



Technical Bulletin for Histotechnology

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Pathology Research in the Nation's Capital

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"Send it to the AFIP" is a familiar phrase heard in histology laboratories around the United States, and in the laboratories of military facilities around the world. But what is the AFIP and what does it do?

The Armed Forces Institute of Pathology (AFIP) is a Department of Defense funded organization devoted to education, consultation, and research in pathology. Located on the grounds of Walter Reed Army Medical Center in Washington, DC, the AFIP employs over 700 personnel including members of the Armed Forces, Department of Veterans Affairs, Public Health Service, as well as civilians.

The AFIP is unique in the world of medicine for its concentration of personnel, equipment, and knowledge devoted to the study of pathology. Although best known for its consultative service, that is, in fact, just one aspect of the broad interests of the Armed Forces Institute of Pathology.

The National Museum of Health and Medicine

The AFIP was created in 1862 as the Army Medical Museum by Surgeon General William Hammond. Hammond was worried at the high incidence of disease among federal troops during the Civil War, which was then in progress. More troops died or were incapacitated due to disease than due to enemy action. Hammond wanted to assemble a team to study these diseases and combat them. Museums in those days were centers of research.

The Museum, now known as the National Museum of Health and Medicine, is located in the basement of the AFIP building and is visited by over 100,000 people each year. The museum collection includes over 17,000 anatomical specimens, 2.2 million archival documents and photographs, as well as 325,000 other medical artifacts. Due to its cramped quarters in the AFIP basement, less than 2% of these items are displayed.

IN THIS ISSUE

No reader should utilize or undertake procedures in Hino-Logic atticles unless the reader, by reason of education, training, and experience, has a complete understanding of the chamical and ghysical properties of all materials to be atilized and the function of each material by which ary procedure is accomplished. The procedures discussed in these atticles typesent the optimist and argerisences of the individual authors. Miles Inc. assumes no responsibility or liability in connection with the use of any procedure discussed hereis. () 1944 Miles Inc. As the only Federal Medical Research Facility at that time, museum personnel conducted the autopsy on slain President Abraham Lincoln in 1865. The fatal bullet is on display, as is a lock of Lincoln's hair and impressions of his face and hands. A later museum team conducted a similar autopsy on assassinated President Garfield in 1881.

Displays in the museum reflect its interests down through the years. A 3,000-piece dry bone collection from the Civil War period shows the effects of missiles on bone. Wax models of disease processes, a popular teaching aid in Victorian times, are also displayed, as are models of early ambulances and old surgical instruments, and the more common pathological specimens in jars (plastination is a modern-day option). Some of the most recent acquisitions are from "Operation Desert Storm," including US Army rations (MREs — "Meals Ready to Eat") and an Iraqi personal medical kit.

While the focus of the museum has been on trauma care and medical transportation, the medical staff has had breakthroughs in other areas through the years in wound healing, amputation techniques, and systems for transporting war wounded. The Spanish American War and the building of the Panama Canal were periods when museum personnel improved the understanding and treatment of infectious diseases. Major Walter Reed, after whom the Medical Center is named, conducted research into the role of the mosquito in yellow fever. He later worked on the disease typhoid. The museum continued its preventative medicine role in World War I, focusing on education and vaccinations, and producing a preventative medicine film, "Fit to Fight."

Several collections of medical and scientific interest have been passed on to the museum to be made available for further research. The Billings microscope collection in the museum is the most comprehensive collection of microscopes in the world. It includes models of Anton van Leeuwenhoek's microscope of 1676, many brass microscopes and modern-day binoculars. The electron microscope collection, possibly the largest of its kind in the world, includes the first electron microscope made in North America in 1940; the first electron microscope made by *Siemens*; and the first electron microscope made in the United States by RCA. Also on display is an unusual desktop electron microscope with fixed lenses made by RCA in 1953. Microtomy is also represented. Paraffin sectioning microtomes are displayed, as is the prototype ultra microtome originally used by Professor H. E. Huxley. Huxley, together with others, studied the ultrastructure of muscle and developed the sliding membrane theory. Other collections at the AFIP include the Vorwald collection of industrial medicine, the McGee collection assembled by a nurse during the Russo-Japanese War, and the Milton M. Helpern collection from the office of the New York City Medical Examiner's Office.

