

Quality Improvement Study: Bone Marrow (BM) Biopsy H&E Staining

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I. Background

A Quality Assurance program is an integral part of any anatomic pathology laboratory. As in many industries, processes are established to ensure that high levels of practice standards are met in order to reduce errors and improve patient safety. To be successful, a program should be customized to meet the needs of the individual organization. An effective quality assessment evaluation must encompass all steps involved in the testing process. For example, it is of little value to assess a special staining technique without including the preparation of the section and the H&E staining process that accounts for 95% of the procedure.¹ In a pathology laboratory, procedures must assure adequate specimen identification, safe and accurate performance of the test, and effective communication of the results. While difficult to measure, outcome indicators are the most relevant in assessment of how the tests contribute to patient diagnosis and treatment.²

Industry standards are put forth by the College of American Pathologists (CAP) to promote excellent outcomes in medical care. The CAP defines “Quality Assurance”, “Quality Control” and “Quality Improvement”.³ Quality Assurance in the pathology laboratory is the practice of assessing the performance of all steps of the testing cycle including pre-analytic, analytic, and post-analytic phases. Key quality indicators include testing “turn-around-times” (time elapsed from inception to completion of a test), identification of errors, clinical correlation of pathologist findings, diagnostic accuracy and completeness of information.¹

Quality Control is an integral component of Quality Assurance, and is the aggregate of processes and techniques to detect, reduce, and correct deficiencies in

an analytical process. Quality Control, when thought of in terms of a measurement of test accuracy and precision, most often applies directly to the clinical laboratory. In the anatomic pathology laboratory, Quality Control measures do not result in a number but rather in a qualitative and quantitative assessment. Some examples of parameters measured for Quality Control include accuracy in accessioning of specimens, prosection, and the embedding, cutting and staining of completed slides. Other parameters include instrument checks, temperature logs for water baths, and qualitative controls for special stains, immunohistochemistry stains, and *in situ* hybridization. The quality of the stained slide is not easily quantifiable, and is best judged under a program of external appraisal and benchmarking.⁴

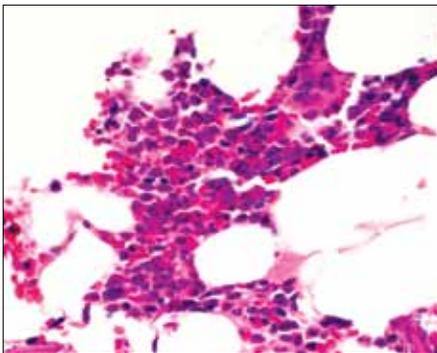
Quality Improvement is the practice of continuously assessing and adjusting performance using statistically and scientifically accepted procedures. The goal of the activities is to improve outcomes. Representative indicators or processes are chosen for evaluation. The current level of performance is measured, and targets or desirable levels of performance are set. Interventions are designed and implemented and the process is re-evaluated. Subsequent activities involve ongoing checks or monitors of systems and processes. Once an issue is identified, steps need to be taken to implement change. The programs are developed in concordance with work that is being performed in the various areas of the laboratory. The 2000 report from the Institute of Medicine, *To err is human: Building a Safer Health System*,⁴ calls for medical laboratories and other healthcare providers to focus on Quality Improvement practices for error reduction and improved patient safety.

II. Goal of Study

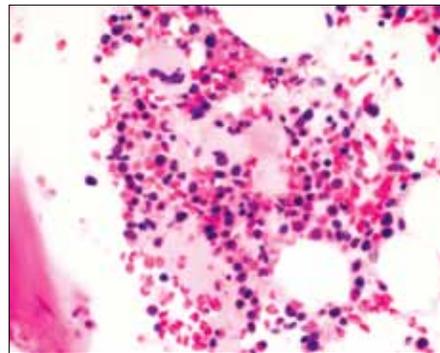
This paper addresses a Quality Improvement opportunity that was identified at the anatomic pathology laboratory at the University of Pittsburgh Medical Center (UPMC). The study was approved by the Quality Assurance Committee at UPMC Presbyterian Hospital. During the process of evaluating the Ventana SYMPHONY discrete slide stainer for possible acquisition, a particular artifact was seen in bone marrow core biopsies processed using the lab's standard H&E process on a Leica Autostainer XL. Highlighted because it did not appear in the SYMPHONY stained slides, the artifact consisted of focal areas in which the nuclei showed a

“waxy” homogenization of the chromatin which gave the appearance of pseudonuclear inclusions. When present, the artifact was seen in localized areas only, not throughout the entire tissue. Figures 1, 2 and 3 show examples of three of the cases showing the artifact. It was also appreciated that there were differences in morphology and color balance between tissue sections stained on the Autostainer XL and those stained on the SYMPHONY instrument. These changes are also illustrated in Figures 1, 2 and 3 outside the areas containing the artifact.

