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Advances in the Staining of Ground Section Histology

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Ground section histology is challenging, time-consuming, and at times frustrating. However, with the aid of variable-speed grinders, automated grinding systems, and advances in techniques, the art of grinding continues to progress. By using a variable-speed grinding system, such as the Exakt Grinding System, technologists can grind sections containing bone/metal implants to 10 to 50µm in thickness. It is now common in dental and orthopedic research laboratories for technicians to routinely process teeth or bone with metal implants in situ.

Orthopedic researchers have been observing the bone/metal implant interface since the early 1970s. Because advances have been made in laboratory equipment and techniques, new knowledge has been gained in understanding the biological response of the tissue at the implant interface. One of the problems still facing investigators is the ability to observe the cells in direct contact with the implant interface. Currently, there are limited stains available that can permeate plastics. Plastics are necessary for embedding to protect the bone or teeth while maintaining the metal implant in situ during the grinding process.

There are many published protocols for the staining of 5-µm plastic sections of bone or teeth with Goldner's Trichrome, methyl green pyronin, and hematoxylin and eosin.[1] However, these stains, when applied to ground sections, are difficult to reproduce consistently. At this time, there are several ways that laboratories are staining ground sections. The first is to prestain the bone prior to embedment.[2-5] Although this method can demonstrate relationships between the implant, fibrous connective tissue layers, and bone, there is a lack of cell and nuclear detail in these sections.

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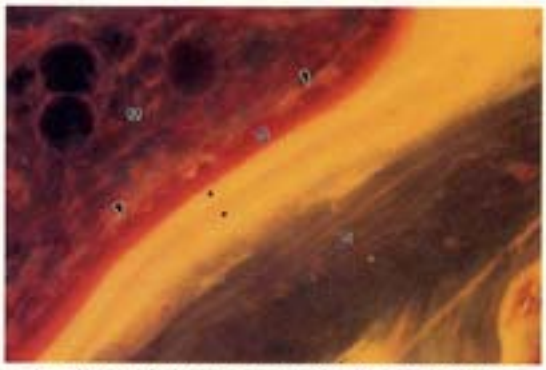

Fig 1. — Double-labeled human cancellous bone (C) showing osteoblasts (arrows), osteoid (O) red and the two fluorochrome labels (*). Note the vibrant colors using the Villanueva mineralized bone stain (MIBS). Marrow (M), magnification (× 200).

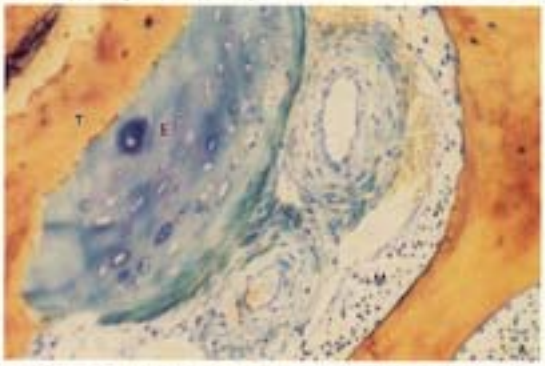

Fig 2. — This low-power field shows two arterioles in a figure-of-eight pattern adjacent to endochondral bone (E) that has formed along the trabeculae (T) of canine bone (orange). Note the excellent cell detail of the surrounding marrow cells (M). Rapid bone stain (RBS) with Van Gieson counterstain (× 200).

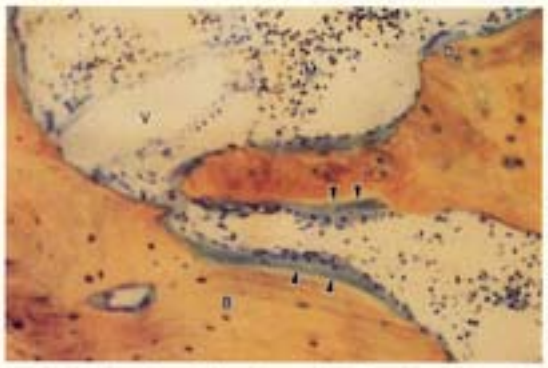

Fig 3. — Cancellous bone (B) showing osteoclast (O) and osteoid (arrows) with osteoblasts. Vessel (V) with marrow cells (M). Bone is orange. RBS with Van Gieson counterstain (× 200).

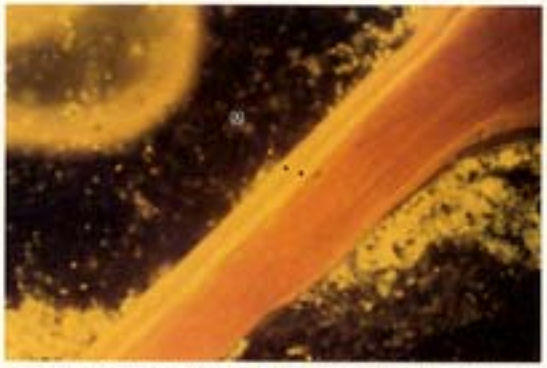

Fig 4. — Double fluorochrome labels (*) seen along the edge of human trabeculae (orange). Marrow (M). RBS with Van Gieson counterstain. Magnification (× 100).