Museum personnel continue to use state-of-the-art techniques in their studies. Recent anthropological projects have included excavation of a Civil War site at Antietam, Virginia, a War of 1812 cemetery at Fort Erie in Canada, and a cemetery high in the Andes in Peru. Continuing its role in education, a current display at the museum is on the disease AIDS (Acquired Immune Deficiency Syndrome), its mode of infection and epidemiology.

With its continuous growth and acquisition of new specimens, the museum is looking for a new site in which to expand. A committee, headed by former Surgeon General C. Everett Koop, MD, is studying the problem. A possibility would be a return to the Mall in central Washington, DC, where the museum was located until 1968. This would be more convenient to tourists and would also provide much needed space for expansion. The museum may become separated from the Department of Defense at that time.

The Center for Advanced Pathology

The shortage of pathologists during World War II prompted Colonel Ash to start the consultation service by which the AFIP is known today. The Center for Advanced Pathology at the AFIP is a group of 22 departments that study disease on an organ systems approach. It is in these departments that the majority of the 65,000 specimens received annually are studied. Over 125 medical professionals are involved in examining these surgical pathology and autopsy cases, for which a modest fee is charged.

Department of Scientific Laboratories

Of interest to the histotechnologists are the 10 laboratories that comprise the Department of Scientific Laboratories. It is these laboratories that provide the technical support for other departments in the Center for Advanced Pathology. Large numbers of microscopic slides are prepared each year from cases submitted to the AFIP for consultation and teaching. Over 50 histotechnologists work in these laboratories.



Fig.1. — The Armed Forces Institute of Pathology is located on the grounds of Walter Reed Army Medical Center in Northwest Washington, DC.



Fig.2. — The museum dry bose collection includes the shattered leg bore of Civil War General Sickles, together with the offending rannonball.



Fig.3. — The museum's electron microscope collection contains many firsts, including the first successfully operating electron microscope in the United States, initial models of German- and Americaa-made commercial microscopes, and a powerful I-million-volt electron microscope.



Fig.4. — Using a motorized sledge microtome, complete sections of long bones, joints, and bone tumors are prepared in the Orthopodic Histology Laboratory.

Five of the laboratories are general in nature and provide technical support for those departments whose needs are ordinary in nature. The five remaining laboratories specialize in certain aspects of histotechnology.

The Orthopedic Histology Laboratory specializes in bone histology. Both paraffin and plastic sections are prepared and stained with a variety of special stains. Wet tissues arriving in the laboratory are x-rayed upon arrival, and then daily during decalcification until the end point is reached. This decalcifier is also used to solubilize small calcium deposits on the surface of contributed blocks. For wet tissues, a lengthy 2-day paraffin processing schedule is followed to ensure adequate penetration of tough collagenladen tissues. For large bone tumors, a motorized LKB sliding microtome is used.

The Laboratory of Veterinary Histology is unusual in regard to the wide variety of specimens it receives, which basically are all organs, any species, domestic or wild, vertebrate or invertebrate. This laboratory supports 14 veterinary pathologists and has the only veterinary pathology residency program in the Department of Defense.

Eye specimens are sent to the Ocular Histology Laboratory. While these may often be small biopsics or paraffin blocks sent in consultation, the specialty of the laboratory is whole sections of eye. A specialized protocol for dissection and processing of eyes is followed carefully. The Laboratory of Parasitic and Infectious Diseases studies "old" diseases such as leprosy and sickle cell disease and is involved with "newer" diseases such as AIDS and drug-induced pathological states. The Neuropathology Lab specializes in central nervous system cases and their techniques.