Figure 1. A&B show bone marrow sections for case 10

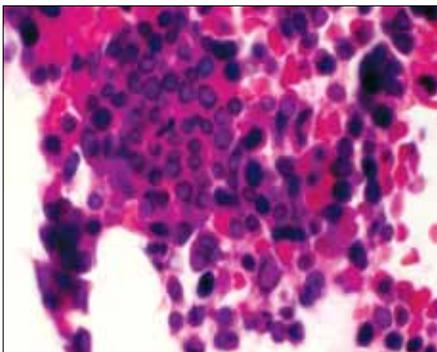


a. UPMC stained bone marrow section (60X) shows artifactual pseudonuclear inclusions

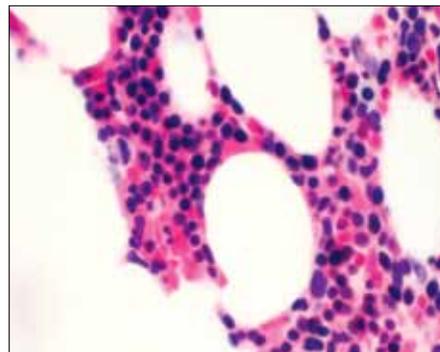


b. SYMPHONY stained bone marrow section (60X) without artifact

Figure 1. C&D show bone marrow sections for case 30



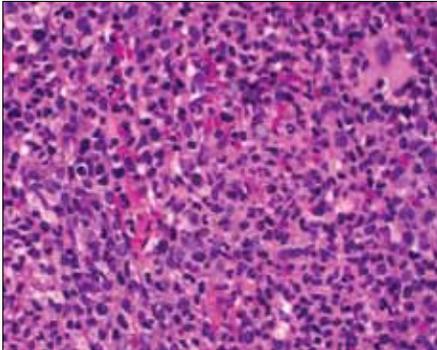
c. UPMC stained bone marrow section (60X) shows artifactual pseudonuclear inclusions



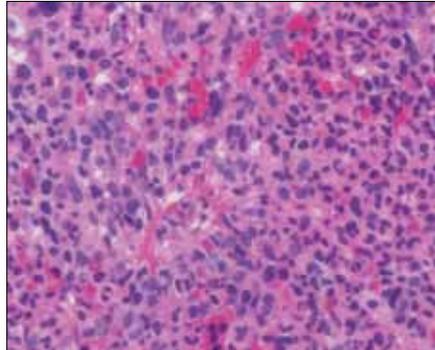
d. SYMPHONY stained bone marrow section (60X) without artifact

Figure 1. E&F Show bone marrow sections for case 31

A study was performed to evaluate prevalence of the artifact on the UPMC slides, as well as to observe its absence on the SYMPHONY stained slides.



e. UPMC stained bone marrow section (60X) shows artifactual pseudonuclear inclusions



f. SYMPHONY stained bone marrow section (60X) without artifact

III. Materials

The study included four micron thick sections of bone marrow biopsies stained on a Autostainer XL at UPMC from July-August of 2007. All tissue was processed on a Thermo Shandon Excelsior, in 10% neutral buffered formalin containing 5% Ethanol. A total of 120 cases were reviewed, and recuts were prepared for staining on the SYMPHONY. A smaller cohort of the biopsy cores were evaluated for nuclear morphology and color balance. The pathologists at UPMC were asked to evaluate 69 of the cases for these characteristics in a blinded fashion.

IV. Methods

A Harris hematoxylin protocol was used for nuclear staining on the Leica Autostainer. The hematoxylin incubation time was 3.5 minutes, with acetic acid/water differentiation. The N2C2 staining protocol used on SYMPHONY was selected to optimize nuclear detail. This protocol produces a lighter hematoxylin stain than that used at UPMC. It has been demonstrated in previous assessments at Ventana Medical Systems, Inc. that a lighter hematoxylin stain allows for better identification of the marrow elements. The scoring system used to evaluate the sections focused on the staining only and is as follows:

- “2” indicates no staining artifacts present
- “1” indicates less than optimal staining due to artifact: artifact COULD impede interpretation
- “0” indicates an unacceptable slide for reasons other than presence of the artifact

All sections were studied and scored.

A hematoxylin and eosin staining intensity was recommended that is lighter than the standard protocol used on the Leica linear stainer. The biopsy cores were evaluated for nuclear morphology and color balance on a scale of poor, fair and good. Each characteristic was assessed separately. A number grade was assigned these characteristics as follows.