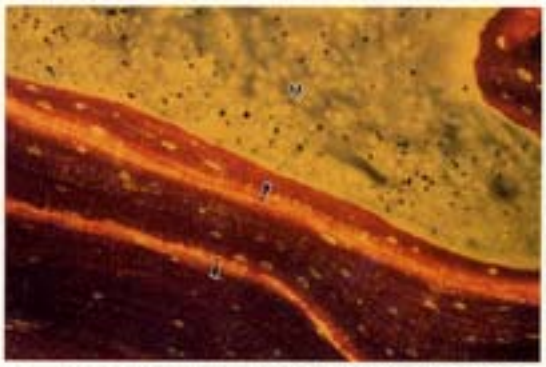

Fig 5. — Double-labeled cancellous bone (arrows). RBS with acid fuchsin counterstain. Bone marrow and cells (M). Magnification (× 200).

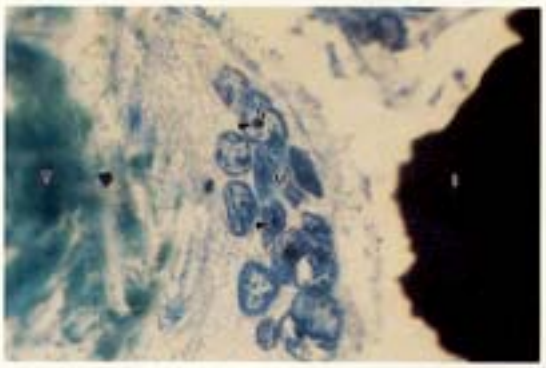

Fig 6. — Large giant cell with nucleus (N) and nucleoli (arrows) clearly seen. Note the mitotic division (M) occurring within the cytoplasm of the nucleus of this tumor giant cell. Implant (I), fibrous tissue (T). RBS with Van Gieson counterstain. Magnification (× 1000 oil immersion).

Another problem associated with prestaining bone prior to embedding is that prestaining can interfere if the investigator needs to do backscattered electron imaging (BSE) and elemental analysis.[6]

An alternate staining procedure is to stain the ground section after the grinding process. Some labs mount their sections on white acrylic plastic.[4,7,8] Schenk grinds to a thickness of between 100 and 400 µm, etches the section, then surface stains the section.[4] Sanders and Sterchi advocate etching the section and, if surface staining is to be done, then it is not necessary to grind to a specific thickness.[7,8]

In 1989 Villanueva et al introduced a mineralized bone stain (MIBS) that allowed the staining of fluorochrome-labeled ground sections of bone/metal implants.[5] Using a modified polymethylmethacrylate (PMMA) protocol and other published protocols[9,10,11] from our laboratory in conjunction with the advancement of the MIBS stain, our laboratory was able to observe stained sections under fluorescent microscopy (Fig 1). Light microscopy observations of sections stained with MIBS stain demonstrate osteoid, and cells within the marrow, while the bone matrix remains unstained. With a slight modification of the MIBS, the bone matrix now reproducibly stains a light apple green. To achieve these results we must place our ground sections in the stain overnight. Although cell observation at the implant interface can be done, the cells routinely appear either over- or understained.

In our laboratory we always strive to improve the histological techniques that are produced. Recently, a stain was developed in our laboratory for staining ground-section histology. While the primary reason for the development of the stain was for ground sections, the stain is excellent when 5-µm cut sections of undecalcified bone embedded in PMMA are needed. The objective of the stain was to improve cell detail, decrease staining time, and preserve fluorochrome labels.

What resulted was a rapid bone stain (RBS) that allows viewing of ground and 5-µm sections in only 12 minutes. Sections can be ground to a thickness between 25 and 70 µm without compromising staining ability. The rapid bone stain demonstrates osteoid, osteoclasts, osteoblasts, and various marrow cells. The RBS stain is versatile because a choice of three counterstains (Van Gieson, acid fuchsin, and modified light green) can be selected by the investigator. Van Gieson is the counterstain of choice in our laboratory (Figs 2 and 3).[12] Fluorochrome labels are preserved using Van Gieson or acid fuchsin (Figs 4 and 5).[12,13] If the investigator prefers the bone matrix to be green, then modified light green can be used.

At present, our lab prefers the MIBS stain when photographing fluorochrome-labeled bone. The MIBS stain is more vivid and impressive when presenting histological results at meetings. Nevertheless, the RBS gives excellent cell detail for the cells that are in intimate contact with the implant interface (Fig 6). We are now able to observe cells such as tumor giant cells with nuclei in mitotic division that, before the arrival of the RBS, were obscured by other staining techniques.

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Classification and References for Staining Procedures for Microorganisms

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Editor's Note:
Contained herein is some basic information on the classification of some microorganisms. Also included are some references for the staining procedures. The paper is to provide the histotechnologist with useful information on the classification of microorganisms. Included in the classifications are bacteria, fungi, mycoplasma, rickettsiae, and viruses.

