

## Recent Hematoxylin Shortage and Evaluation of Commercially Available Substitutes

Ashley Groover, BS; Carlette Geddis, BS, HTL(ASCP);  
Amanda Finney, BS  
Medical University of South Carolina  
Charleston, SC  
grooverj@musc.edu

### Abstract

In 2008, some laboratories reported difficulty obtaining hematoxylin stain solutions or dye powder from their usual commercial sources. Discussions on the listserv Histonet quickly revealed rumors of a hematoxylin dye shortage. Vendors with a short supply of hematoxylin were

offering their customers substitute nuclear stains as they were unable to predict when hematoxylin would once again be available. While some predicted that the shortage would ease "sometime in the fall," the uncertainty left laboratories scrambling for an alternative nuclear stain.

We evaluated different substitute stains that were available through various commercial sources, and in a blinded study compared their staining results with the Gill's hematoxylin #2 solution we routinely use in our laboratory.

### Introduction

Hematoxylin, a derivative of the logwood tree *Haematoxylon campechianum*, is one of only a few dyes derived from nature that is still in use in the modern histology laboratory.<sup>1</sup> Because the tree is found in just a few regions of the globe, supply of this natural commodity may fluctuate as a result of climate, political, or economic forces. Although there were once 4 logwood

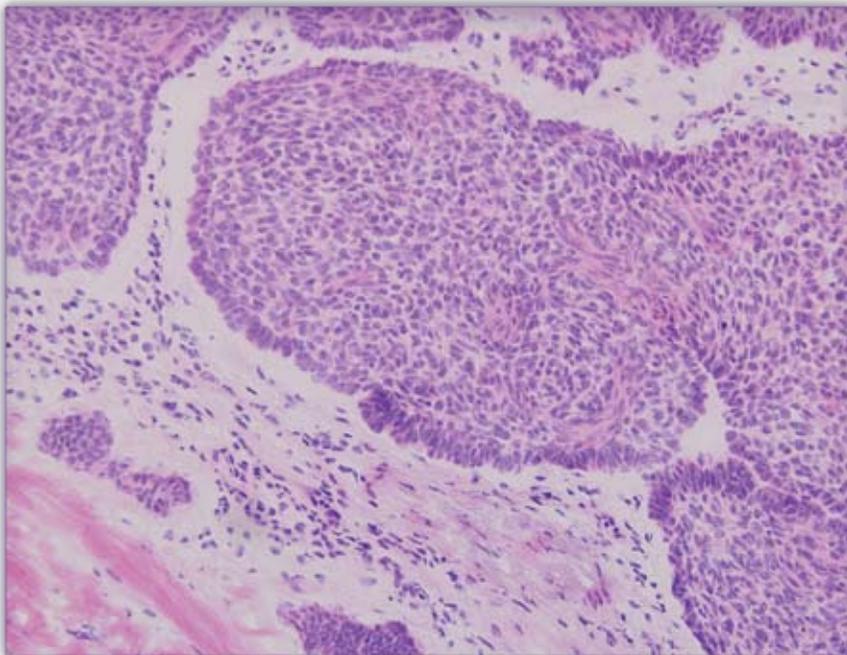


Fig. 1. Basal cell carcinoma in human skin, Gill's hematoxylin #2 stain. 100X

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**Table 1: Comparison of Manufacturer's Stain Protocols Used for Frozen Sections**

Gill's Hematoxylin #2	Tango™	Newly Blue™	Phoenix Blue™
100% ethanol	100% ethanol	100% ethanol	100% ethanol
Water rinse	95% ethanol	Water rinse	95% ethanol
Gill's hematoxylin #2 40 seconds	70% ethanol	Newly Blue* 4 minutes	Water rinse
Water rinse	Water rinse	Water rinse	Phoenix Blue† working solution 3 minutes
0.25% acid alcohol 20 seconds	Tango mordant 2 minutes	0.25% acid alcohol 3 dips	Water rinse
Water rinse	Water rinse	Water rinse	Bluing reagent 30 seconds
Bluing reagent 20 seconds	Tango stain 2 minutes	Bluing reagent 30 seconds	Water rinse
Water rinse	Water rinse	Water rinse	95% ethanol
Eosin phloxine 20 seconds	70% ethanol	70% ethanol	Eosin Y 30 seconds
95% ethanol	Eosin Y 1 minute	Eosin phloxine 1 minute	100% ethanol
100% ethanol	95% ethanol	95% ethanol	Xylene
Xylene	100% ethanol	100% ethanol	Mount
Mount	Xylene	Xylene	
	Mount	Mount	

\*Newly Blue stain consists of equal parts solution A (1% celestine blue) and solution B (4% ferric ammonium sulfate).  
 †Phoenix Blue is prepared by pouring solution A into the bottle of solution B. The manufacturer does not reveal the components of these solutions.

processing plants in 1943, the increased profitability of sugar cane in the 1970s, as well as the clear felling of forests in Central America, resulted in a drastic decrease in production of hematoxylin.<sup>1</sup> Price per volume went to record highs. Today, there remains only one true supplier of logwood chips, Mexicana De Extractos, in Campeche, Mexico.<sup>1</sup> Thus, those in need of hematoxylin depend on the Mexican market for a steady supply of logwood chips at a fair price. Because greater profit is achievable with the production of various chemicals, the supply of hematoxylin is dwindling, creating the need for good synthetic substitutes.

Indigenous to Central America, the logwood tree extract was originally used as a dye by the Aztecs and was imported to Spain by Hernando Cortez. It was later introduced to the European market by the Spaniards, creating competition for the already established violet dye, indigo.<sup>2</sup> As exploration continued, competition developed between countries vying to control the valuable export supply. By the 16th century, the logwood extract trade was highly established. Hematoxylin remained highly valued as a source of pigment for clothing until the rise of aniline dyes in the 1850s.<sup>1</sup> The use of different metal salts as mordants to bind to the hematoxylin allowed for a spectrum of color to be established for use on fabrics. As a result of this mordant-dependent color range, the role of hematoxylin within the medical community was established and later flourished in the field of pathology.

### Materials and Methods

We evaluated Tango™ (Anatech LTD, Battle Creek, MI), Newly Blue™ (Newcomer Supply, Middleton, WI), and

Phoenix Blue™ (ThermoFisher Scientific, Kalamazoo, MI) against our routinely used Gill's hematoxylin #2. Substitute dyes were utilized according to the manufacturer's protocol on 8 micron, air-dried frozen sections of a previously diagnosed basal cell carcinoma. The block was serially sectioned to obtain a sufficient number of slides to allow the tissue to be stained in each substitute as well as the Gill's hematoxylin #2. Each product was evaluated for ease of use, shelf life, cost, and staining characteristics. Slides were evaluated microscopically and graded in a blind study by 4 board certified dermatologists utilizing the diagnostic criteria of our laboratory.

### Discussion

Our objective was to identify a synthetic hematoxylin substitute stain that is cost effective, is readily available commercially, and that achieves staining results similar to natural hematoxylin. We routinely utilize the hematoxylin and eosin (H&E) stain on frozen sections of integument in our Mohs laboratory to evaluate abnormal growth patterns. Sections stained with hematoxylin substitute dyes in this study were evaluated microscopically for their suitability in diagnosing carcinoma. Gill's hematoxylin, the stain routinely employed in our laboratory, is not a proprietary stain formulation, so it can be obtained from numerous commercial sources. Available in multiple strengths, our lab uses double strength Gill's hematoxylin (Gill's #2), which we purchase as a ready-to-use 4-liter container costing approximately \$23 a liter. Due to the addition of ethylene glycol within the Gill's formulation, filtration is not needed as oxidation of the dye is reduced, which also allows for an extended shelf life of up to 2 years. The major disadvantage of using Gill's hematoxylin is its increased sensitivity to acid, which makes it unsuitable for use with low pH counterstains.<sup>3</sup>

**Table 2: Comparison of Stain Shelf Life**

Product	Stock Solutions/Kit	Working Stains
Gill's hematoxylin	2 years unopened	1 month
Tango	1 year unopened	1-2 weeks
Newly Blue	1 year unopened	5 days
Phoenix Blue	1 year unopened	5 days

**Table 3: Comparison of Cost**

Cost in US dollars per liter of solution	Gill's hematoxylin	Tango	Newly Blue	Phoenix Blue
	\$23	\$10	\$43	\$53

Eosin is used as the counterstain in our staining procedure, staining the cytoplasm pink-orange while leaving the nuclei a contrasting blue-purple. We utilize a regressive staining method; the section is deliberately overstained and then excess dye is selectively removed (differentiated) in acid alcohol. The stain is blued in ammonia water prior to counterstaining in eosin (Table 1). Not unexpectedly, the physicians who viewed the unidentified slides rated the tissue stained with natural hematoxylin as excellent (Fig. 1). It was unanimously chosen for the best cell clarity and crisp background staining. It yields cells with well-resolved nuclei and distinct visible chromatin. Precipitate was not observed microscopically. Of those compared in this study, the physicians rated hematoxylin as their dye of choice.

Tango, according to the manufacturer's product sheet, is a proprietary formulation containing the dye mordant blue 3 (Anatech LTD, Battle Creek, MI), which uses sulfuric acid to adjust the pH of the stain. The Tango stain set is composed of two components that are used consecutively in the protocol: the first is the positively charged mordant, ferric alum, which binds with negatively charged DNA or RNA. Mordant blue 3 is a negatively charged red stain that binds to the mordant or any cationic tissue elements. The Tango-mordant complex turns blue following a water rinse.<sup>4</sup> Our cost for Tango is approximately \$10 a liter, making it the most cost-effective substitute evaluated in this study. The kit did not specify to filter the mordant or stain before initial use. We used the reagents directly from their containers and found no precipitate on the slides. Tango does, however, suggest daily filtration for both stock reagents after initial use. The stain set has a shelf life of 1 year. The working reagents are stable for approximately 1 to 2 weeks after initial use.