The School of Histotechnology in the Department of Scientific Laboratories is the only such school in the Department of Defense. It is attended by members of the armed forces. Two classes are held each year. Upon graduation, members are sent to military histopathology centers around the world. The curriculum of the school undergoes frequent changes to reflect modern trends in histotechnology. The late Lee Luna, former editor of this newsletter, was Chief of the Department of Scientific Laboratories at the AFIP until his retirement.

Center for Advanced Medical Education

Each year, nearly 3,000 medical professionals attend AFIP-sponsored programs in Washington and around the nation. These include nearly 50 short postgraduate courses, longer 6-week courses in radiology, lectures, seminars, and fellowships. A wet workshop is held annually for histotechnologists.

Additionally, microscope slide sets, 35-mm projection slides and motion pictures are available for educational purposes. Over 200 foreign nationals attend the AFIP each year.

Supporting the research and educational aspects of the AFIP is the Center for Scientific Publications. This center reviews research proposals, provides editorial review of manuscripts, edits the annual report and co-edits the AFIP fascicle series Atlas of Tumor Pathology. This center also is involved in the publication of the World Health Organization's book. International Histological Classification of Tumors.

The many wet tissues, slides, patient reports, and histories reviewed each year are stored by the Center for Records and Information Management. New material arriving on a patient is assigned a new accession number, while additional material arriving on a previously studied case is given the old number. Accession numbers follow all blocks, tissues, slides, and reports through to their filing. More than 2 million cases have been accessioned since 1862.

American Registry of Pathology

In 1922 a tenant agency, the American Registry of Pathology, was formed to act as a link between the AFIP and the civilian medical community. With this cooperative effort, the two organizations work together to formulate the long and short educational courses and expand research facilities. Histotechnologists can purchase control slides through the American Registry of Pathology. They have also recently published the updated AFIP staining manual. Both the American Registry of Pathology and the Center for Advanced Pathology have their own laboratories for research and development. In addition to classical correlation studies, these laboratories are involved in new uses of RNA and DNA probes, Artificial Intelligence, Quantitative Pathology, and Image Analysis.

The Division of Immunology in the Department of Cellular Biology performs many of the immunoperoxidase stains for the institution. Over 50,000 are performed each year. Automated stainers are used for some of the work, although with such a high number of slides to be stained, working by hand has often been found to be more convenient. Other immunoperoxidase methods are performed in ARP laboratories. Elmer's Glue[®] is used as a slide adhesive throughout the institution. Research in this area includes comparisons of DNA Hybridization and Immunoperoxidase, sensitivity and specificity studies, and comparison of commercial antibodies.

The Future

In its position in the forefront of Pathology, the AFIP is in a unique position to study new trends and needs in the pathology laboratory of the future. Several projects are underway on different fronts. For the United States Air Force, the AFIP has developed a quality assurance program that studies CAP returns from each Air Force laboratory worldwide. The AFIP also has developed a quality assurance program and proficiency testing for United States military drug testing facilities.

For surgical pathology, the AFIP is developing a video transmission project of microslides. This will enable military pathologists worldwide to discuss difficult cases with AFIP experts, and for the military, the AFIP is developing a DNA Identification Laboratory to support the United States Armed Forces around the world.

References

- Micorzi MS, Townsend FM, Koop CE. From Army Medical Museum to National Museum of Health and Medicine. Arch Pathol Lab Med. 1990; 115:1290-1295.
- Z. Dreyfus BT. Mining medical treasures. Am Mod News. June 22, 1990.