Microorganisms

A minute living organism, usually a microscopic entity capable of carrying on living processes. It may be pathogenic. Those of medical interest are bacteria, rickettsiae, viruses, yeasts (fungus), and protozoa.

The important thing about microorganisms is that they infect and cause diseases. Most textbooks of medical microbiology deal with the subject either microbe by microbe or disease by disease. It should be understood that the centrally significant aspect of the subject is the mechanism of microbial infection and pathogenicity, and that the principles are the same, whatever the infectious agent; that is, the entry of microorganisms into the body, their spread through tissue, and the role of immune response, toxins, and phagocytes are generally the same for bacteria, rickettsiae, viruses, and protozoa.

Classification of Some Microorganisms[1]

Bacteria
Bacteria are unicellular organisms that have cell walls and reproduce asexually. Their genetic information is DNA, although it does not organize into chromosomes, and they synthesize enzymes through the usual DNA-to-RNA-to-protein mechanisms. Their enzyme content enables them to support themselves wherever the environment offers the necessary raw materials. While most bacteria support themselves independently in a free-living state, a few are capable of surviving and multiplying inside other living cells.

Bacteria vary in their need for oxygen. Some cannot exist without oxygen and are called obligate aerobes. Some cannot exist where there is oxygen; these bacteria are called obligate anaerobes. Still other bacteria, called facultative anaerobes, are capable of growing either with or without oxygen. Cell shape is the most widely used basis for classifying bacteria. The categories are: cocci (singular, coccus), which are spherical; bacilli (singular, bacillus), which are rod-shaped; spirochetes, which are spiral-shaped; and vibrios, which are curved like an S or a comma. The reactions that cell walls display with different stains provide another descriptive trait. Bacteria that stain with Gram's iodine are called gram-positive, while those that do not stain are gram-negative. Some organisms retain dye even after acid treatment; these are called acid-fast.

Fungi
Fungi (singular, fungus) are more complex structurally than bacteria. Although most fungi reproduce asexually, they have a nucleus and their DNA condenses into chromosomes during cell division. Fungi can assume many different shapes (including mushrooms), but the fungi important in human disease come in two forms: yeast, which are spherical or ovoid unicellular organisms that reproduce by budding; and molds, which consist of long, branching filaments, called hyphae (singular, hypha), made up of numerous cells placed end to end. A mass of hyphae clumped together is called a mycelium. Some pathogenic fungi can grow in either yeast or hyphal form. Fungi with this property are called dimorphic. They generally assume the yeast form when growing deep in tissues, whereas on surfaces, mycelial growth is the rule.

Mycoplasma and Rickettsiae
These two groups of organisms are difficult to classify. They do not share all the properties of bacteria, but do not clearly belong to any other category.

(continued on page 6)

Mycoplasma are the smallest cells capable of independent existence. The generalized average bacterium can be considered to have a diameter of 1 µm (one-millionth of a meter), while mycoplasma have a diameter less than 15% as large. Mycoplasma lack cell walls and may assume a variety of shapes. It is difficult to grow mycoplasma in the laboratory. In man, they infect primarily the lungs, the genitourinary tract, and the oropharynx.

Rickettsiae are irregularly shaped but usually appear as gram-negative rounded or elongated organisms about 0.3 to 0.5 µm in diameter. They are obligate intracellular parasites, able to multiply only inside living cells. Since they cannot survive free in nature, they must parasitize other organisms. The natural habitat for rickettsiae is the insect world, usually ticks and mites; man becomes a host only if bitten by an infected insect. Insects and rickettsiae survive in a commensal relationship, but man, when parasitized, becomes sick.

Viruses

Microbiologists used to argue whether or not viruses should be considered living organisms. They have no energy-generating or biosynthetic mechanisms of their own, being completely dependent upon the metabolic support of a host cell. They do, however, contain genetic material and reproduce themselves, and it would be difficult to exclude them from the category of infectious agents.

Viruses are the smallest living particles, some (polio virus, for example) being only 10 nm in diameter. (A nanometer is one-billionth of a meter or one-thousandth of a micron.) Others, like the pox viruses, are up to 300 nm in diameter. Marked morphologic and antigenic differences exist among viruses, but all viruses consist of a nucleoid, a central nucleic acid core that comprises 5% to 25% of the virus, and the capsid, an outer protein coat that contributes 75% to 95% of the complete organism.

Methods for Demonstrating Microorganisms

Because microorganisms are pathogenic, giving origin to disease or morbid symptoms, it is important that they be identified, not only by the physician, but also by the laboratorian. The following references will provide an array of excellent methods for demonstrating microorganisms in microscopic sections taken from tissue specimens. Staining procedures are not being provided because it would be difficult to decide which are the most appropriate or useful, since most laboratories select their procedures based on the methods that work best in their laboratory (not all methods work the same in all laboratories for some unknown reason) and for the individual histotechnologists in the laboratory.

Regardless of the procedure one uses, the staining quality can only be determined by following this motto: There is nothing consistent in the science of histotechnology. What performs well today most likely will not tomorrow. "Therefore, one must use today's results to determine tomorrow's quality."[2] This should be practiced on all facets performed daily in the histology laboratory.