Test slides were stained in Tango according to the manufacturer's protocol (Table 1). This is a progressive stain, which avoids the need for acid differentiation. Also, no bluing agent is needed in

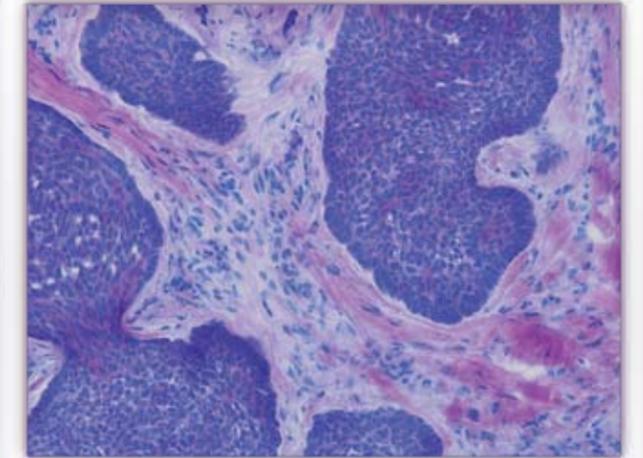


Fig. 2. Basal cell carcinoma in human skin, Tango stain. 100X

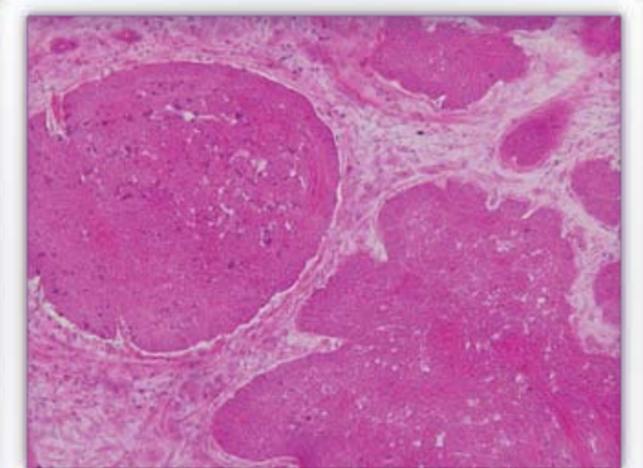


Fig. 3. Basal cell carcinoma in human skin, Newly Blue stain. 100X. Image displays severe understaining of nuclei.

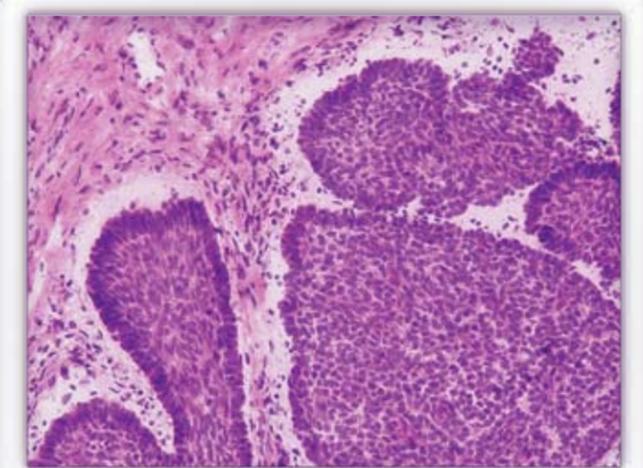


Fig. 4. Basal cell carcinoma in human skin, Phoenix Blue stain. 100X

this staining protocol. Upon microscopic examination, nuclei stand out as a bold blue whereas the cytoplasm remains a purple hue (Fig. 2). There were mixed opinions as to the quality of the staining. Our reviewers noted that the stain was distinctly different than a traditional H&E stain, and all four indicated that Tango would not be their first choice if hematoxylin was available. They did acknowledge, however, that they can effectively diagnose basal cell carcinoma with Tango, but not with the same ease and timeliness that they can with traditional hematoxylin. Their dislike of the stain seems to be unanimous in that the tumor, epidermis, and appendages are all blue, whereas only the dermis remains pink. Also, the specimens appear to be overstained with no discernable chromatin visible within the blue masses of nuclei. Although the manufacturer's protocol does not offer a range of staining times with the kit, it is hoped that with some manipulation, this stain could be lightened to reveal clearer nuclei with distinct chromatin.

Newly Blue is available as a stain kit composed of two separately packaged solutions that are mixed together prior to staining. Solution A is 1% celestine blue solution. Solution B is 4% ferric ammonium sulfate. According to Carson, celestine blue was commonly used as a substitute during the hematoxylin shortage of the 1970s.<sup>5</sup> When combined with iron alum, it is thought to be resistant to the effects of acid differentiation<sup>6</sup>; however, the manufacturer suggests that it may be used as either a progressive or regressive stain. The product insert indicates that the working stain is only stable for 5 days. Stain precipitate must be filtered off of the surface daily, increasing the set-up time.

Our cost for the Newly Blue stain is approximately \$43 a liter, almost double the cost of Gill's hematoxylin #2. Test sections were stained with the regressive protocol according to the manufacturer's staining procedure (Table 1). Nuclei were surprisingly understained, especially given that our sections were thicker (8 microns) than the 4 to 6 microns recommended by the manufacturer. Due to the disappointing initial result, we repeated the stain two additional times, each time starting from scratch with the manufacturer's directions in hand. Despite several attempts, the slides stained with this product were predominately pink, without any sign of adequate differentiation of nuclei (Fig. 3). All 4 dermatologists concluded the sections were undiagnosable as basal cell carcinoma. Tumor was evident but cell type was not. Neither nuclei nor keratinocytes were distinguishable in the tumor or epidermis. Overall the specimen was simply too pink.

It is unclear whether manipulation of staining times would yield a more acceptable result. While the manufacturer did not specify to filter the working solution, there proved to be a considerable amount of precipitate on the slides, which further obscured microscopic evaluation. The precipitate persisted even after filtering.

The last substitute we evaluated was the Phoenix Blue stain. The product insert does not reveal the name of the dye contained in this stain kit and the manufacturer was unwilling to reveal this information over the telephone.<sup>7</sup> Our cost for this kit is approximately \$53 a liter, more than double the cost of Gill's hematoxylin #2, making it the most expensive alternative of those we evaluated. The stain set consists of two solutions, A and B, which are mixed before use. Once mixed, the working solution is stable for 5 days. Phoenix Blue resembles an H&E, staining nuclei a purple-blue hue with pink cytoplasm. Test sections were stained according to the manufacturer's recommended protocol (Table 1).

This stain earned high marks from our reviewers for appearance of nuclei, cell, and background clarity (Fig. 4). However, the cytoplasm was a darker pink than we prefer and our sections appeared slightly overstained. The manufacturer suggests a stain time of 2:30 to 4:00 minutes in the Phoenix Blue stain. We stained the slides for 3 minutes, but a shorter stain time might have produced a more satisfactory result. It is quite possible that the stain can be lightened, allowing for more nuclear detail and chromatin visibility. Our reviewers unanimously rated Phoenix Blue as quick and easy to use in diagnosis. The manufacturer makes no mention of the need to filter the working solution after preparation, which caused visible precipitate on our slides with our initial stain attempt. After filtering the working solution, the precipitate was eliminated.

## Conclusion

Hematoxylin shortages will probably come and go and there is no telling how long they may last; however, the price for this resource continues to climb. It is prudent for laboratories to identify an effective replacement that will meet their needs in order to prevent interruption of patient care services. It is hoped that with some modifications, the synthetic substitutes evaluated in this study can be optimized for use on frozen sections. In the fast paced world of Mohs surgery, a quality stain is crucial for quick (same day) and accurate diagnosis of tissue so that immediate removal and closure are possible. Of the three products tested, Phoenix Blue seems to best suit our needs. While the increase in cost compared to hematoxylin is significant, it offered results that were considered comparable to those attained with Gill's hematoxylin #2.

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## Acknowledgments

The authors wish to acknowledge the generous support of the reviewers who participated in this study: Joel Cook, MD, Carin Litani, MD, Rocky Baceleiri, MD, and Richard Marchell, MD.

All photos courtesy of Rocky Baceleiri, MD.



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# After 24 Years in Formalin, It Should Be Fixed

Robert A. Skinner, HTL(ASCP)\*; William T. Bellamy, PhD†; Frances L. Swain, HT(ASCP)\*; Larry J. Suva, PhD\*  
\*Center for Orthopaedic Research

†Department of Pathology and Laboratory Medicine  
University of Arkansas for Medical Sciences  
Little Rock, AR

skinnerroberta@uams.edu

## Introduction

Dr. Carl L. Nelson, Chairman, Department of Orthopaedic Surgery at the University of Arkansas for Medical Sciences, passed away on January 14, 2005. In the aftermath of his passing, we were left with the decision of what should be done with the various materials that accumulated during his many years with the department. One such sample consisted of a slabbed specimen of proximal tibia dating back to 1981 that was found in a container of formalin. Periodically over the years, he was asked if the sample could be discarded but he always responded, "Leave it there. Some day I'll get to it." His "some day" to look at it never came. But rather than simply discarding the tissue, we decided to examine how 24 years of formalin fixation would affect a bone/tumor composite such as this.

## Materials and Methods

The specimen consisted of a longitudinally gross-cut slab of proximal tibia measuring nominally 90 x 75 x 5 mm in largest dimension containing an area of tumor/cancellous bone composite measuring approximately 60 x 35 mm in surface area abutting one aspect of the cortex. The specimen was prepared for microscopic evaluation using our standard protocol. It was decalcified to endpoint in 5% formic acid as confirmed by ammonium oxalate chemical test, then dehydrated through graded ethanols, cleared in methyl salicylate, infiltrated, and embedded in paraffin. The resulting block was sectioned at 5 to 6 microns and affixed to plain glass slides. Slides were initially stained with hematoxylin and eosin (H&E) (Gill's hematoxylin #2 for 5 minutes/eosin Y phloxine for 1 minute) for cellular and architectural evaluation.<sup>1</sup> Tissue was subsequently stained with safranin O-fast green and CD-34 with primary antibody clone QBEnd/10 (Ventana Medical Systems, Tucson, AZ, catalog #760-2620) and Envision plus™ detection (Dako, Carpinteria, CA).