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Minimizing Laboratory Waste

Joanna Brady, HTL (ASCP) Colmery O'Neal VAMC Topeka, KS

In this new era of reserve, preserve, and recycle, the pressure is on to conserve our environmental resources. The laboratory contributes a lot of waste that cannot be eliminated, but there are a few steps that can be followed to cut down on needless laboratory waste. Listed below are some helpful ways we can avoid excess waste and show care for the environment:

Minimizing Chemical Waste in the Laboratory

- Use the minimum amount of chemical needed for the procedure or test performed. For example, only 10 times the tissue volume of 10% formalin is needed for tissue fixation. There is no need to fill a specimen container to the brim for a small GI biopsy.
- Filter reusable solutions back into the container and cap tightly to avoid evaporation.
- Whenever possible, use solutions that have a greater stability and shelf life.
- Unless a fume hood is required, keep all open solutions away from air currents, eg, air conditioner vents, air ducts, to prevent evaporation into the atmosphere.
- Buy chemicals in the dry form and mix only what is needed when it is needed. Dry chemicals have a longer shelf life than premixed solutions.
- Check with your engineering department to see if waste xylene can be used in the boiler plant. Some systems pour the xylene into the fuel tanks for moisture absorption.
- Invest in a distillation apparatus to reclaim xylene, alcohol, and other solvents used in the laboratory.

Minimizing General Laboratory Waste

- Use washable, laboratory-grade glassware instead of disposable plastic. If plastic is used, consider washing and reusing.
- Unused paraphernalia, such as gauze pads, from biopsy trays can be used for unsterile procedures or teaching service.
- Recycle all nonbiohazard glass, plastic, cardboard boxes, paper, etc.
- Purchase chemicals and solutions in the economysize package instead of one-use individual containers. Then recycle the containers.
- Old reagent containers can be washed and used for mixing and storing new solutions.

Differential DNA-RNA Staining of Formalin-Fixed Paraffin Tissue: A Modified Luna-Parker Giemsa Stain

William F. Barlow, HT (ASCP) Riverside Hospital Wilmington, DE 19802

Traditionally, the MGP (Methyl Green - Pyronin) stain has been used to demonstrate DNA and RNA. Optimal differentiation of the methyl green and pyronin components is only possible with Carnoy's fixed tissue. Carnoy's is inferior to formaldehyde as far as artifactual shrinkage is concerned. A modified Giemsa stain is described here which demonstrates differential staining of DNA and RNA in formalinfixed tissue. DNA stains a light turquoise blue, whereas RNA stains purple. We routinely use this stain in our laboratories for lymph node and bone marrow biopsies.

 Fixation
 10% buffered neutral formalin

 Processing
 Paraffin

 Microtomy
 Cut sections at 4 to 6 µm

Solutions

Gurr¹ Giemsa Stain Improved R66 (Stock Solution)

10% Stock Solution Rosin Alcohol

Rosin ² (violin)	10.0 g
Reagent alcohol	

Phosphate Buffer, pH 6.25

Sodium phosphate, monobasic	
Sodium phosphate, dibasic	0.5 g
Deionized water	_500.0 mL
Add 5 drops 90% phenol (carbolic acid) of buffer and refrigerate.	

0.4% Sodium Acetate Trihydrate

Sodium acetate	1.0 g
Deionized water	

Giemsa Stain Working Solution

Phosphate buffer, pH 6.25	
Methanol	
Stock Giemsa Solution	
Add the Giemsa stain to the methanol, add buffer.	

Working Solution Rosin Alcohol

10% Ros	sin Alcoh	ol		mL
Reagent	Alcohol		100.0	mL

¹ Biomedical Specialitics, Dox 1687, Santa Montes, CA 90406 (213-938-7515)

² Rosin (Scherl and Roth), United Musical Instruments, 1000 Industrial Packway, Elkhart, IN 46516 (900-759-9124)

Staining Procedures

- Decerate slides in solvent of choice, place in absolute alcohol, and rinse in graded solutions of hydrated alcohol to deionized water.
- 2. Place in working Giemsa solution overnight.
- Rinse sections repeatedly for 5 minutes in sodium acetate water.
- Place sections in working Rosin alcohol and dip up and down frequently. Proper differentiation is usually complete in 4 minutes. However, depending on tissue thickness and type of tissue being stained, differentiation may require up to 6 minutes.
- Rinse in one change of absolute reagent alcohol and check quickly under 10× on the microscope to determine proper differentiation. If differentiation is complete, dehydrate slides in absolute alcohols, clear in solvent, and coverslip.