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Tissue Flotation Bath

Monterey Proves Attractive Site for NSH Symposium/Convention

Brent Riley
Managing Editor

Several adventurous histotechnologists were seen rollerblading, bicycling, and kayaking in Monterey during the 1992 Symposium/Convention for the National Society for Histotechnology. But they weren't "playing hooky" from the workshops, lectures, and meetings that took place. They were just releasing a little pent-up energy after a hard day of learning, participating, sharing, and networking. After a productive day of improving their skills and knowledge about histotechnology, they simply wanted to take on a different type of challenge — have a different kind of fun. And their exuberance was motivated not just by the immediate activity, but also by the overall success of this 18th annual convention.

The 1992 symposium/convention was held September 12-18. "Set Sail Into the Future With Histotechnology" was the theme of the meeting, which attracted 1,044 registrants to the California resort city to attend some of the 70 workshops, 19 lecture sessions, and miscellaneous meetings and social events.

"The workshops were all well-attended," reported Phyllis Boris, NSH convention chairman. "We had a lot of people waiting to get into our limited workshops. And we're always sorry that we can't accommodate everyone in every workshop, but some of the hands-on workshops are just not suited to large groups."

The convention was held in three different facilities: the Monterey Convention Center, The Doubletree Hotel, and the Monterey Marriott Hotel. The convention center and Doubletree Hotel share the same building, while the Marriott is attached by an overhead walkway. Workshops and meetings were conducted in all three facilities and, according to Boris, it was very easy to get around.

Although the Monterey area offers plenty of tempting opportunities for recreation and sightseeing, it did not

interfere with workshop attendance. "They came to the workshops in droves," Boris observed. "We always strive to offer workshops that are on the cutting edge. We cover all the advanced areas for those who are ready, but we'll never overlook the basics."

The lectures were also well attended. They included both veterinary, industrial, research (V.I.R.) and clinical scientific sessions. The Professor C.F.A. Culling Memorial Lecture was given by Clive R. Taylor, M.D., Ph.D., of the University of Southern California School of Medicine. His talk was titled "Diagnostic Immunohistochemistry — Standards of Practice." The NSH International Lecture, "Resins and Their Application in Diagnosis," was delivered by Keith R. Cole, F.A.I.M.L.S., of the University of Western Australia, The Queen Elizabeth II Medical Centre in Perth, Australia. And the Swiss Society Exchange Lecture featured Jakob Zbaeren of the University Clinic of Medicine, Inselspital, in Bern. Zbaeren's talk was titled "Immunocytochemistry — Problems — Suggestions for Improvement."

Panel discussions were also held for both clinical and V.I.R. topics. The panels consisted of experts in many areas. Attendees submitted questions to the panels in advance and at the sessions. "Panel discussions went better than ever," Boris said. "We try to have a variety of different fields represented on the panels so almost everybody can get their questions answered. This was one of the better ones because we received a lot of questions ahead of time. That's always a big help." In fact, they had more questions than time allowed at the Wednesday afternoon V.I.R. session. Questions that weren't answered Wednesday were answered at the clinical session on Friday morning. "Friday's session is usually not well attended because most of the attendees prefer to get an early start on their return home," Boris explained, "but there were a lot of people this time."

Sixty-three companies were represented at the scientific exhibits, which began after the last workshop on Tuesday afternoon. These exhibits gave histotechnologists an opportunity to see the latest in histology equipment and supplies.

"Every year, many of the exhibiting companies want bigger booths to display their products," Boris remarked. "This year, we had to limit the number of companies participating because there just wasn't enough space available. We even removed the NSH education booth and the food services in the exhibit hall to make more room. And we still had six or seven companies on the waiting list. But starting next year, our conventions will be held in large convention centers that can accommodate all of the exhibitors." Boris observed that the booth designs get more sophisticated every year, using more state-of-the-art technology to display their products and communicate their messages. "It's really gratifying to see that," she said.

As anyone who has attended a national convention knows, social events are an important part of the experience, providing an opportunity to exchange ideas and get to know histotechnologists from around the country. Perhaps the most notable social event is the Thursday evening Awards Banquet, where a number of prestigious awards and scholarships are presented. These include the Histotechnologist of the Year Award, the J.B. McCormick, M.D. Award, and the Golden Forceps Award. This year's banquet was preceded by a cocktail party, sponsored by Miles Inc.

In addition, Miles Inc. sponsored an "MTV Night" party on Tuesday night. Guests were asked to come as their favorite musical entertainer, and a number of "famous" faces were seen roaming around the party scene. "The Miles party was very good," Boris exclaimed. "That's the one thing everybody looks forward to because you never know what Miles is going to do."

Another important social event was the welcoming reception held on Friday night for newcomers. People attending the convention for the first time were invited to get acquainted with NSH officers and other histotechnologists. The event featured an ice cream sundae bar. There were more than 300 newcomers at the convention.

"As usual, the local group did a real fine job," Boris said. "This meeting can't be done without a lot of people — national people, local people, and the people at the NSH office."