## Results

There was no significant difference in the sectioning characteristics of the 1981 specimen when compared to contemporary specimens of similar size, composition, and relative density that are fixed and prepared with our routine protocol.<sup>2</sup> We encountered typical compression for

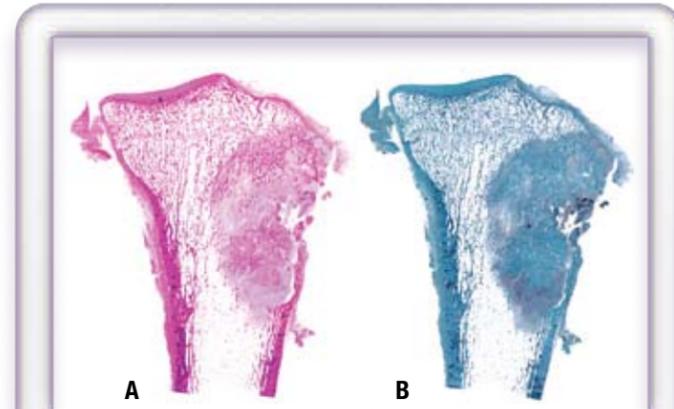


Fig. 1. H&E (left) and safranin O-fast green (right) stained slides of a proximal tibia slab section that was immersed in formalin for approximately 24 years prior to 5% formic acid decalcification. Note the overall section quality is quite good for each. The H&E staining is adequate. The tumor field is readily apparent. The safranin O component is grossly lacking.

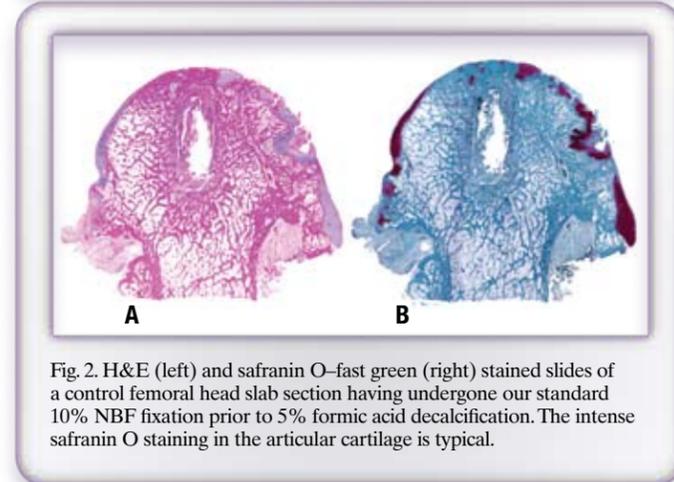


Fig. 2. H&E (left) and safranin O-fast green (right) stained slides of a control femoral head slab section having undergone our standard 10% NBF fixation prior to 5% formic acid decalcification. The intense safranin O staining in the articular cartilage is typical.

a specimen with a block face of this size containing a similar percentage area of cortical bone and relatively little chatter; the marrow-to-trabeculae transitions remained intact. In fact, we were able to produce a 6-section ribbon with 5 usable sections before the cortex began to unacceptably chatter. There was little in the way of "exploding marrow fields" on the waterbath, which allowed for sufficient flotation time to deal with most of the cortical wrinkles. Standard slide drying was adequate.

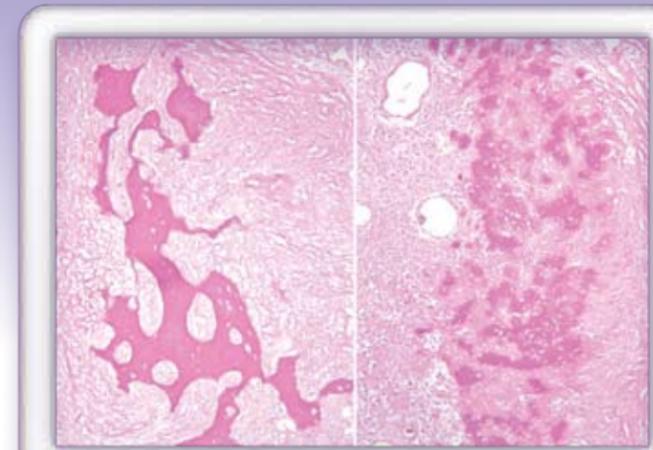


Fig. 3. Two fields within the tumor field of the 24-year formalin-fixed proximal tibia consistent with typical osteosarcoma. H&E, 40X

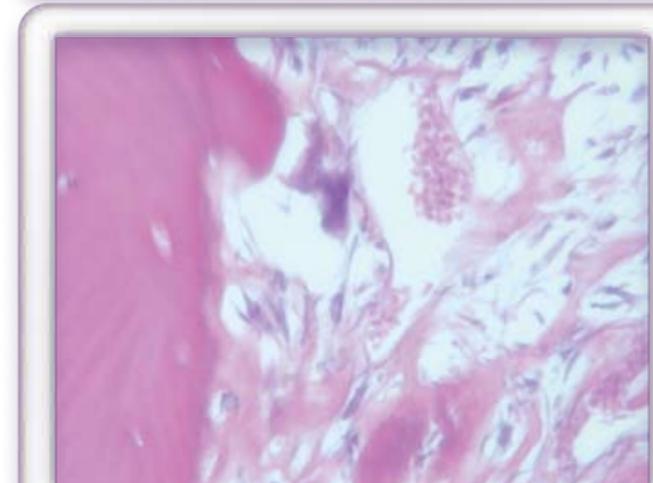


Fig. 4. Multinucleated osteoclast adjacent to a scalloped region of bone resorption in 24-year formalin-fixed proximal tibia. Staining is adequate for recognition of cement lines and cells within lacunae. Interfaces vary but are generally intact. H&E, 400X

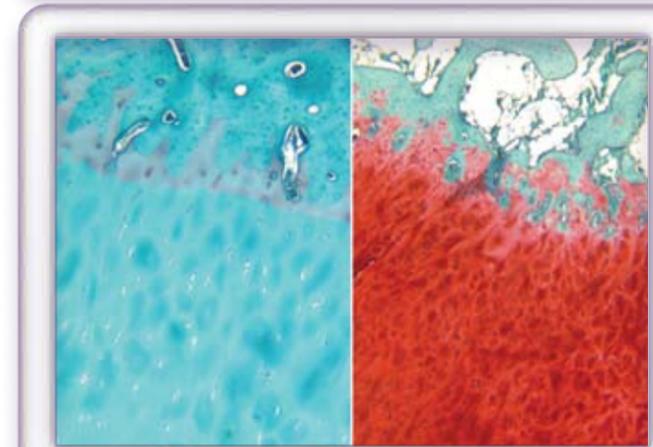


Fig. 5. Safranin O-fast green stain on region of articular cartilage/bone interface in 24-year formalin-fixed specimen (left) and control femoral head (right). The anticipated strong staining intensity in the articular cartilage and color transition at the bone cartilage interface is clearly visible in the control sample. There is a distinct absence of safranin O staining in the articular cartilage of the 24-year sample; however, some staining is present in the interface. 16X

During H&E staining, there was no significant difference in adhesion of the sections between the 1981 specimen and other sections of similar size and composition (Fig. 1A, 2A). The H&E stained slides were assessed for overall microscopic quality. It was immediately noted that the staining of the articular cartilage, especially at the bone/cartilage junction, was not as intense as usually seen in our standard fixed specimens. Filled bone lacunae were easily recognizable. Osteoid seams, or cement lines, were clearly delineated. There was slightly more than expected cortical bone "lifting" observed microscopically than was noted by eye. The slide was then subjected to a blinded review for diagnosis by our histopathology core director pathologist. He was able to identify the tumor as osteosarcoma (Fig. 3) without any difficulty and offered additional positive comments on the staining, pointing out several osteoclasts that appeared to be active at the time of excision (Fig. 4).

Speculation arose about how immunohistochemical (IHC) staining might be affected by the extended amount of time the specimen remained in formalin. There was doubt that staining for CD-31 or CD-34 would be successful if in fact the section remained adhered to the slide. Of course, the slide was too large to be stained on an instrument, so special accommodation was made to attempt doing the IHC.

Before committing the necessary resources to an immuno run, we decided to try a safranin O-fast green stain to see if a representative section would remain on the slide through a more aggressive staining process and to determine if the cartilage staining could be enhanced. No modifications were made to the standard stain protocol.<sup>3</sup>

Section-to-glass adhesion was comparable to that noted on the H&E slide. The Weigert's hematoxylin used in the protocol stained as expected, but the safranin O and fast green stains were significantly weaker in the 1981 sample when compared to the control slide (Fig. 1B, 2B). Review by our section's bone biologist concurred with the initial assessment of the H&E stained slide, which confirmed that after 24 years in formalin, morphologic studies are not compromised. A review of the safranin O-fast green stain revealed, however, that there was significant leaching of the glycosaminoglycan (GAG) side chains and/or the core protein, primarily from the cartilage. Safranin O staining in the subchondral bone immediately below the cartilage/bone interface was noted, but no safranin O was seen in the articular cartilage where the most intense staining would be expected, as illustrated in the control slide (Fig. 5). It was speculated that proteoglycan leaching occurred at some time during the 24-year immersion in formalin.

These results led us to question just how long proteoglycan will survive in formalin fixative. We decided to return to our collection of archived samples to evaluate both 5- and 10-year-old specimens to explore this question. We found diminished stain intensity for the 5-year sample and even less staining in the 10-year sample (Fig. 6). Structures such as chondrocytes and chondrocyte lacunae were easily identifiable, with the most intense staining located in close proximity to these cells. However, the overall staining was not as intense or as uniform as that seen in the Figure 5 control.