Results

DNA	light blue
RNA	purple
Bacteria, fungus, parasites	various shades of blue and purple
Background	orange

Remarks

In order for the proper color hues of DNA and RNA to be produced, overnight incubation in the working Giemsa solution is required. The traditional May Grunwald Giemsa method does not differentiate DNA and RNA. This stain may have many other uses in different fields of biotechnology.

References

 Lillie RD, Amres A, B, C. In: Hatopathologic Technique and Practical Hatochassistry. New York, NY: McGraw-Hill: 1967;84:85
 Luna LG, Parker A. Mcdified Gierma for demonstrating Nocardia

 Luna LG, Parker A. Modified Giemia for demonstrating Nocardia anomides filaments. Am J Med Tech. October 1970;36:10.

 Luna LG. Personal communication domoistrating Nocardia asteroider filaments, 1977.



Fig.1 --- Plasma rells staining in lymph node section, 100×, MLPG Stain.



Fig.2. — Cysts of Entamoscha histolytica in colon biopsy, 1000 ×, MLPG State.

Tyler's Modification of Glycogen Digestion: A Faster, Simpler, and More Reliable Way to Digest Glycogen

Aaron R. Tyler, HM1, US Navy Histology Supervisor Naval Hospital Subbase New London Groton, CT 07349

For years there has been a problem in numerous histology laboratories with glycogen digestion procedures. This article suggests a reliable method to dissolve glycogen.

The most common glycogen digestion procedure is the one listed in the Armed Forces Institute of Pathology Laboratory Methods in Histotechnology manual. In this procedure, 0.1 g of diastase of malt is dissolved in 100.0 mL of a phosphate buffer solution and the solution is preheated to 37'C before using. After sections have been incubated in this solution for 1 hour, the glycogen should be totally digested, and the sildes are stained using the Periodic Acid Schiff (PAS) stain. Strict adherence to this procedure does not always produce adequate glycogen digestion.

A second common procedure involves the use of human saliva. Instead of using diastase of malt, the technician will spit in the coplin jar. The alphaamylase in human saliva digests the glycogen and yields very good results. In fact, this method works so well that instructors at the Tri-Service School of Histotechnology located at the Armed Forces Institute of Pathology teach students to spit in their coplin jars during the didactic portion of the program. Although this method of glycogen digestion works well, it has its obvious drawbacks, not the least of which is that no pathologist wants to look at a specimen with squamous epithelial cells from the technician's mouth lying on top of the tissue section. Alpha-amylase digestion is referenced as a third glycogen digestion method in Sheehan's *Theory and Practice of Histotechnology*, 2nd edition. A 0.5% solution is preheated for 1 hour, and the slides are placed into the solution for an additional hour. Sheehan also mentions the use of human saliva as an alternative procedure.

I felt there had to be a more reliable method for glycogen digestion than either the use of diastase of malt or human saliva that would be faster than 1 hour for preheating and digestion. For my studies, 1 obtained both type IX-A alpha-amylase (derived from human saliva) and type II-A (derived from the *Bacillus* species). Both chemicals are available from several chemical companies. Tissue samples were obtained from colon, small intestine, stomach, esophagus, and liver for staining with the PAS stain both with and without glycogen digestion using six different methods of glycogen digestion:

- 0.1% diastase of malt in phosphate buffer preheated for 1 hour at 37°C
- 0.5% type IX-A alpha-amylase in distilled water for 1 hour at 37°C
- 0.5% type II-A alpha-amylase in distilled water for 1 hour at 37°C
- 2% type II-A alpha-amylase in distilled water for 15 minutes at 56°C
- 2% type II-A alpha-amylase in distilled water for 30 minutes at room temperature.
- Phosphate buffer containing human saliva for 1 hour at 37°C

The five different tissues and six different digestion procedures were performed 25 times. The diastase of malt digestion showed little difference in staining between the digested and undigested slide. The other five methods yielded results more or less identical with total digestion of the glycogen. I was surprised to discover that the slide digested at room temperature in the type II-A alpha-amylase showed adequate digestion in half the time recommended for digestion by diastase of malt. And, although the IX-A and II-A alpha-amylase solutions were both excellent, the IX-A alpha-amylase is as much as 200 times as expensive as the II-A alpha-amylase.