The success of the meeting was evidenced by the evaluation forms returned. "The response was very positive," Boris said. "And we had many more forms returned this year than in previous years." Specific results of the evaluation survey will be released soon in an issue of *NSH In Action.*

Next year, the NSH Symposium/Convention will be held October 2-8 in Philadelphia. Future years will see the meeting in Nashville, Tennessee; Buffalo, New York; Albuquerque, New Mexico; and Columbus, Ohio. "We try to go back and forth between the eastern and western part of the country," she explained. "That way, everybody can have a chance to attend one of the conventions."

Scheduling the symposium/convention is especially difficult because the ASCP and CLMA meetings are also held in the fall and it is important not to schedule a convention within one week of those popular meetings. "That doesn't leave a lot of viable dates," Boris explained. "It's a real puzzle to try to work it all out."

Boris encouraged those interested in attending future meetings to get their reservations in as early as possible. Overall, reservations were made late this year, perhaps because of the uncertain economy. "I hope this won't be a trend," Boris said, "because it is more difficult to plan for the meeting and assure that we have enough rooms."

Variety is the key to the success of this symposium/convention. So when someone tells you they "set sail into the future" at this year's event, they could be talking about workshops, exhibits, parties...or rollerblading.

Dr. Clive Taylor accepts appreciation award for presenting the 1992 Professor C.F.A. Culling Memorial Lecture.

Light Green-Picric Acid as an Alternative Counterstain

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Traditionally, light green SF (yellowish) has been used as a counterstain for many special stains, including Grocott's Methenamine Silver[1] and Periodic Acid-Schiff.[2] Used in combination with picric acid, light green gives a well-differentiated background with decreased risk of overstaining.

The original formula called for 5% light green in 1% alcoholic picric acid.[3] This solution required 10 minutes for staining.

The modified, aqueous light green-picric acid solution offered here stains in 1 minute. It is formulated from commercially available saturated aqueous picric acid, thus reducing the safety hazards of using dry picric acid. Not only does this stain provide differentiation among many tissue background structures, it is also more consistent in depth of color than the traditional light green (Figure).

Solutions

Light Green (Stock)

Light green SF (yellowish) (CI#42095)	0.1 g
Distilled water	200.0 mL
Glacial acetic acid	0.2 mL

Saturated Picric Acid, aqueous

Light Green-Picric Acid (Working)

Light green (stock)	25.0 mL
Saturated picric acid	25.0 mL

This stain loses its potency after use with 30 to 40 individually stained slides. It is suggested that the working solution be combined as needed, although its shelf life is approximately 3 months.

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Staining Procedure

1. Decerate slides in usual manner and stain with primary stain procedure.
2. Rinse slides with distilled water.
3. Stain in working, light green-picric acid solution, 3 minutes.
4. Rinse slides quickly in 2 changes of 95% alcohol.
5. Dehydrate slides in absolute alcohol, clear, and mount with appropriate medium.

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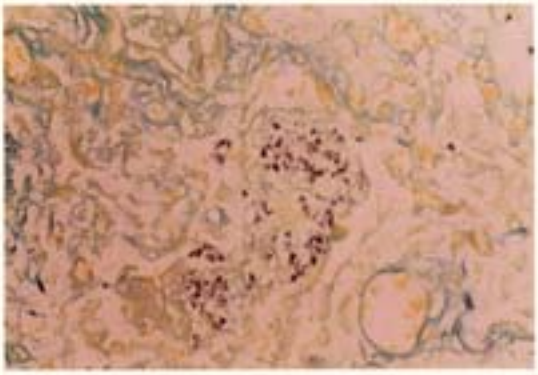

Pneumocystis carinii stained with Grocott's Methenamine Silver with Light Green-Picric Acid counterstain.

Histo-Logic Applauds Award-Winning Histotechnologists

The following awards were presented at the 18th NSH Symposium Banquet on September 17, 1992, in Monterey, California.

Golden Forceps Award
Sponsored by Miles Inc., Diagnostics Division

Janice R. Herring
Indianapolis, Indiana

J.B. McCormick, M.D. Award
Sponsored by Dr. J.B. McCormick

Billie L. Swisher
Atlanta, Georgia

Histotechnologist of the Year Award
Sponsored by Shandon/Lipshaw, Inc.

Cathy Sanderson
Salt Lake City, Utah

Diamond Cover Award
Sponsored by Miles Inc., Diagnostics Division

Marcelo L. Larramendy
Helsinki, Finland

Diamond Cover Merit Award
Sponsored by Miles Inc., Diagnostics Division

Julie F. Foley
Research Triangle Park, North Carolina

The Editor's Award
Sponsored by Miles Inc., Diagnostics Division

Robert A. Skinner
Little Rock, Arkansas

The Lee G. Luna Foreign Scholarship Award
Sponsored by Surgipath Medical Industries, Inc.

Sheron C. Lear
Louisville, Kentucky

Sakura Student Scholarship Award
Sponsored by Sakura Finetek, USA, Inc.

Rebecca L. Moore
Cobleskill, New York

N.S.H. Convention Scholarship Award
Sponsored by Histology Control Systems, Inc.

Sandra L. Thamer
Buffalo, New York

Instrumentation Laboratories Educational Scholarship Award
Sponsored by Instrumentation Laboratories, Inc.