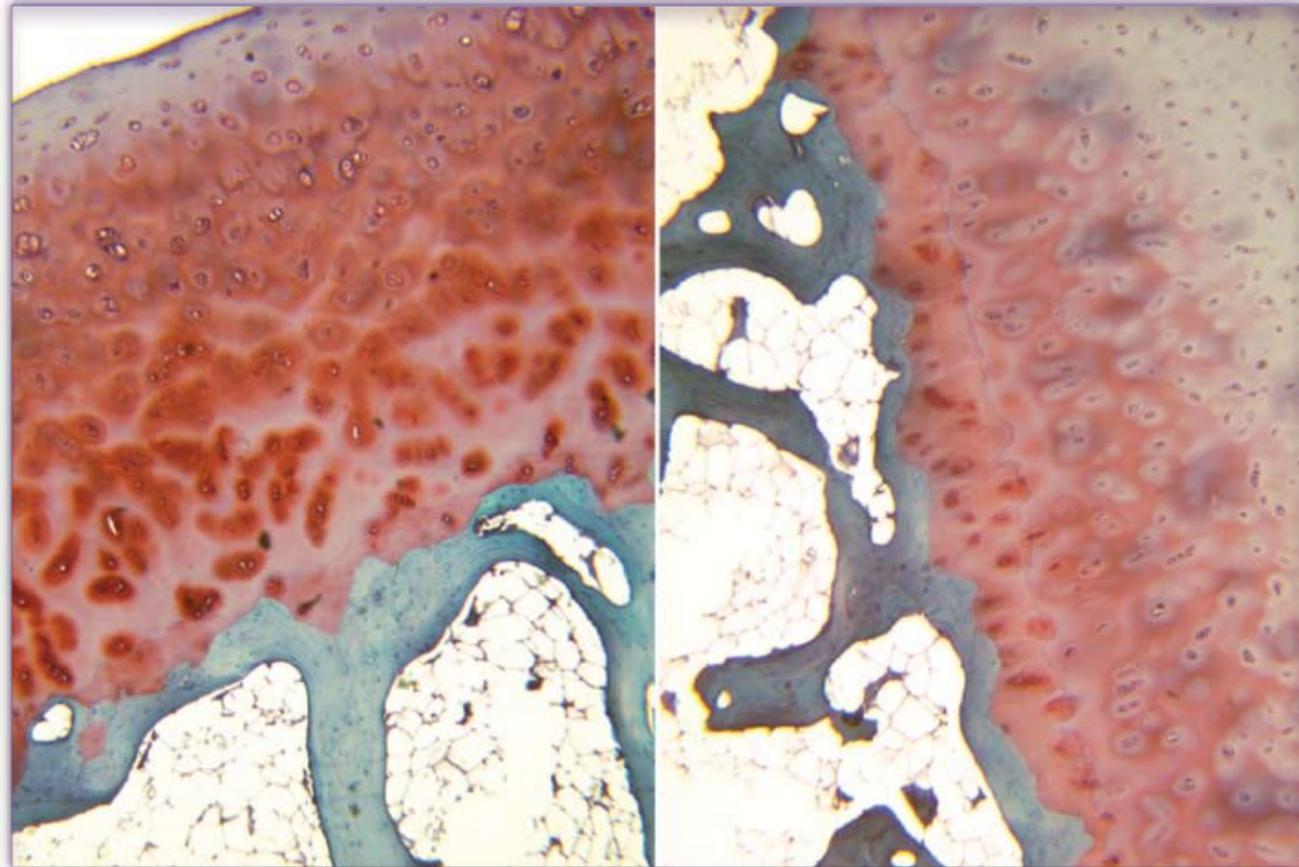


Fig. 6. Safranin O-fast green stain on region of articular cartilage/bone interface femoral head fixed in formalin for 5 years (left) and 10 years (right). Structures are easily identifiable in each prep. The intensity in the 5-year fixed sample is clearly more intense than the 10-year sample. The diminution of intensity appears to proceed from the articular surface inward. 16X



Fig. 7. CD-34 stained slide of proximal tibia slab section that was immersed in formalin for approximately 24 years prior to 5% formic acid decalcification. Note the overall section quality has deteriorated during the CD-34 procedure as compared to the end quality of the sections subjected to H&E and safranin O-fast green staining.

We decided to attempt CD-34 immunohistochemistry. This marker was selected for its affinity to label endothelial cells that are associated with the diagnosed tumor. Included in the stain run was a positive control slide from a “normal” fractured femoral head that was fixed per our standard protocol (minimum 72 hours but generally 3 to 5 days) and decalcified as previously described. The slides were incubated at room temperature in a sealed humidity chamber. Slides were covered with a Kisol-Folie (Delaware Diamond Knives, Wilmington, DE) barrier to ensure maximum antibody coverage with minimum fluid. The results were mixed.

The 1981 section displayed significant damage as far as cartilage “lift,” and in many fields there was total trabecular detachment. Much of the marrow, especially fattier marrow, remained. Surprisingly, the tumor area displayed very good adhesion throughout the stain process (Fig. 7). The 1981 section showed no specific staining for CD-34, only nonspecific marking of blood cells, while the control section exhibited positive staining for CD-34, as expected, in the lumen of vessels, along with an acceptable level of background staining (Fig. 8). Due to the absence of positive staining in the 1981 slide, we considered this effort to be a failure.

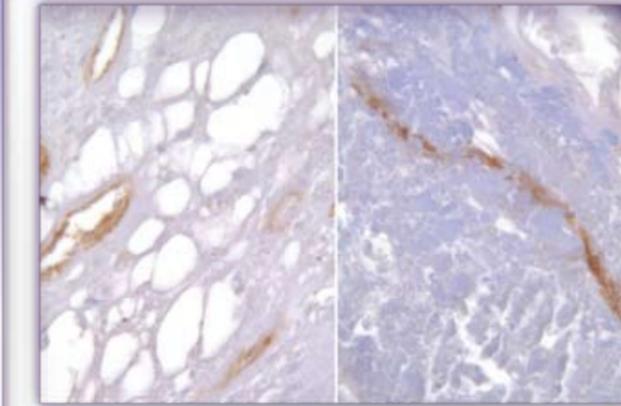


Fig. 8. Two regions of positive CD-34 staining in section of control femoral head. 400X

## Discussion

There has been much discussion in scientific publications and on the listserv Histonet regarding immersion time frames—specifically, what might be considered adequate fixation, inadequate fixation, or overfixation in formalin, especially when applied to immunohistochemistry. There appears to be a consensus that fixation in formalin begun within 30 minutes of excision and carried out for 24 hours but not more than 48 hours should be adequate for most tissue types and procedures.<sup>4</sup> Our standard formalin fixation protocol for specimens we processed as described in this report is 48 to 72 hours prelab (whole) and 72 hours postlab (for a 5-6 mm slab) before initiating 5% formic acid decalcification. This same protocol was applied to the stain controls referred to in this report. Our fixation and processing protocols have been utilized on numerous specimens that have been successfully stained and reported in the literature by us or our collaborators for such markers as VEGF, BMP, TGF, multiple collagens, OPN, TWIST 2, PCNA, TUNEL, and TRAP, among others.<sup>5-7</sup> Because our group has extensive experience working with nonstandard bone samples and modifying procedures intended for routine sized and treated material, we felt well prepared for the challenge of assessing the effects of long-term fixation.

With respect to the leaching of the safranin O-fast green stain, the process appears to be more likely related to tissue chemistry and not merely exposure. So, while one would look at the femoral head views in Figures 5 and 6 and perhaps draw the conclusion that the pattern of diminished staining is due to gradual formalin penetration from the articular cartilage inward, this is likely not the case. It seems more likely that there is a specific decrease of proteoglycan staining from the periphery of the specimen inward to the subchondral bone. This could be a function of tissue density/retentive properties as one moves from the cushioning makeup of the articular surface to the more supportive makeup of that transitional cartilage/bone interface. Or it might be a function of the degradation/complete loss of either the core protein or the

attached glycol groups, which comprise the structure of the proteoglycan itself.

Those of us who labor in teaching facilities or research institutions are prone to dropping items of interest into a container of formalin and sticking it on the shelf with the intent of someday going back to it for further study. Then other things come up and the specimen may sit there for years. Our archives currently contain samples in formalin dating back to 1990. A recent addition consists of a half-grossed femoral head that has been sitting in formalin for 9 months because the batteries ran out in the surgeon’s camera and the surgeon got called back to the OR, and other reasons that have kept this specimen from rising to the top of the “to do” list.

## Conclusion

The work leading to this report began in 2005 as a simple exercise in curiosity and then expanded to include many more specific applications, such as the incorporation of Syndecan markers as an indicator for proteoglycans, for example. As a research group, we are well aware of the pitfalls of reporting data based on a study group size of one. Understandably, some readers might be curious about the pH of the formalin found in the archived samples. Since we do not know the initial pH of any of the formalin fixative solutions used for sample preservation, we have been unable to assess the impact pH may have had in the staining results we observed. Perhaps we can drop tomorrow’s femoral head into formalin of known pH and get back to you in 10 years or so. The observations we’ve reported here seem to contest the widely held notion that tissues stored indefinitely in formalin are safely preserved for all manner of further study.

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## Acknowledgments

The authors wish to acknowledge Dr. Richard W. Nicholas, Chairman, UAMS Orthopaedic Surgery, for advice and support; Dr. J. Michael Gruenwald, Head, UAMS Orthopaedic Trauma Section, for supplemental material used in this report; Ms. Kristy Nicks and Ms. Nisreen Akel, UAMS Department of Physiology, for their photomicrography assistance; Mr. William R. Hogue for macro photography and computer assistance.

Funding provided by the Carl L. Nelson Chair of Orthopaedic Surgery.

Dr. Carl L. Nelson was an innovative surgeon, a respected researcher and an effective teacher. We appreciate this opportunity to share with you this potential once-in-a-lifetime specimen he left behind for us to think about.

# Large Mount Trichrome Method for Quantification of Ischemic Tissue in a Porcine Cardiac Model

Angela L. Smith, HT(ASCP); Matthew D. Galovic, BS;  
Stephen L. Smith, PhD  
Baxter Healthcare Corporation  
Deerfield, IL  
we3smitty@yahoo.com

## Abstract

The porcine heart is similar to the human heart in function, size, and anatomy. Therefore, it is an appropriate model for the study of cardiovascular disease, specifically myocardial ischemia. Myocardial ischemia can be induced in pigs by ligating the left anterior descending artery (LAD) or the left circumflex artery (LCX). Both methods of ligation create a regional infarction, which allows for evaluation of the efficacy of angiogenic and neovascular agents for which histology is indispensable. Traditional-sized sections remove infarcted tissue from healthy tissue, preventing a uniform assessment of regional angiogenesis and neovascularization. This report describes the use of large mount sections (4 x 6 cm) to process cardiac cross-sections and the use of Masson's trichrome stain to quantitate areas of ischemia. Large mount sections, together with Masson's trichrome, can be used to quantify changes, if any, in infarcted tissue, which are the result of angiogenic and neovascular agents.

