be introduced if proper techniques were not followed.[13] It was observed in this study that there was a high proportion of artifacts which emanated during this stage of tissue processing in relation to the total number of total artifacts observed, the patterns of microtome artifacts observed in this study were either scratch and knife lines artifacts which are both due to damaged knife edges. The cause of these artifacts was also confirmed in another study.[14] The artifact with the highest frequency in this study was fold artifacts, this pattern of artifact has been observed to be introduced during lifting of the tissue section from the water bath, this was verified in a review that this pattern was seen with alarming regularities due to the thin sections being eventually stretch around other structures having different constituencies,[13] so careful flotation and microtomy have been shown to help reduce the occurrence of these artifacts.[4] Special care should be adhered to, this will help reduce the occurrence of these artifacts, also distilled water rather than tap water should be used and the water bath should be emptied and dried after every sectioning and flotation.

Staining and mounting artifacts which arise during staining fall into two groups: Incomplete or patchy staining and precipitates or contaminants derived from the staining solution.[14] Regarding the presence of various patterns of artifacts which can be attributed to staining, it was observed that artifacts due to residual wax were seen as areas devoid of staining was due to failure to completely remove wax from sections accounted for quite a high percentage of artifacts observed during staining. The mechanism of the occurrence of this artifact was also documented in a study.[15] Prolonged xylene treatment and restaining can overcome this problem. Stain deposits were observed on the prepared histological sections, and this pattern was the highest in this stage which might be because an automatic strainer was used during staining, used open racks, this cause was also confirmed in another study.[4] Hence, the use of closed racks or containers and filtering of the staining solutions can help reduce this artifact. Complete removal of wax, ideal temperature and timing can help curb the occurrence of staining artifacts. Finally, air bubble entrapment during mounting which was observed as circular areas on the prepared tissue section. It contributed one-fourth of the total number of artifacts reported in this section; careful mounting should be adhered to.

Most studies did not have frequencies for fixative, tissue processing, staining, and mounting artifacts, which caused restraints in comparison of this study with other studies.

There is a high occurrence of artifacts which occur during fixation, tissue processing, staining, and mounting have not been statistically analyzed by most authors, so I recommend that future researchers should evaluate the percentage frequencies of the various patterns of artifacts that occur during those stages and over a longer period of time. Finally, there should be more awareness to enlighten students, histoscientist, and pathologists at large on the presence of these artifacts as they cause pitfalls in diagnosis.

CONCLUSION

Artifacts are encountered in most microscopic sections, and these cause misinterpretation of tissue sections, whereas fold artifacts were the most prevalent patterns observed in this study due to the thin sections which easily stretch around other structures having different constituencies if the tissue is not carefully lifted from the water bath.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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