In conclusion, the II-A alpha-amylase showed good glycogen digestion in half the time and proved to be more economical than the other methods. I have implemented this method of glycogen digestion into my laboratory's standard operating procedures for the past year and have never encountered problems with glycogen digestion. The cost of the Bacillusderived alpha-amylase is relatively low, so there is no cost increase in performing the test. The time saved versus the use of diastase of malt and the unorthodox use of human saliva has led me to believe that this is the way to go when digestion is required. Although I have only experimented with tissues fixed in 10% buffered neutral formalin. I do not believe that other fixative methods would not be acceptable for use with the Bacillus-derived II-A alpha-amylase.

References

- Sheehan DC, Hrapchak BB. Theory and Practice of Hinterechnology. 2nd ed. St. Louis, Mo: Mosby; 1987-186.
- Armed Forces Institute of Pathology. Laboratory Methods in Historychaology, 3rd ed, Washington, DC: American Registry of Pathology, 1992;171.

Answers to "Questions in Search of an Answer"

I am responding to the query about celloidin-paraffin double embedding in the September 1993 issue of *Histo-Logic*. Many years ago, when I needed to make thick celloidin sections of crayfish tissue, I tried methods in which the final concentration of celloidin used for embedding was 2%. After the celloidin block was soaked in paraffin-saturated chloroform, it was embedded in paraffin. All of the tissue blocks were soft and difficult to cut on a rotary microtome, which was the only kind I had.

Success was finally mine when I tried a method used by Inman for brain (Inman O. Nongraded dehydration and low-pressure infiltration for rapid celloidin embedding of brain tissue. *Stain Technology*. 1968;43:69-73). A more concentrated celloidin solution gives stability to the tissue and terpineol allows the blocks to cut nicely on a rotary microtome. Although the paper states that 5-µm sections can be cut. I made no mention in my notes of trying to section at less than 25 µm.

1 quite agree with (the Scientific Editor) that paraffin or plastic processing is preferable; the celloidin procedure does give beautiful sections, but it is tedious. If celloidin must be used, try soaking the block in terpineol or possibly expose it to chloroform fumes. Inman's procedure may still be in use where it originated. If so, they would be able to give Ms. Saccaro helpful tips.

Joan Roach, Room 250 Kenneth Norris Jr. Cancer Hospital University of Southern California Los Angeles, CA 90033

Questions in Search of an Answer

Please examine the enclosed H&E slide. I am unable to identify or eliminate the dark artifact found in the mucin. This seems to happen quite frequently in our mucinous tissue. I would appreciate any light you could shed on this small, but irritating, problem.

Kathy Tooker, HT (ASCP) Histology Supervisor Portland Adventist Medical Center Portland, OR

Editor's Note: I have seen this problem numerous times in my own laboratory. Correcting the problem is not always a one-step deal, since there may be numerous causes for the artifact, including incomplete paraffin removal, improper fixation, or incomplete removal of the plastic polymers from the paraffin mixture. Readers are welcome to respond with possible solutions to this problem.





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MILESSTONES

Procedures Old and New – What Goes Around Comes Around

Terri C. Staples Scientific Editor

Not a single day passes that I do not read of a new "modification" of an existing procedure that is meant to make life easier in the histology laboratory. The ideas are really fantastic, and I often ask myself why I did not think of that solution. While looking through old issues of *Histo-Logic*, I came across numerous articles and helpful hints that are as "hot" today as they were when first published. Here is a short list of "new ideas" from previous editions:

Use of Zinc Chloride in Zenker Type Fixatives (Vol. VI, No. 4, October 1976):

Carolyn Barszez from Narragansett, RI, wrote of using zinc chloride as a substitute for mercuric chloride in zenker fixative. "The main advantage of zinc chloride is that it is less toxic than mercuric chloride. It also does not cause the brown to black mercury precipitated pigment artifact that is found in mercuric chloride fixed tissues... Sectioned tissues retain their brilliant staining ability because of the mordant action characteristic of Zenker type fixatives....The use of zinc chloride seems to be a reasonable step toward protecting ourselves and our environment from possible mercury poisoning."