## Introduction

Masson's trichrome stain on large mount paraffin sections of porcine heart is useful for quantifying induced ischemic tissue in this myocardial infarction model. Routine hematoxylin and eosin (H&E) stains the nuclei blue and other cellular elements shades of pink<sup>1</sup> (Fig. 2, 4), while Masson's trichrome stains the nuclei black, cytoplasm and muscle fibers red, and collagen blue<sup>2</sup> (Fig. 3, 5). The three colors of Masson's trichrome visually distinguish healthy cardiac tissue (red) from infarct tissue (blue). The use of trichrome to distinguish healthy tissue from damaged tissue is standard for myocardial infarct.<sup>3</sup>

The porcine heart has coronary artery circulation similar to humans, which makes the pig a favorable myocardial infarction model for study.<sup>4,5</sup> The size of the heart is unsuitable for routinely sized sections because the small sections remove ischemic tissue from healthy tissue. The limitation of the 1.5 x 1.5 cm sections are overcome by using large mount sections, 4 x 6 cm. The combination of the Masson's trichrome and the large mount technique can be used to quantify areas of healthy cardiac tissue and infarcted tissue.

## Materials and Methods

### Tissue Collection and Processing

Five pig hearts, approximately 4 cm x 6 cm x 8 mm, were received fixed in 10% neutral buffered formalin (NBF). The LCX of each heart was ligated 4 to 8 weeks prior to collection and 2 slices were taken for processing (Fig. 1). Slices were placed into Super Cassettes (Surgipath Medical Industries, Richmond, IL) and processed with pressure and vacuum on a Tissue-Tek<sup>®</sup> VIP<sup>®</sup> 5 processor (Sakura Finetek, Torrance, CA).

Tissues were exposed to each of the following solutions for 2 hours: 10% NBF, 80% alcohol, 95% alcohol, 100% alcohol, 100% alcohol/xylene (50:50 mixture), xylene (2 changes), and paraffin (4 changes at 60°C). Processed tissues were embedded in paraffin using Surgipath Super Molds (Surgipath Medical Industries, Richmond, IL).

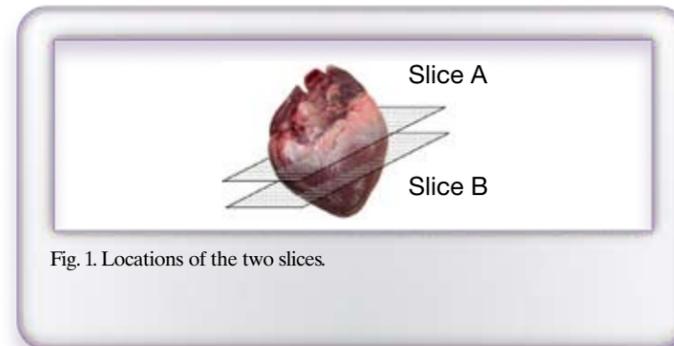


Fig. 1. Locations of the two slices.

### Microtomy

Blocks were faced (trimmed) and soaked on wet ice after which they were sectioned at 4 microns on a RM2255 rotary microtome (Leica Microsystems, Bannockburn, IL). Sections were floated on a warm water bath and collected onto 3 x 4 inch glass slides (Brain Research Laboratories, Cambridge, MA). Slides were dried at 58°C for 2 days in a laboratory oven prior to staining.

### Masson's Trichrome Staining Procedure

Slides were stained with the Masson's trichrome stain protocol (Poly Scientific R&D Corp, Bay Shore, NY).

1. Deparaffin slides in xylene and hydrate to distilled water
2. Postfix slides in a coplin jar filled with Bouin solution at 60°C for 60 minutes
3. Wash slides in running warm tap water to remove yellow color from sections
4. Stain with Weigert's iron hematoxylin solution for 10 minutes
5. Wash in running cold tap water for 5 minutes and rinse in distilled water
6. Stain in Biebrich scarlet-acid fuchsin for 10 minutes



Fig. 2. Pig 1, Slice A, H&E.



Fig. 3. Pig 1, Slice A, Masson's trichrome.



Fig. 4. Pig 1, Slice B, H&E.

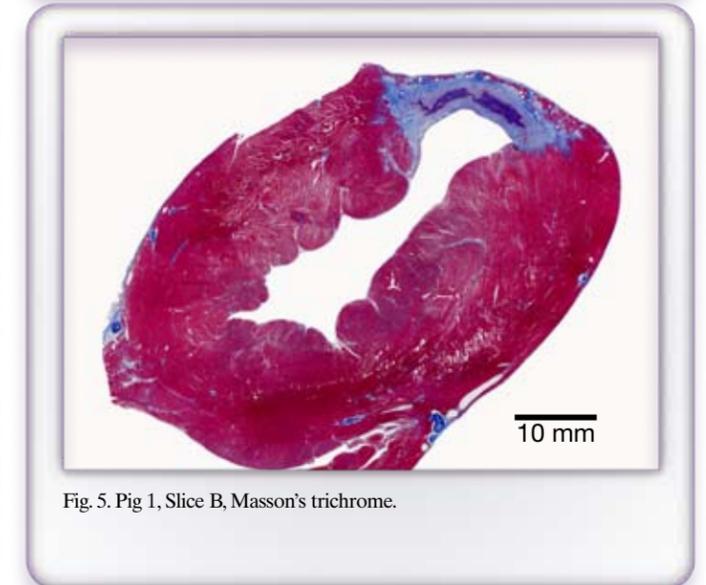


Fig. 5. Pig 1, Slice B, Masson's trichrome.

7. Rinse in distilled water
8. Place slides in phosphomolybdic-phosphotungstic acid solution for 10 minutes
9. Stain in aniline blue solution for 10 minutes
10. Rinse in distilled water
11. Place slides in 1% acetic acid for 1 minute
12. Dehydrate with 2 changes of 100% alcohol and clear in xylene
13. Apply coverglass with mounting medium

## Results

The large mount trichrome slides (Fig. 3, 5) were scanned and processed using ImageJ software.<sup>6</sup> Regions of interest were drawn around the infarct area, the left ventricular (LV) chamber, and the left ventricular walls of the heart. Using the three regions of interest, the infarct percentage was calculated:

$$\% \text{ Infarct} = \frac{\text{Area of Infarct}}{\text{Area of LV Walls of Heart} - \text{Area of LV Chamber}}$$

Table 1: ImageJ Software Measurements				
	Area of LV Walls of Heart (mm <sup>2</sup> )	Area of LV Chamber (mm <sup>2</sup> )	Area of Infarct (mm <sup>2</sup> )	% Infarct
Pig 1				
Slice A	1880.27	426.49	37.81	2.6
Slice B	1966.30	355.04	129.12	8.0
Pig 2				
Slice A	2017.44	426.49	260.70	16.2
Slice B	1969.60	355.04	24.64	1.4
Pig 3				
Slice A	1896.83	426.49	117.05	7.9
Slice B	1724.40	355.04	171.54	11.9
Pig 4				
Slice A	2307.72	426.49	113.03	5.9
Slice B	1929.74	355.04	121.25	7.2
Pig 5				
Slice A	2161.44	426.49	41.88	2.6
Slice B	1898.17	355.04	0.00	0.0

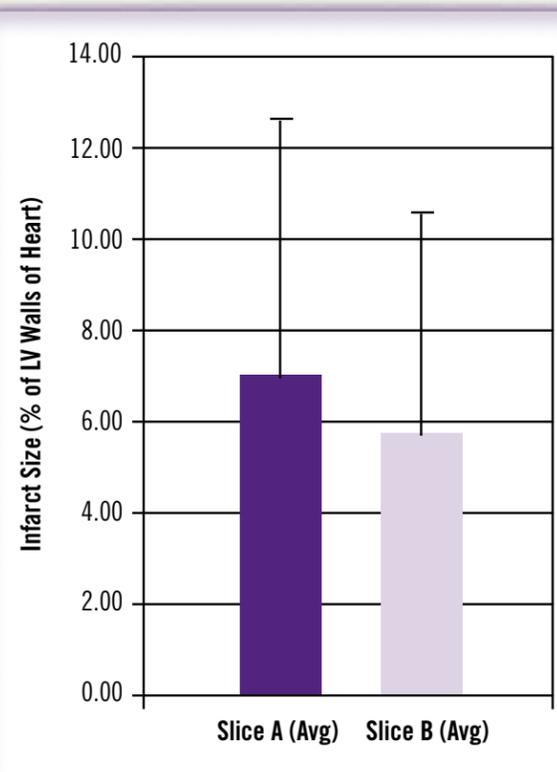


Fig. 6. Average infarct sizes for slices A and B.

### Conclusion

This study demonstrates that the combination of Masson's trichrome and large mount slide technique can be used to quantify healthy and infarcted tissue. Quantification of healthy and infarcted tissue can demonstrate variability of myocardial infarct models and the effects of angiogenic and neovascular agents. The use of Masson's trichrome stain to identify infarcted tissue is well accepted.<sup>3</sup> The use of large mount slides for cardiac histology is new, as is the computer software to quantify areas of infarct. The ImageJ software is an alternative to traditional hand-traced overlays.<sup>7</sup> Masson's trichrome stain with the large mount slide technique allows for a uniform assessment of cardiac tissue and the treatment effect of angiogenic and neovascular agents.