Joyce Trout
Des Moines, Iowa

Miles Educational Scholarship Award
Sponsored by Miles Inc., Diagnostics Division

Jeralyn E. Mace
Ames, Iowa

Dezna C. Sheehan Memorial Educational Scholarship Award
Sponsored by the National Society for Histotechnology

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Miles Inc., Diagnostics Division, is pleased to announce an important *CHANGE* in the distribution of our Histology and Cytology instrument products.

Beginning January 1, 1993, you may purchase Miles Histology and Cytology instrumentation directly from Miles. These products include:

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Continue to order all Miles Histology/Cytology accessories and disposables exclusively from Scientific Products Division, Baxter Diagnostics Inc., a subsidiary of Baxter Healthcare Corporation. This change in distribution will help Miles maintain product pricing and will provide each and every customer stronger sales and service support. We have already organized our sales and service staff for greater efficiency and better customer service.

Although this is an important change in the way we do business, rest assured, some things have not changed:

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- Easy order processing by facsimile, phone, or mail

Remember, this important change in product distribution begins January 1, 1993. Please make a note of this date on your calendar.

Miles and Baxter are looking forward to meeting your Histology and Cytology needs even better in 1993.

Brent L. Riley
Senior Marketing Manager
Cellular Diagnostics

Zinc Facts

Charles J. Churukian
Department of Pathology
University of Rochester Medical Center
Rochester, NY 14642

Zinc chloride is a satisfactory substitute for mercuric chloride in preparing Zenker's and Lillie's B-5 fixatives. Tissue sections mordanted with Zenker's fixative prepared with zinc chloride will stain well with Mallory's PTAH method.

Zinc formalin is an excellent fixative for histochemical and immunohistochemical stains.

The formula for zinc formalin is:

Formalin, 37%	10.0 mL
Distilled water	90.0 mL
Zinc sulfate	1.0 mL

Zinc formalin has a pH of 4.2, which may cause the formation of formalin pigment in tissues. Commercially available zinc formalin, which is buffered to pH 5.5 to 6.0, will eliminate the possibility of formalin pigment forming in tissues. Formalin pigment in tissue sections may be removed by treatment with 10% ammonium hydroxide in 95% alcohol for 10 minutes.

A Simple and Effective Mounting Medium for Preserving Alcohol-Soluble Chromogens

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Abstract
A mounting medium with the following advantages has been developed for preserving alcohol-soluble chromogens: (1) It can be made inexpensively and is very simple to use. (2) It can keep the intensity of the chromogen for a significantly longer period of time. (3) It does not interfere with or distort the observation of tissue samples.

Introduction
Since diaminobenzidine hydrochloride 3'3 (DAB) has been found to be a potential carcinogen, many new chromogens have been introduced. Among these, 3-amino, 9-ethyl carbozole (AEC), and 4-chloro-1-naphthol (4CN) have shown some advantages and are widely used. However, because of their solubility in alcohol and tendency to fade rapidly in conventional mounting media, an issue of preserving samples emerges. Although Crystal Mount has been invented[1] and solves this problem, it is more time-consuming and difficult to use. We introduce a simpler method, used in our lab, that demonstrates excellent results.

Materials and Methods
A. Reagents:

The following reagents were utilized in preparing the mounting medium:

1. Gelatin	Fisher Scientific
2. Glycerin	Fisher Scientific

The formula utilized was:

Gelatin	5.0 g
Distilled water	50.0 mL
Glycerin	45.0 mL

B. Preparation of Medium:

Dissolve gelatin in distilled water at 70 to 80°C (use stirring bar), add glycerin, and stir until all bubbles are removed. Preservatives, such as sodium azide, may be added to prevent contamination by bacteria. The medium can be stored at 4°C or at room temperature. Before use, warm solution to between 50 and 60°C. The slides can be mounted either wet or dry by dropping 1 to 2 drops of medium on the surface of the sample prior to covering with coverglass. The medium will dry in about 30 seconds to 1 minute. Slides should be totally dry in about 10 minutes. The slides can be stored in a sealed plastic bag at 4°C or at room temperature in a conventional slide file. If bubbles are found between the sample and the coverslip after drying, the coverslip can easily be removed by dipping the slide in warm to hot water (50 to 60°C) and remounted as described above. If the slides are stored at 4°C, they should be left at room temperature for 10 to 15 minutes or between 50 and 60°C for 2 to 3 minutes before observation or photography. To photograph at high magnification, warm slides to between 50 and 60°C for 2 to 3 minutes or simply put the slide on the microscope stage with strong light for about 30 seconds in order to achieve excellent resolution.

Results
Our medium can be made with little expense, is easy to use, and preserves alcohol-soluble chromogens for a long period of time. Fig 1 shows a slide that was photographed an hour after coverslipping. Fig 2a shows a slide that was coverslipped and photographed in July 1990 using AEC and our mounting media; Fig 2b shows the same slide photographed in June 1991. Fig 3 shows a slide that has been stored at room temperature for 10 months with no change in the intensity of the chromogen. Fig 4 is a slide that has been stored at 4°C for $2^1/_2$ years and was photographed at × 1000 to show that this medium does not interfere with observation of the sample.