Vacuum Processing of Small Biopsies (Vol. VII, No. 2, April 1977):

Joyce Moore of Little Rock, AR, discusses several rapid processing schedules for processing small biopsies. The schedules range from 2 to 4 hours in length, depending on the size of the specimen. "The use of a tissue processor which incorporates vacuum has made it possible for us to provide the pathologist with microscopic slides three hours after surgery."

Solutions to Amylase Problems (Vol. X, No. 3, July 1980):

Bill Barlow from Wilmington, DE, discusses the use of alpha-amylase dissolved in a buffer solution (pH 6.0). "Complete digestion of glycogen takes place in 20 minutes or less at 37°C, or preferably at room temperature in 1 hour." The procedure is much more reliable than mult diastase.

Negative Controls – Useful Ideas (Vol. XI, No. 1, January 1981):

Mack Alexander of Omaha, NE, discusses the use of brain tissue ("the head of the caudate nucleus") as a negative control for special stains since "...this tissue is less reactive to a large number of special stains." The section is placed on the same slide as the positive control, and is, therefore, stained the same way as the positive control.

Histoids – Helpful Hints for Histology Hackers

Editor's Note: The following tips were gleaned from old issues of Histo-Logic.

- > Tissue sections containing keratinized areas, such as toenails or keratinous horns, can be softened by placing the block face down in a container of permanent wave solution or depilatory cream. The epithelial tissue and connective tissue structures are not damaged by this technique, and the keratin is much easier to section. (Lewis Shapiro, New York, NY, 1977)
- > Treated slides can be prepared easily by filling a "Press-to-Seal Moistener," similar to the type used by secretaries for scaling envelopes, with the adhesive solution. The adhesive flows to the sponge and can be easily spread on a clean glass slide. (William Dotson, Chapel Hill, NC, 1981)
- > Liquid embroidery paint can be used to circle tissue sections to be stained with immunohistochemical procedures. The liquid embroidery provides a mark

for the location of the tissue, a well to keep reagents confined, and a separator that will allow multiple sections to be stained differently on a single slide. (Patricia Shook, Cleveland, OH, 1982)

- > Keep a bottle of O.C.T. in the refrigerator for frozen sections. When a frozen section is needed, the O.C.T. is already cool and will not take as long to freeze. (Barbarn Lilly, Bluefield, WV, 1987)
- > Sections that have been held in formalin for extended periods of time will lose their ability to stain properly with hematoxylin. To restore the basophilic properties of these sections, treat the slide in 0.5% Periodic Acid prior to staining with the routine H&E stain. (Annamae O'Neal, Morgantown, WV, 1989)
- > Silver deposits on the inside of plastic coplin jars can be removed by soaking the container in 5% sodium thiosulfate. Pour the thiosulfate into the coplin jar and allow it to stand overnight. (Frank Razzaboni, Wilmington, MA, 1990)

Challenge and Opportunity Confront NSH President

During 35 years in the histopathology laboratory at Baylor University Medical Center in Dallas, Freida Carson has faced a lot of challenges. But as president of the National Society for Histotechnology, she may be taking on her biggest challenge yet. Marilyn Gamble and other past NSH presidents have helped to raise the organization to a point that presents many opportunities. Now, it's up to Carson to take full advantage of those opportunities.

Fortunately, Carson has the desire and competence to do just that. In fact, she has already begun. After her first year in office, Carson's most notable accomplishment is the implementation of strategic planning to the Society.