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### Acknowledgments

The authors thank Shirley Powell (Mercer University) for sharing her wealth of knowledge on large mount histology; Sharon Kelly (University of Michigan Histopathology Laboratory) for sharing her Masson's trichrome technique; Jackie Kunzler and Dolores Fischer (Baxter Technology Resources Pathobiology Department) for their continued support; Amy Carpenter (Baxter Technology Resources Pathobiology Department) for her continued support and staining the H&E large mount slides; Jim Diorio (Baxter Technology Resources Particle Science Department) for his guidance and for capturing the large mount images; Dr. Kevin Lewis and Eileen Kaminski (Baxter Bioscience Experimental Surgery Department) for their support, critique, and ideas for this study.

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# Dyeing to Be Safe

**Maureen Doran, HTL(ASCP)**  
**Southern Illinois University School of Medicine**  
**Histology Center**  
**Carbondale, IL**  
**mdoran@SIUMED.edu**

Special stains are the name of our game. However, they represent a spectrum of techniques that can be problematic when developing appropriate safety procedures for their use. Many of these staining procedures involve reagents and mixtures of chemicals that pose potentially acute or chronic hazards. The acute (short-term) hazards are usually from corrosives, flammables, and oxidizers. We have a heightened awareness of these reagents because the consequences of mishandling them are immediate and often result in personal injury or property damage.

Some of the dyes used in histological stains are characterized as having a potential for high chronic toxicity. Human carcinogens are also considered to exhibit high chronic toxicity. Long-term exposure to these agents increases the risk of cancer. The Occupational Safety and Health Administration (OSHA) Laboratory Standard 1910.1450 defines "select carcinogens" as chemicals that are listed as "known to be carcinogenic" in the Annual Report on Carcinogens published by the National Toxicology Program. Benzidine-based dyes fall into this category because of their ability to metabolize to benzidine. These azo dyes have CI# 20000-35990 and include Congo red, Biebrich scarlet, Bismarck brown, Ponceau S, oil red O, Bordeaux, and trypan blue. Benzidine was one of the first chemicals for which an association between occupational exposure and increased cancer risk was recognized. As early as 1920, industrial exposure to benzidine was linked with bladder cancer.

In 1973, OSHA banned the production of benzidine for commercial sale in the United States. The US no longer allows imports of benzidine, however, benzidine-based dyes may be imported and are commercially available. Current data suggest that these dyes are not produced in significant quantities. The regulating agencies acknowledge that the quantity of benzidine-based dyes that is imported is unknown and admit that this information would be useful in determining the potential for human exposure to benzidine.

In 1980, the National Institute for Occupational Safety and Health (NIOSH) published a special communication on the review of benzidine-based dyes. This report concluded that the carcinogen benzidine is present in benzidine-based dyes both as the result of the impurities introduced in manufacturing and as products of thermal and enzymatic decomposition. The greatest risk of exposure to workers is through inhalation of dry powders. Although inhalation is the major route of exposure to these

dyes, many of the inhaled dye particles are too large to reach the lungs. The particles are returned to the epiglottis by the ciliary action of the bronchial mucosa. Once in the epiglottis, the particles are then swallowed. Eventually, it is intestinal bacteria that metabolize the benzidine-based dyes back to benzidine by reducing the azo bond. In a study from China, workers exposed to benzidine-based dyes had an increased incidence of bladder cancer. In a NIOSH study, workers in a dye manufacturing facility were monitored for exposure to benzidine-based dyes despite the fact that these workers were using cartridge-type respirators at the time of sampling and that the environment appeared to be dust free. All of the random urine samples tested positive for benzidine.

Since 1980, the use of mixtures containing benzidine at concentrations of 0.1% or higher is permitted only in closed systems. Dye weighing areas should be regulated and access limited to authorized employees who are wearing personal protective equipment adequate to prevent skin contact or inhalation of the dyes. Workers must observe special precautions to reduce exposure.

Absorption through the skin is another source of exposure for benzidine. Evidence indicates that bacteria commonly found on the skin can also reduce the benzidine-based dye complex. This suggests that there is a potentially significant contribution to the overall exposure via the cutaneous route. Benzidine is readily absorbed through the skin, so reuse of personal protective equipment or contaminated work clothes can be a source of exposure. Glove selection is important. Latex gloves may not provide adequate protection, especially when working with dye mixtures that contain alcohols, acids, or solvents. Many benzidine-based dyes readily degrade to benzidine in aqueous solutions at high temperatures. Congo red, for example, is heated when used to stain for amyloid.

In 1983, the National Occupational Exposure Survey reported that 1554 workers, including 426 women, were potentially exposed to benzidine. At that time, educational materials were developed specifically to warn artists of the potential hazards of benzidine-based dyes. In 1986, the *American Journal of Industrial Medicine* published an article issuing this warning statement: "Continued exposure to small amounts of many different chemicals is relevant not only for chemists, but also artists, who may employ different dyes and pigments. For this occupational group, bladder cancer mortality was increased two-fold over that observed in the general population." It is not clear that histologists were included in this survey even though their exposure to these dyes may be greater than the exposure an artist would experience.

In 1983, NIOSH recommended that the production, use, storage, packaging, and distribution of all benzidine-based dyes be discontinued in light of the potential carcinogenic risks. Although eliminating these dyes from the histology laboratory is not practical, adhering to strict safety measures is essential. Dry powders should only be handled inside a containment system that prevents dust dispersal and protects the worker from inhalation hazards. The most effective control of carcinogens is at the source of contamination by enclosure of the operation or a local exhaust system. Commercially prepared solutions can minimize exposure to toxic dusts if adequate containment is



Fig. 1. One type of containment device used to prevent exposure to airborne dye powders.

not possible. Stringent work practices are necessary to ensure that dye particles do not contaminate clothing or the work area. Skin should be protected by impervious material to prevent dermal exposure. Our ability to protect ourselves from hazards depends on the knowledge to recognize the danger and our willingness to employ safety measures to protect ourselves. The bottom line: How important is your health?

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# Picro-Sirius Red Dye, Polarization, and Collagenous Tissue

Vicki Kalscheur, HT(ASCP); Jessica Riehl, BA  
 Comparative Orthopaedic Research Laboratory  
 University of Wisconsin, School of Veterinary Medicine  
 Madison, WI

kalschev@svm.vetmed.wisc.edu

## Introduction

There is a well-known **Sirius** Sunlight Glass Studio in Historic Mineral Point, WI, and **Sirius** is one of the brightest stars in the night sky. In our histology specialty, **Sirius** red F3BA is a complex acid dye developed in Germany that can be adapted for use in a special stain technique to demonstrate collagen, a component of connective tissue.

The role of connective tissue is, as the name implies, to connect cells within a tissue. It also provides strength and support to organs and muscular, vascular, and nerve tissues. Connective tissue consists of cells separated by a jelly-like extracellular substance that is composed of salts, water, protein, and carbohydrates. It is this extracellular substance, consisting of highly organized fibrillary proteins (protein fibers), that we are interested in when we employ connective tissue stains. Connective tissue fibers can be divided into 3 types: collagen, elastin, and reticular fibers. Of these 3 types, collagen is the dominant fiber in most connective tissues. The primary function of collagen fibers is to add mechanical strength to the connective tissue. This is achieved by collagen molecules being densely packed together.

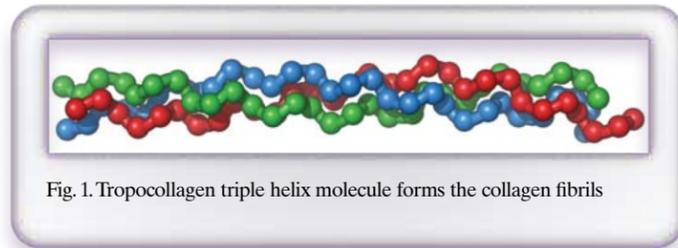


Fig. 1. Tropocollagen triple helix molecule forms the collagen fibrils

### Important Connective Tissue Facts

- Connective tissue may contain *fixed cells*, such as fibrocytes and fat cells, which are unable to move about freely within the tissue, and *wandering cells*, including macrophages, lymphocytes, mast cells, eosinophils, and neutrophils. A specialized cell, the fibroblast, is involved with tissue repair after injury has occurred
- Loose connective tissue allows blood vessels to pass through it, assisting in nutrition to all tissues
- Tendons and ligaments are formed from dense connective tissue
- Other specialized connective tissues include adipose, cartilage, bone, blood, and lymph

## Materials and Methods

Sirius red (also known as direct red) F3BA is an acid dye that selectively stains collagen and can be used to replace acid fuchsin in the van Gieson connective tissue stain. In the scientific literature, one can find it used to stain amyloid, basement membrane, and reticulum. Following staining with picro-sirius red (PSR), the key is to visualize it with polarized light microscopy, as the natural birefringence of collagen is greatly enhanced by the binding of long, aligned fiber molecules dyed with sirius red. With polarized light, the collagen fibril orientation shows orange/red bands against a dark background.

In order to see all of the fibers with polarized light, the slide is typically rotated on the microscope stage so that all levels can be visualized; if left in only one orientation, important areas can be missed. Rotating the slide allows incident light to diffuse through the tissue, which allows the emission wavelength to demonstrate changes in the components of the tissue. The collagen fibers appear red when the same slide is viewed with brightfield microscopy; other tissue elements will be a bright yellow. Different filter combinations result in different polarized color—experienced researchers select which combinations are optimal for the results they hope to achieve.

Collagen type and organization changes as bone heals and matures. It is very organized in mature bone, and less so during the healing process. In our musculoskeletal research laboratory, we use PSR as a connective tissue stain for bone collagen (Type I), which is coarsely fibered (woven bone), or osteoid, present in the early stages of bone healing. In later phases, the mineral components, hydroxyapatite, consisting of calcium, phosphate, and hydroxyl ions (Type II, III) are laid down on the matrix. We have found the birefringent properties of the PSR stain useful for decalcified dental tissues, tendon studies, collagen bundles in the growth plate, tissues from thermal energy studies, and in those with ocular abnormalities.

