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Incomplete Staining Artifact: A Confounding Frozen Section Pathology Artifact Encountered During Mohs Micrographic Surgery

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ABSTRACT

The intent of this brief communication is to describe a unique incomplete staining frozen section pathology artifact encountered during Mohs Micrographic Surgery. At the authors' institution, an amorphous, eosinophilic artifact that obscured cellular architecture was observed multiple times during histological interpretation. It was determined that incomplete tissue staining was likely caused by weak staining, possibly related to an interaction between hematoxylin dye solution and acetone. We adjusted our SLS stain line protocol by adding a 15 second water rinse between the acetone and hematoxylin pots and then compared the old fixation protocol with our new fixation protocol. This artifact, which was regularly found intraoperatively at five separate MMS laboratories has sustainably resolved.

Mohs Micrographic Surgery (MMS) is a dermatologic procedure that includes tumor extirpation, tissue grossing, slide preparation, and microscopic histologic interpretation. Tissue grossing and slide preparation are vital components of the MMS procedure. There are many steps throughout tissue processing that can result in frozen section pathology artifacts. Frequently encountered frozen section pathology artifacts include vacuolation of cytoplasm or "freeze artifact," overstaining and understaining with hematoxylin and eosin, incomplete dehydration, and splaying of collagen in the dermis.[1-3] We describe a unique incomplete staining frozen section pathology artifact.

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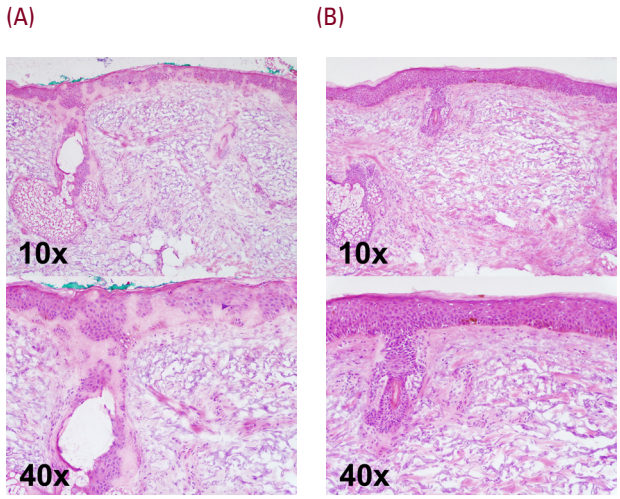
INTRODUCTION

On histologic interpretation during MMS, the authors observed numerous areas of incompletely stained tissue where cellular architecture was preserved, but obscured by an amorphous, eosinophilic artifact that eliminated contrast between the hematoxylin and eosin-stained cells. The incompletely stained areas were rounded areas of decreased staining intensity that crossed cellular boundaries (Figure 1A). Because of the importance of cellular variegation in MMS histologic interpretation, the staining artifact proved challenging when discerning between benign and malignant cellular features. The reliability of MMS in preventing skin cancer recurrence relies on the precision of slide preparation and histologic interpretation. Technical and interpretational errors may account for greater than 75% of local skin cancer recurrence after MMS with technical laboratory problems accounting for the largest portion of those errors.[4]

SOLUTION

After troubleshooting with the *ASMH frozen section manual,* it was determined that incomplete tissue staining was likely

FIGURE 1. (A) Hematoxylin and Eosin-stained images of the incomplete staining artifact at 10 and 40x magnification after going through the original staining protocol. (B) Hematoxylin and Eosin-stained images at 10 and 40x magnification after going through the new staining protocol.



*The subtle variation in histologic appearance between A and B is due to the images representing successive sections of the same tissue block.