(continued on page 16)

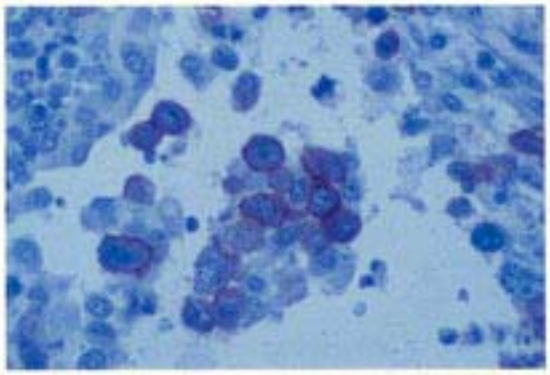

Fig 1. — Photographed one hour after coverslipping.

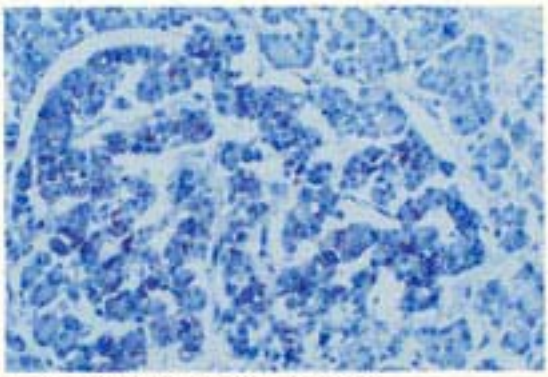

Fig 2a. — Coverslipped and photographed July 1990.

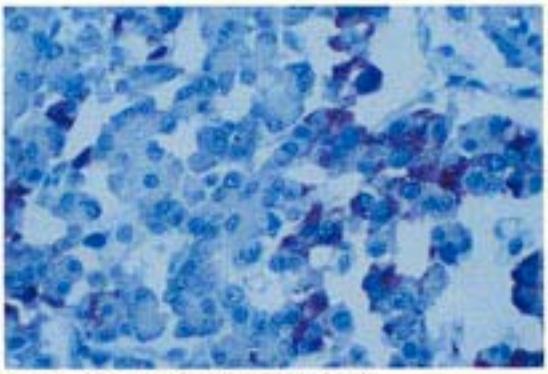

Fig 2b. — Same slide as 2a. Photographed June 1991.

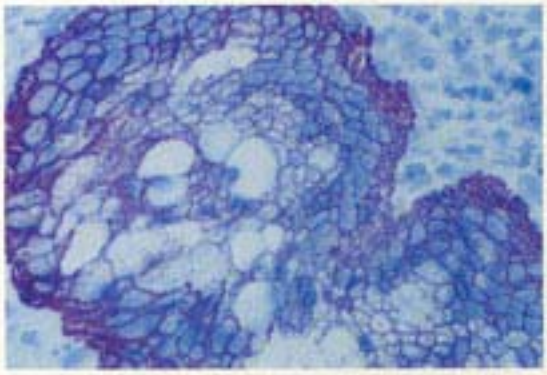

Fig 3. — Photographed after 10 months at room temperature.

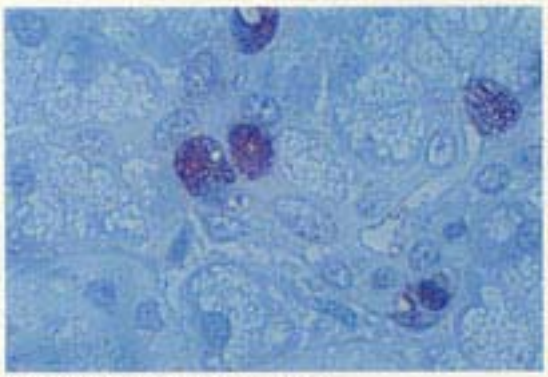

Fig 4. — Photographed after 2½ years at 4°C.

Discussion

With conventional mounting media, long-term preservation of antigens that have been visualized with alcohol-soluble chromogens is difficult. Crystal Mount,[2] developed to solve this problem, works well, but needs to be hardened at 80 to 90°C for 10 minutes. To view a slide mounted with Crystal Mount at high magnification (× 400), one needs to cover the Crystal Mount with a permanent coverslip. The medium we developed preserves the alcohol-soluble chromogens, is easy to make and use, and gives excellent resolution at high magnification for both observation and photography.

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1. New autoprobe universal immunosystems and other Biomeda immunoreagents, Bulletin No. 894FMD/7-0715-06:12. Obtained from: Fisher Medical Division.
2. Nash SJ, ed. *Handbook — Immunochemical Staining Methods.* Santa Barbara, Cal: Dako Corp; 1989:10.

Janice Herring Wins 1992 Golden Forceps Award

Brent Riley
Managing Editor

Scientist…technician…innovator…inventor. Perhaps, of all the words we could use to describe Janice Herring, HT, the name she's most proud of is "Golden Forceps Award Winner."