### Picro-Sirius Red Stain

Fixation: 10% neutral buffered formalin

Technique: Cut paraffin sections at 5 microns

Solutions:

#### 0.1% Picro-sirius solution

Sirius red F3BA ..... 1.0 g  
 Saturated picric acid .....1000.0 mL  
 (Allow solution to ripen for a few days)

#### Mayer's hematoxylin

Hematoxylin crystals.....1.0 g  
 Distilled water.....1000.0 mL  
 Sodium iodate.....0.2 g  
 Ammonium or potassium alum.....50.0 g  
 Citric acid.....1.0 g  
 Chloral hydrate.....50.0 g

Dissolve alum in water. Add and dissolve hematoxylin crystals. Add iodate, citric acid, and chloral hydrate. Stir until components are in complete solution. Stain keeps well for

months.

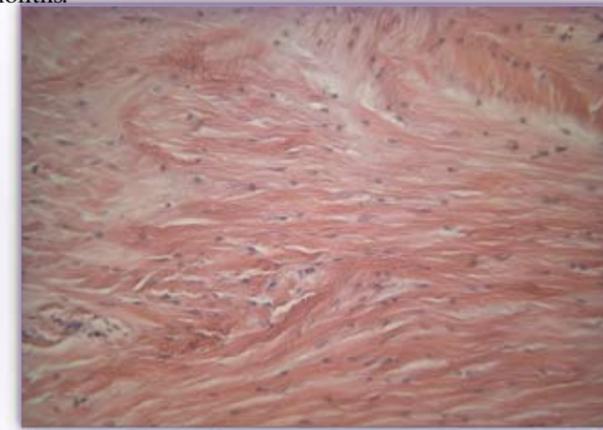


Fig. 2. H&E-stained tendon, viewed with light microscopy. Note the cellular proliferation in the center. 100X

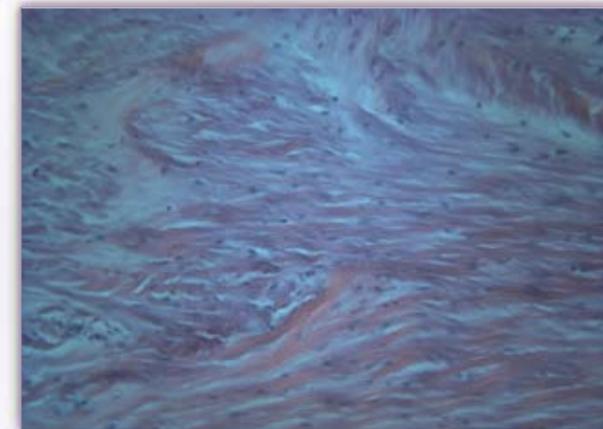


Fig. 3. Same H&E-stained tendon in Fig. 2, using a polarized filter with light microscopy. 100X

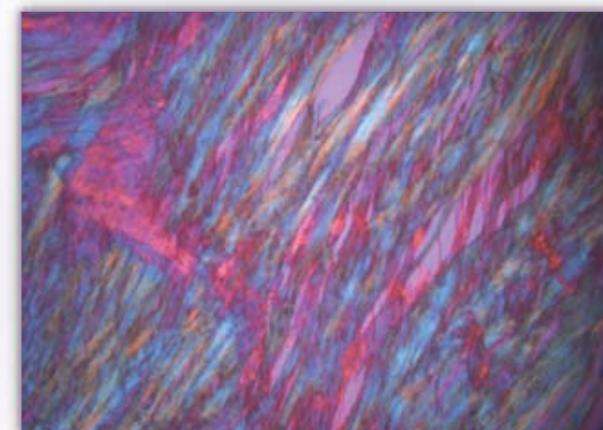


Fig. 4. PSR-stained tendon using polarized microscopy. 100X

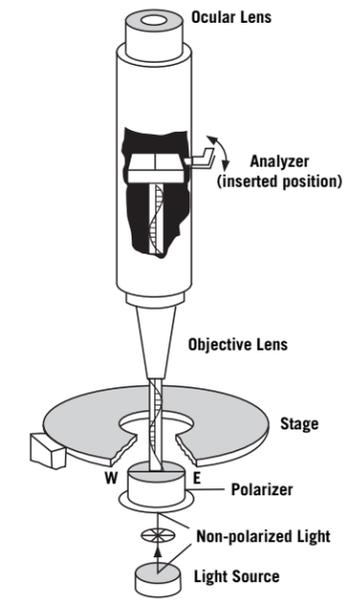


Fig. 5. Polarizing microscope.

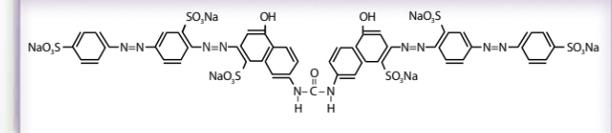


Fig. 6. Acid dye structure sirius red F3BA.

### Staining Procedure

1. Running tap water for 15 minutes
2. 0.1% picro-sirius solution for 30 minutes
3. Wash in water
4. Mayer's hematoxylin for 15 minutes
5. Running tap water for 15 minutes
6. Dehydrate, coverslip

## Results

### Light microscopy

Nuclei	Black if stained with iron hematoxylin
Collagen	Red
Cytoplasm, erythrocytes	Yellow

### Polarizing microscopy

Large collagen fibers	Orange or yellow birefringence
Thin collagen fibers	Green birefringence

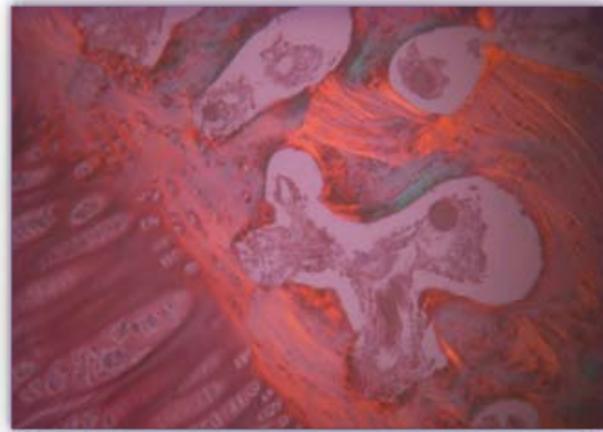


Fig. 7. PSR-stained collagen bundles in growth plate (left) and newly forming osteoid (right) in 3-week-old canine physis using polarizing microscopy. 100X

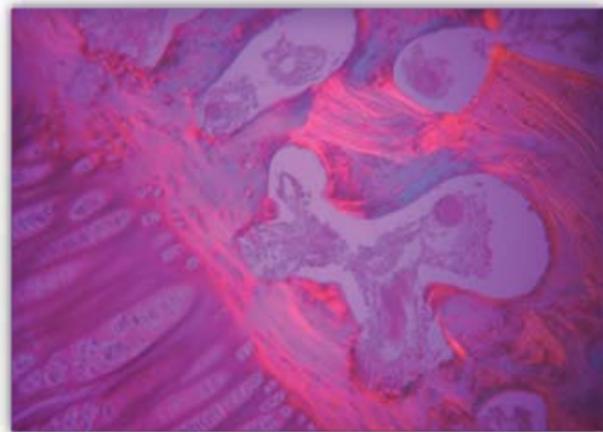


Fig. 8. Another view of Figure 7 using different filters with polarizing microscopy. 100X

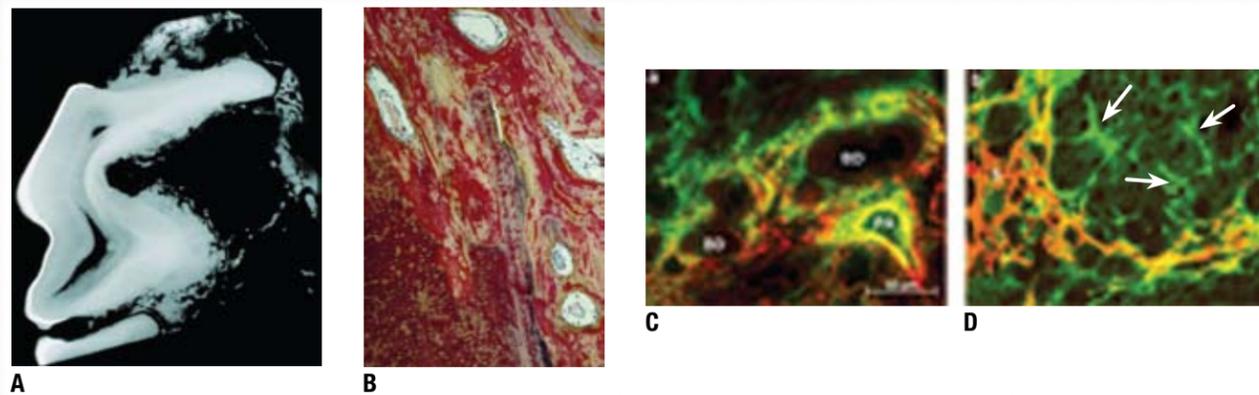


Fig. 9. (A) Dental radiograph; (B) PSR-stained abnormal mandible region viewed with brightfield microscopy, 40X; (C, D) two images of PSR-stained elastic fiber viewed with polarizing microscopy, 40X. Arrows indicate abnormal bone fiber pattern (green) in contrast to abnormal mature bone (orange).

The PSR stain is widely used and highly interpretive. The images in Figures 2 and 3 are of tendon, stained with hematoxylin and eosin showing how light microscopy, enhanced with a polarized filter can show more detail. Ultimately, in Figure 4, we see the tendon stained with PSR and polarized to show best contrast of collagen fiber in tendon. Figures 7 and 8 are from a 3-week-old canine physis where the use of the PSR shows stained collagen bundles in the growth plate. The PSR-stained tissue with polarized light exhibits color varying from green to yellow through red and shades of orange, depending on the cross-linking of the collagen, with older and larger collagen being more cross-linked. Deposition of collagen starts out as fine fibrils that later become cross-linked into larger fibers and bundles. The pattern and time frame of healing is of special interest in these studies.