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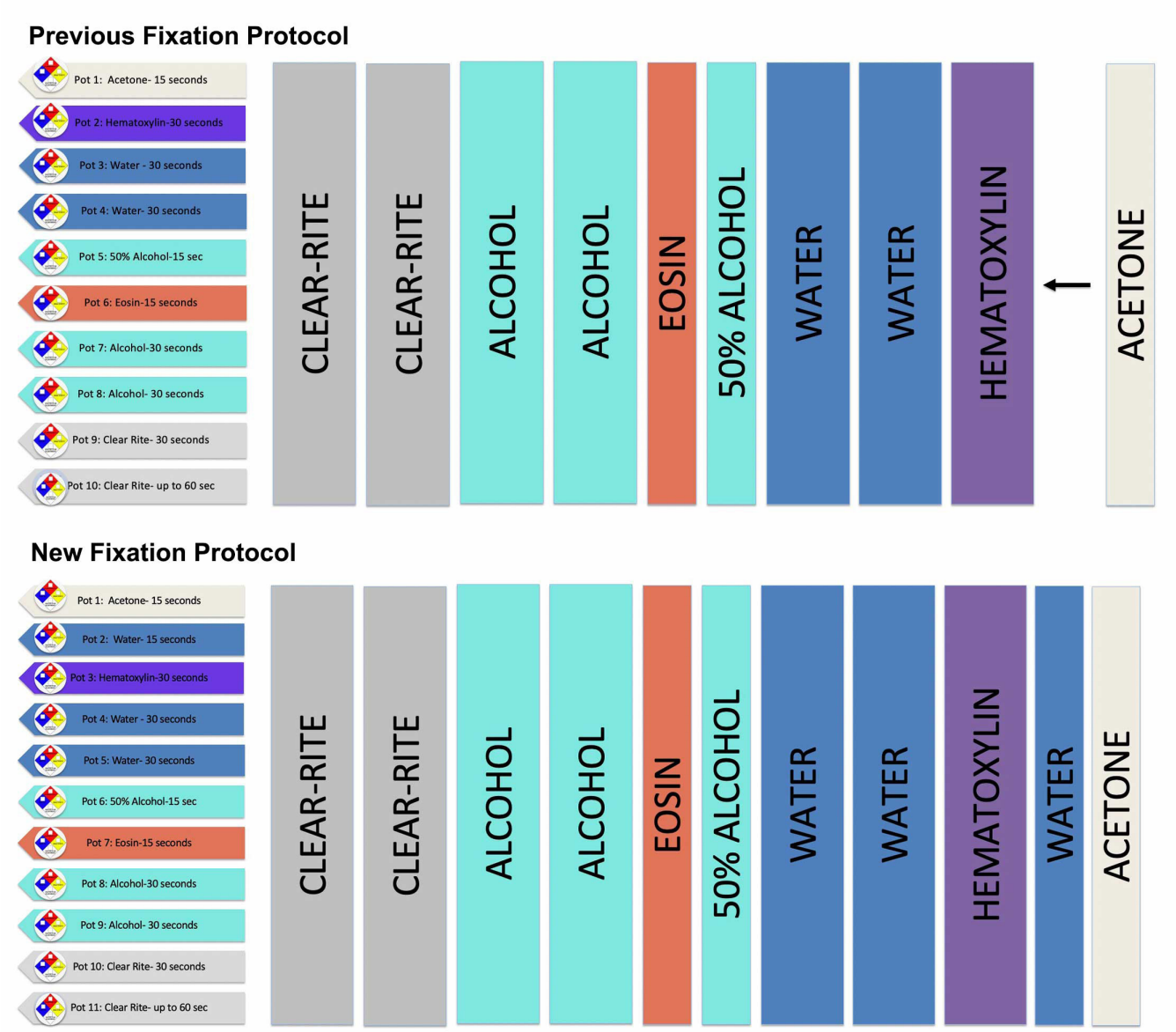
FIGURE 2. Original and new fixation protocols. The new fixation protocol eliminates the Coplin jar and includes acetone in the stain line. Also, it adds a water pot between the acetone and hematoxylin pots.



caused by weak staining, possibly related to an interaction between hematoxylin dye solution, which is water-based, and acetone. Our protocol for the Linistain (SLS) stain line (Thermo Scientific Linistat Linear Stainer) included dipping the slide into a Coplin jar of acetone for ~15 seconds and then dipping it directly into hematoxylin on the SLS stain line (Figure 2). This protocol using acetone in a Coplin jar rather than on the stain line was the traditional protocol at our MMS laboratories at the time. We adjusted the SLS stain line protocol by adding a 15 second water rinse between the acetone and hematoxylin pots (Figure 2). Also, we eliminated the Coplin jar and placed the acetone on the SLS stain line. In order to validate our new fixation technique, we tested the same specimen tissue and compared the old fixation protocol with our new fixation protocol.

Internal sections cut at 4-5 µm thickness were standardized for our assay. The first section was stained with the new fixation protocol and the second section was stained with the original fixation protocol. Both slides moved through the entire SLS stain line, were cover-slipped, and viewed microscopically by separate MMS surgeons and dermatopathologists to validate the quality of slide preparation. Sixty slides were run through this assay, 35 through the new fixation protocol and 25 through the previous fixation protocol. Of the 25 slides that were test-

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ed with the original fixation protocol, 16 (64%) displayed the incomplete staining artifact (Figure 1A). Of the 35 slides that were tested with the new protocol, 0 displayed the incomplete staining artifact (Figure 1B). While using the new stain line protocol, there were three cases where cartilage detached from the slide. In all three cases, the corresponding specimen that was treated with the original stain line protocol did not detach from the slide. Heating slides with a slide warmer helped sections adhere to the slide. As a result of this assay, the new fixation protocol was incorporated at our MMS laboratories. This artifact, which was regularly found intraoperatively at five separate MMS laboratories has sustainably resolved.

LIMITATIONS

Cartilage processing was limited by the new fixation protocol. Acetone hardens tissue to the slide, and it is important to note that the section may detach from the slide during the new fixation process. We found that skipping the fixation steps of the stain line (acetone and water) with cartilage-containing tissue was the best method to avoid tissue detachment. Skipping tissue hydration and fixation does not significantly affect morphologic detail.[5] Also, we used a plate warmer and positively charged slides, but with less success than skipping the fixation steps altogether. This limitation leaves room for further troubleshooting.

CONCLUSIONS

Tissue fixation is the most important step of the staining process during frozen section pathology. In our assay, over 60% of slides treated with the original fixation protocol had incomplete staining artifact, while 0% of the slides treated with the new fixation protocol had incomplete staining artifact. Adding water between Acetone and Hematoxylin as a step of the tissue fixation process is necessary to produce high quality stained slides. This alteration in our tissue fixation protocol eliminated the incomplete staining artifact which improved consistency in tissue staining, overall slide preparation quality, and histologic interpretation. Further studies are needed to fully elucidate the etiology of this staining artifact.

DISCLOSURES

All authors have no conflicts of interest to declare.

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