Herring won the coveted award for her article, "In Situ Hybridization Detection of Type I Collagen mRNA in Neonate Rat Bones: Effects of Decalcification," which appeared in the January/February 1992 issue of *Histo-Logic*. It was the first award she had ever received for publishing an article. In fact, it was the first article she had ever published.

Herring is a biologist/histotechnologist for the Skeletal Diseases Division of Lilly Research Laboratories, Eli Lilly & Company, in Indianapolis. The Golden Forceps Award is presented for the best paper published in *Histo-Logic* during the past year.

"I was totally surprised when I was told that I had won the award," she recalled. Herring was also surprised when Lee Luna, editor of *Histo-Logic* at the time, expressed an interest in publishing her article. She first told Luna about the paper at the 1990 NSH Symposium/Convention in San Antonio. "He was really excited about it and asked if he could publish it for me. He said it was very innovative and even said it could be the lead article in an issue."

Her article discusses the effects of several decalcifying agents on type I collagen mRNA detection levels and localization in long bone metaphyses of neonate rats by in situ hybridization at the light microscope level.

Herring has been with Lilly for nearly fourteen years. "When I first started at Lilly, I was doing routine electron microscopy where I was examining samples taken from studies using growth hormones and insulins being developed by recombinant DNA technology," she explained. "In the pharmaceutical area, you go from one research project to the next. If a treatment is needed for a certain disease, that's what we work on. I've been in three different departments here, and I like the variety." She currently works with about 40 people in her department, although she is the only histotechnologist. "Right now, it couldn't be better as far as using my knowledge and experience in one place," she said.

Herring started working in a laboratory at an age when most of us are just working on passing ninth grade. At 14, she worked at the Indiana University dental school every day after school. A neighbor worked at the dental school and they needed someone to clean glassware and do other odd jobs around the lab. Herring worked in the lab throughout high school.

"I started out cleaning slides in a histology lab where I worked my way into histology," she continued. "So it was only natural that I developed an interest in biology." In pursuing that interest, Herring studied

biology at Indiana University and Butler University, receiving a BS degree in biology from Butler. While earning her degree, she worked full time in a tissue culture lab at Indiana University Medical Center.

After graduation, Herring returned to the dental school to do histology again. But she had also developed a strong interest in electron microscopy, which she later learned while at Indiana University Medical Center.

Her combination of experience in histology and electron microscopy made Herring an ideal candidate for her current position. She works on in situ histohybridization and also does some immunology work. Her department at Lilly is working to find new ways to treat or prevent osteoporosis. In particular, Herring looks for expression of proteins in bone that has been treated with Lilly compounds.

Herring explained that there is a tremendous interest in osteoporosis today because of the aging population. "It's becoming a highly competitive field of study now," she said. "But it presents unusual challenges because of the nature of bone. I'm working now to get equipment to cut frozen sections."

One of Herring's goals is to look for messenger RNAs for different bone growth factors. "We're trying to understand the mechanisms involved in the development of osteoporosis so we can prevent it," she continued.

Most skeletal histology is performed decalcified. But when left undecalcified, bone is very difficult to section. "It's very hard," Herring explained. "It takes very specialized equipment. In some cases, the bone is infiltrated with very hard plastics and then cut. But, if we're looking for a messenger for a particular protein, we have to do frozen sections so we can access the messenger RNA for a protein. The equipment must have special drives and gears that can cut the hard tissue. A motorized mechanism is absolutely necessary. It is impossible to cut good frozen sections with an instrument that does not have a motor."

One way to prepare sections that are embedded in plastics is to use a milling machine to grind them down. "But that is very slow," Herring said. "I use a tungsten carbide steel knife to cut frozens. It has to be a very hard steel or you wouldn't be able to section it." Undecalcified bone will shatter if not sectioned properly. "It's almost as hard as a rock," she said.

Another common problem in cutting undecalcified bone is that the bone marrow tends to separate away from the bone. "This isn't good because we need to see the relationship of the bone marrow to the bone," Herring said. "We must maintain the relationship between the soft and hard tissues."

One particular challenge faced by Herring is preparing sections of vertebrae from rats. And that's where Janice Herring, the inventor, comes in. She has developed a bone-holding device for an instrument with a bandsaw blade. "A lot of people don't like to work with mouse or rat bones because they can't hold on to the bone to cut it down for embedding," she explained, "so I came up with a bone-holding device that you can use with the Exakt cutting-grinding system." She hopes to publish a paper on that device.

So, where does Herring go from here? "My next goal is to learn as much as I can about molecular biology," she explained. "That seems to be the future — gene engineering. I want to continue my in situ histohybridization work, localizing messenger RNAs and studying the expression of proteins in bones to develop a treatment for osteoporosis. I'll be a molecular biologist who does histology on bones."

"For me, winning the Golden Forceps Award is the most exciting thing to happen in my career. I'm a technique developer as well as a researcher," she said, "and I have always agreed with Lee's philosophy that, if you have a technique, give it to the world. I see myself writing a number of articles in the future."

With such a unique combination of talent and experience, Janice Herring will always have plenty to give.

Having fun at the Miles "MTV Night"

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