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Artifacts: A menace in histopathology

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Abstract
Introduction: The word 'artifact' is derived from the latin words, 'Ars' (art) & 'factum'(made). The study was undertaken to know the prevalence and patterns of artifacts.
Materials & methods: This is a cross-sectional observational study. 250 consecutive hematoxylin and eosin (H&E) stained sections were retrieved from the archives and analyzed for artifacts under light microscope.
Results: Among 250 slides, 227(90.8%) showed the presence of artifacts. The folding artifact was the most prevalent constituting 98(43.17%) and excess mountant 6(2.64%) was least common.
Conclusion: Folding artifacts were the most prevalent pattern in this study. Proper technical measures need to be employed in order to prevent/minimize the occurrence of artifacts in a skillful manner, as they may pose diagnostic difficulties.

Keywords: Artifacts, histopathology, section folding, H&E stain

Introduction
As we all know, Histopathological examination of tissues still remains as gold standard for final diagnosis of various lesions.
This diagnostic method requires preparation of tissue sections and staining. The procedure is laborious and starts from the time the tissues are fixed till the sections are mounted on the glass slides. This work requires skilled technical staff. The primary aim of this is to preserve the morphology of cells as close to how they were within the body before surgical removal.
The various steps in slide preparation are fixation, tissue processing, embedding, microtomy, staining and mounting. Inspite of automated tissue processors that are used for preparation of sections and staining, many artifacts are encountered which may interfere with diagnosis.
Accurate diagnosis of the lesions under the microscope requires high quality sections. This depends on the skills and experience of laboratory technicians also. Artifacts can occur at any of the above mentioned stages in slide preparations. It is important to know the various artefacts.
The present study was undertaken to know the prevalence of artifacts that occur in the slides because of procedural errors and list the various types of artifacts encountered, so that based on the type of artifacts, we can find the cause for the same and undertake appropriate remedial measures [1, 2].

Materials and Methods
This is a cross sectional study involving analysis of 250 consecutive histopathology slides. Slides for study were retrieved from archives of previous month. These were the slides prepared routinely using leica automated tissue processor and stained with hematoxylin and eosin from specimens sent to the department for histopathology. These slides were observed grossly and then microscopically for the artefacts encountered due to problems in tissue processing i.e from fixation to mounting of sections.

Results
Among 250 consecutively reviewed slides, 227(90.8%) showed the presence of artifacts. Of the 227 slides with artifact, the section folding was the most common constituting 98(43.17%) of the total slides with artifacts and the least common was excess mountant constituting 6(2.64%).

Folding artifact appeared like folded south Indian dosa. Scoring 37(16.30%), dry mounting 33(14.54%), split sections 29(12.77%) were the next common artifacts. Stain mucks was seen in 24(10.57%) slides. There were no artifacts in 23(9.2%) of the slides.

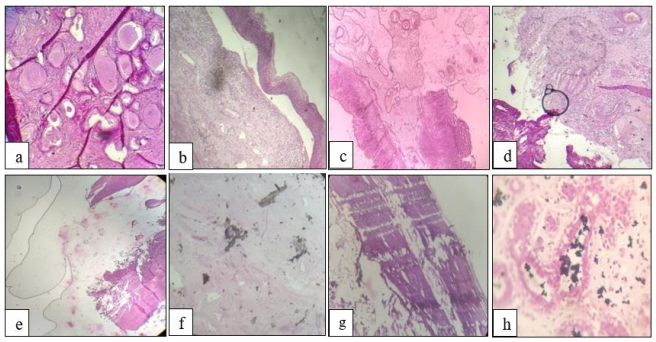

Fig 1: a: Folding of sections, b: Split sections, c: Dry mounting, d: Air bubbles, f: Excess mountant, g: Dust particles, h: Scoring, i: Stain mucks.

Table 1: Types of artifacts expressed in numericals & percentage

Type of artifacts (n=227)	Number of slides	Percentage
Folding of sections	98	43.17%
Split sections	29	12.77%
Dry Mounting	33	14.54%
Air Bubbles	18	7.93%
Excess mountant	6	2.64%
Dust particles	10	4.40%
Scoring	37	16.30%
Floaters	7	3.08%
Stain Muck	24	10.57%

Discussion

To recognize the artifacts and taking measures to overcome them is a biggest challenge in histopathology laboratory. Ekundina VO, Eze G have given a detailed account of common artifacts encountered in histopathology, their recognition and remedial measures, with the aim of promoting awareness on the same [3].

Artifacts in histopathology i.e. technical artifacts are introduced into tissue specimen from the time of fixation to processing, section cutting, staining and mounting. Inspite of using automated tissue processor and experienced technicians, prevalence of artifacts was found to be more than 90%. Zegarelli in 1978, published first article regarding artifacts [4]. Since then there are few articles discussing the same along with remedial measures.

Igho OE & Aimakhume A observed 406 artifacts in 388 tissue sections over a period of one year. Most of the tissue sections had more than one pattern of artifacts which is similar to the present study. They observed artifacts in 94.58% of sections similar to the present study and fold artifact accounted for majority of the cases [5].

Though the use of DPX an mountant media has an advantage that it can be peeled off early, it can give a foggy appearance. Mounting media of proper consistency along with using adequate amount prevents the artifact. This was the least common artifact in our study.

Formation of air bubbles under the coverslip was less common. Usage of inadequate mounting medium or too thin mountant can be the cause for the same. Tiny air bubbles trapped during mounting should be removed carefully by applying mild pressure using blunt needle. Other cover slipping artifacts are contamination of slides with pollen, spores, eggs, dust, insect parts, use of unsized coverslips, insufficient or excess mountant, positioning of coverslips etc. [6].

Wyunchuk M studied artifact of H&E staining and published the problem and its solution and designed a compressive quality assurance program to correct problems as they occurred [7].

Tissue folding artifacts occur during lifting of tissue sections. They can be avoided by transferring sections to new water bath and adding small amount of detergent to it [8].

According to Chatterjee S, folds in sections is a common artifact in tissues containing hard component and is difficult to avoid even with greatest care [9].

Small pieces of unrelated tissue in the slides are floaters or gross contamination artifacts. They appear either during grossing, processing or floatation of tissue sections. To avoid this artifact, only one specimen should be grossed at a time, the cutting board should be cleaned after grossing each specimen and water in the water bath has to be changed often [10].

Active alum hematoxylin solution develops a fluorescent sheen on the surface of the hematoxylin stain daily, after standing for few hours overnight. This has to be removed daily before immersing sections in hematoxylin by using a wet blotting/filter paper and pushing the sheen to one side of container and lifted off when they come over the filter paper. Otherwise they remain as dark bluish precipitates on section. This is commonly seen in tissue wherever there is mucoid or myxoid areas [4].

Conclusion

Section folding artifacts were the most prevalent pattern of artifact observed in this study. Proper technical measures need to be employed in order to prevent/minimize the occurrence of artifacts in a skillful manner, as they may pose diagnostic difficulties. Regular monitoring of tissue processing and staining is adviced to minimize the occurance of these artifacts and our laboratory has entered into an extended quality assurance program.

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