Connective tissue studies utilizing the PSR stain may be carried out on dental, orthopedic, and other tissues that have been sufficiently preserved prior to processing. We fixed our samples in 10% neutral buffered formalin for at least 48 hours. Five-micron sections were prepared and stained per the procedure published by Sweat et al, 1964. Stained slides were examined with brightfield microscopy. The PSR-stained slides were also examined using polarizing microscopy with an analyzer set at 60° to the plane of the light with a first-order-red plate inserted into the analyzer. Morphology and staining patterns as well as evidence of birefringence were assessed. Other connective tissue stains that offer good contrast of collagen are Masson's trichrome and the modified Russell-Movat pentachrome method. Both result in elastic fiber, collagen, mucin, and muscle differentiation.

Immunohistochemical staining with antibodies to collagen III and collagen I can provide additional information. Along with histologic evaluation of slides, fine detail radiographs (Fig. 9A) offer correlary information for evaluation of tumor or dental abnormalities when compared to the microscopic findings (Fig. 9B). Additionally, Figure 9C and 9D show mandibular histology images of abnormal mature bone (orange) with elastic fiber (green, white arrows) under polarized light. The contrast assists in interpretation of specific dental abnormalities.



Fig. 10. Star Sirius.

## Conclusion

There is much to be learned when conducting connective tissue evaluations for pathological and research needs. The PSR stain with polarizing microscopy can yield very useful information to the researcher and pathologist regarding collagen type and structure.

The PSR stain may be used in many areas relative to the study of collagen diseases, and more. Take time to look at the stars, visit the galleries, and enjoy the beauty of **Sirius**, whatever form it may take.

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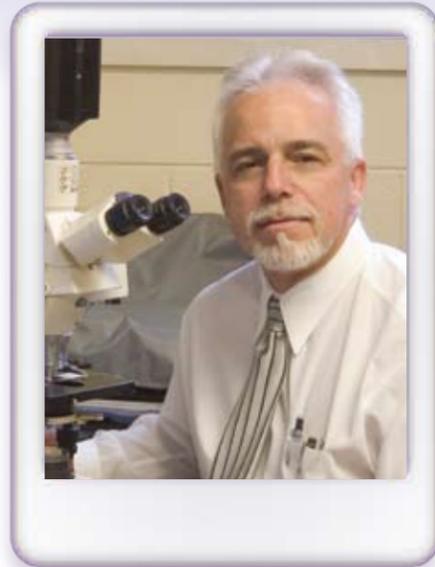
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The *HistoLogic*® Archives. Another resource that demonstrates Sakura dedication to histology.

## Letter From the Editor

### Submitting articles to *HistoLogic*®



On behalf of *HistoLogic*, I would like to express my appreciation to all of you who have supported this publication through the submission of manuscripts over the past 38 years. *HistoLogic* was created in 1971 in an attempt to keep histology practitioners around the globe connected to one

another through the sharing of relevant, practical scientific and technical information. Since that time, it has been read worldwide by more than 1,000,000 individuals. It is my sincere hope that the information that has appeared within these pages during my editorship has been of benefit to you in your work.

We understand that many of our colleagues may not have the opportunity to further their professional development through attendance at symposia and workshops. For this reason, the appearance of sound scientific information in your mailbox becomes all the more important to aid you in keeping your theoretical knowledge complete and up to date. As a result, we at *HistoLogic* remain committed to providing you with the highest caliber publication possible.

Few things contribute more to the advancement of science and technology than the sharing of knowledge and ideas with our colleagues. Indeed, this communication is the very foundation upon which new ideas are stimulated and new developments and discoveries are built. I believe that as scientists we have a responsibility to share what we know. It matters not whether you publish your work in *HistoLogic*, the *Journal of Histotechnology*, or any other scientific publication, only that you do, in fact, share your knowledge. Knowledge shared in print will always remain one of the most effective tools for reaching large numbers (often tens of thousands) of individuals.

This is where you come in. If you have never considered submitting your work for publication, I urge you to consider

the opportunity. I know that some may be daunted by the perception that it is very difficult to get one's work published. *HistoLogic* is interested in any manuscript that is scientifically valid. I've conversed with some colleagues who believe that they don't write well enough to successfully publish an article. However, as the scientific editor, I am available to assist you in any way possible. I am happy to help you construct your article, so please do not allow this concern to discourage you from coming forward with your ideas.

For those who are interested in submitting a manuscript to *HistoLogic*, I urge you to contact me directly at [dellav@musc.edu](mailto:dellav@musc.edu), or you may correspond with me via the mailing address included on the back of this issue. I prefer that manuscripts be prepared using word processing software such as Microsoft® Word or Corel® WordPerfect.®

Articles that appear in *HistoLogic* are required to have relevant scientific or technical references that should be listed in the order in which you cite them in your article. References must be sufficiently detailed as to allow the reader to locate the information you used when constructing your manuscript. We prefer that references are formatted according to the *AMA Manual of Style*, and I can assist you with getting your references into that format, if necessary. We encourage you to include color images that serve to illustrate key points in your article for the reader. While we prefer these in electronic format, including jpeg and tiff, we can also work with high-quality color prints and slides. All slides should have a caption that includes such relevant information as tissue type, stain used, magnification, and any remarkable features to be brought to the attention of the reader.

Most important of all, please be sure to include the most expedient way for me to communicate with you. Email is preferred over postal mailing, but if email is not available, please include a telephone number where you can be reached.

I hope that you will seriously consider making a contribution to the histology community by submitting a manuscript to *HistoLogic*. I look forward to hearing from you.

Vinnie Della Speranza, Scientific Editor



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## ✓ Mark Your Calendar! Educational Opportunities in 2009

### JUNE

- 19 **Teleconference Network of Texas/UT Health Sciences Ctr**  
Teleconference 12:00 pm Central Time (800) 982-8868  
Title: **Bringing FISH Testing In-House**  
Speaker: Bonnie Whitaker, HT(ASCP)QIHC  
Ohio State University Medical Center  
Columbus, OH
- 
- 24 **NSH Teleconference 1:00 pm Eastern Time**  
Title: **Troubleshooting IHC**  
Speaker: Jim Burchette, HT(ASCP)  
Duke University Health System  
Durham, NC  
Phone: (443) 535-4060  
Email: [histo@nsh.org](mailto:histo@nsh.org)
- 
- 25-26 **Tennessee Society for Histotechnology**  
Site: Doubletree Hotel  
Memphis, TN  
Contact: Charlene Henry  
Email: [Charlene.henry@stjude.org](mailto:Charlene.henry@stjude.org)

### JULY

- 17 **Teleconference Network of Texas/UT Health Sciences Ctr**  
Teleconference 12:00 pm Central Time (800) 982-8868  
Title: **Melanomas: A Diagnostic Approach Using Immunohistochemistry**  
Speaker: Elizabeth Sheppard, MBA, HT(ASCP)  
Ventana Medical Systems, Inc.  
Tucson, AZ
- 
- 22 **NSH Teleconference 1:00 pm Eastern Time**  
Title: **Staining and Identification of Pigments and Minerals in Tissue**  
Speaker: Debra Wood, MS, HT(ASCP)  
Indiana University School of Medicine  
Indianapolis, IN  
Phone: (443) 535-4060  
Email: [histo@nsh.org](mailto:histo@nsh.org)

### AUGUST

- 21 **University of Texas Health Sciences Ctr/San Antonio**  
Teleconference 12:00 pm Central Time (800) 982-8868  
Title: **Microwave Technology: When, Where, Why and How**  
Speaker: Donna Willis, HTL(ASCP)HT  
Milestone Medical  
Grand Prairie, TX
- 
- 26 **NSH Teleconference 1:00 pm Eastern Time**  
Title: **Role of Immunohistochemistry in the Diagnosis of Lymphoma**  
Speaker: Ann Marie Blenc, MD  
William Beaumont Hospital  
Royal Oak, MI  
Phone: (443) 535-4060  
Email: [histo@nsh.org](mailto:histo@nsh.org)

### SEPTEMBER

- 18 **Teleconference Network of Texas/UT Health Sciences Ctr**  
Teleconference 12:00 pm Central Time (800) 982-8868  
Title: **Small Specimen Management in a Large Volume World**  
Speaker: Herbert Skip Brown, HT(ASCP)  
Leica Biosystems LLC  
St. Louis, MO

### OCTOBER

- 3-8 **National Society for Histotechnology Symposium/Convention**  
Site: Birmingham, AL  
Contact: Aubrey Wanner, NSH Office  
Phone: (443) 535-4060  
Fax: (443) 535-4055  
Email: [Aubrey@nsh.org](mailto:Aubrey@nsh.org)
- 
- 16 **Teleconference Network of Texas/UT Health Sciences Ctr**  
Teleconference 12:00 pm Central Time (800) 982-8868  
Title: **Histology Process Improvement: Defect/Error Tracking**  
Speaker: William DeSalvo, BS, HTL(ASCP)  
Sonora Quest Laboratories  
Gilbert, AZ
- 
- 28 **NSH Teleconference 1:00 pm Eastern Time**  
Title: **Getting Through CAP Inspections**  
Speaker: Christine "Charlie" Dornier  
Celerus Diagnostics  
Flippin, AR  
Phone: (443) 535-4060  
Email: [histo@nsh.org](mailto:histo@nsh.org)

### NOVEMBER

- 18 **NSH Teleconference 1:00 pm Eastern Time**  
Title: **Muscle Case Studies**  
Speaker: Jon D. Wilson, MD  
William Beaumont Hospital  
Royal Oak, MI  
Phone: (443) 535-4060  
Email: [histo@nsh.org](mailto:histo@nsh.org)
- 
- 20 **Teleconference Network of Texas/UT Health Sciences Ctr**  
Teleconference 12:00 pm Central Time (800) 982-8868  
Title: **Process Improvement: A Beginner's Guide**  
Speaker: Tim Webster  
Seacoast Pathology, Inc.  
Exeter, NH

### DECEMBER

- 16 **NSH Teleconference 1:00 pm Eastern Time**  
Title: **Handling the Big "C's" in Histology**  
Speaker: Maureen Doran, HTL(ASCP)  
SIU School of Medicine  
Carbondale, IL  
Phone: (443) 535-4060  
Email: [histo@nsh.org](mailto:histo@nsh.org)



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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Vinnie Della Speranza, *HistoLogic*<sup>®</sup> Editor, 165 Ashley Avenue, Suite 309, Charleston, SC 29425. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.