- “1” Poor
- “2” Fair
- “3” Good

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V. Results and Discussion

Evaluation of the 120 slides resulted in the elimination of one case because the clot section was pulled instead of the bone marrow biopsy. Of the remaining 119 cases, 37 (31%) cases from UPMC linear staining process showed the nuclear artifact. Of these 37 cases, none showed the nuclear artifact in the section stained on SYMPHONY (see Table 1).

The artifact can put the Pathologist at risk of limitations in diagnosis. These findings strongly suggest that the artifact resulted from the linear staining process, while the SYMPHONY staining process eliminated the artifact

in this study. The SYMPHONY staining technology differs significantly from that of the automated linear stainer used at UPMC in several key areas.

The SYMPHONY system uses an environmentally safe deparaffinization method and fresh reagents on each slide. The study suggests the loss of artifact and enhanced nuclear detail of the tissue stained on the SYMPHONY are, to at least some degree, results of these technological differences.

Evaluation of the 69 cases for nuclear morphology

Table 1: The number of cases selected for evaluation was 120. One case was eliminated because it was a clot instead of a bone marrow biopsy. NA - not applicable

Staining instruments used	LEICA XL	Staining Score	SYMPHONY	Staining Score
Number of cases with artifact	37 (31%)	1	0	NA
Number of cases without artifact	82	2	119	2

showed 50 SYMPHONY stained cases (72.5%) with improved nuclear morphology when compared to those stained on the linear stainer. 18 (26%) Symphony stained cases showed equivalent nuclear morphology, while one case showed inferior morphology.

Color balance was also compared for cases stained on

the SYMPHONY stainer and those stained on the linear stainer, with results favoring the SYMPHONY staining at a rate of 54% or 37 cases (see Table 2).

The cases which showed nuclear morphologic improvement could be further segregated into those

Table 2: The Symphony effect on morphology and color balance. The number of cases selected for nuclear morphology and color balance evaluation was 69.

	Improved	Equivalent	Inferior	Total Cases
Morphology	50 (72.5%)	18 (26%)	1 (1.5%)	69
Color Balance	37 (54%)	32 (46%)	0	69

that showed one or two levels of magnitude difference in the scores between the Symphony stained samples and those from the same cases stained on the Leica linear stainer. Of the 50 cases displaying a notable difference in morphology, 38 (76%) improved by one level (e.g. from a score of 1 to a score of 2), while 12 (24%) cases showed an improvement of 2 levels (e.g. from a score of 1 to a score of 3).

Of the 37 cases displaying an improvement in color balance, 29 (78%) improved by one level, while 8 (22%) improved by 2 levels.

The lighter hematoxylin and eosin intensity staining recommended for bone marrow biopsies allowed

The cases which showed color balance improvement also could be further segregated as stated above.

Table 3: The cases showing improved results in nuclear morphology and color balance from the Symphony process were further evaluated as to the degree of improvement.

	Mild to Moderate Improvement (1 Level)	Substantial Improvement (2 Levels)	Total Cases
Morphology	38 (76%)	12 (24%)	50
Color Balance	29 (78%)	8 (22%)	37

pathologists' better visualization of nuclear morphology as demonstrated in this study. The same principle applies for any tissue in which close evaluation of nuclear morphology is needed as in lymphoid tissue. Also, the selection of an eosin level that complements the hematoxylin intensity provides appropriate color balance and was favored by the evaluating pathologists.

at UPMC, including the pathology staff. Identification of the artifact was discovered inadvertently during a comparison of the quality of staining while evaluating a new staining process. This study illustrates the need for external validation of quality in H&E staining, as well as the need for benchmarking.

VI. Conclusion

The SYMPHONY H & E stainer provides superior staining of H & E slides with fewer staining artifacts, better nuclear morphology, and superior color balance when compared to the linear stainer. In this study where small areas of artifact are present within large tumor areas, there was no obstruction to the assessment and diagnosis. In cases in which the evaluation is centered on early involvement by neoplasm or minimal residual disease, the artifact could obscure the diagnosis. This study presents a Quality Improvement opportunity that was not readily apparent to the laboratory personnel

At UPMC, personnel are open to identifying process improvement opportunities. The Quality Improvement processes already integrated into normal daily laboratory workflow led to identification of this artifact and completion of this study. Their program operates not only to identify areas of improvement as determined by their written plan, but is also flexible enough to allow identification of opportunities for improvement as they present. This integration provides greater efficiency and makes quality improvement an ongoing part of laboratory operations.

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