The process began in October at the NSH Symposium/ Convention in Philadelphia. A half-day workshop on strategic planning was conducted for the Board of Directors and a method called SWOT analysis was used to initiate the process at the board meeting. The method allows the board members to take a good look at the organization's Strengths and Weaknesses, and the Opportunities and Threats that it faces.

"We began to look at the areas in which we needed to set goals," Carson said. "We put some broad goals up for consideration and then voted on which were the most important. Then we looked at objectives that would enable us to meet those goals.

"Unfortunately, you can't adequately develop objectives and strategies in a half day." Carson continued, "so we're holding another half-day workshop at the 1994 Symposium to develop it further."

One hindrance to strategic planning is the inability to meet regularly and often. "It's a long time between conventions and it's easy to lose the momentum created at the last meeting," Carson explained. "We've never had the money to have interim meetings or midyear committee meetings. It would even be nice to just have an interim board meeting."

When the strategic planning process is fully implemented at the board level, Carson plans to integrate the process within the various NSH committees. "We want to get everybody thinking and moving in the same direction over the next 5 to 10 years," she said. "We're not very far along with that process yet, but at least we're beginning."

One major goal that has already been determined is to increase NSH membership to 10,000 by the year 2000. According to Carson, they hope to reach this goal by promoting the many benefits of NSH membership and by increasing the cost differential between members and nonmembers when ordering NSH publications and services.

Carson is also working to improve the image of the NSH and the Histotechnology profession by encouraging training, certification, and licensure programs. The NSH is currently working with those states that are in the process of establishing licensure laws for healthcare laboratory technicians.

The challenge is to get Histotechnologists included in the laws. Many state legislatures exclude Histotechnologists because, typically, they are not involved in performing tests on or analyzing tissue. "Because we don't do tests or sign our name to anything, Histotechnologists are often left out of such legislation," Carson explained. "We would like to see licensure because at least it would set some uniform standards for the field. There are a lot of Histotechnologists out there who have no measure of the competency they have.

"The first step is to find out when such bills are before the various state legislatures," she continued. "Many times they are voted on without our knowledge."

The strength of existing licensure laws is another issue that the NSH is addressing. Some states require only certification to get a license. As long as a Histotechnologist is certified according to the state's certification requirements, no additional education or examination is necessary to obtain a license. In some states, all that is necessary for certification is a high school diploma.

However, many states are adopting education into their licensure laws. "We believe that the minimum education necessary for the practice of Histotechnology is an associate degree," Carson said.

Another strategy the NSH is using to encourage licensure with adequate certification is to encourage the development of more schools and training programs and to help promote the programs that already exist.

"We're also developing more educational tools ourselves," Carson explained. "All of our self-assessment books are being put onto diskettes. The first one is available now, enabling people to sit at their computer and work with self-assessment in several different formats."

Fulfilling her duties as president of the NSH often means putting in a full 8-hour day. And although Carson has taken on some significant challenges, the consensus is that she is headed in the right direction.

NSH Works to Increase Membership

Efforts to increase the membership of the National Society for Histotechnology can translate into opportunities for existing members as well as new members. Existing members can compete for a \$150 annual prize awarded to whoever signs up the most new members. Meanwhile, the new members gain all the opportunities and benefits of membership.

"Our goal is to increase membership to 10,000 by the year 2000," explained Rebecca Massey, chair of the NSH Membership Committee. That means more than doubling the current membership of 4,385 and will require a 14% increase in each of the next 6 years.

In 1993, the member recruitment contest was won by Fred Argilan of Frederick, MD. Argilan brought in eight new members from September 1, 1992 to August 31, 1993.

State societies can also earn a \$150 award by bringing in the most members from their state. The 1993 award went to the state of California.

Other efforts to increase NSH membership include a gift certificate promotion whereby members can buy gift memberships for other Histology professionals. "We're hoping that there are Histotechs out there who will want to introduce their Pathologist to the NSH, or Pathologists who will want to introduce their Techs," Massey said. This promotion will be advertised in various professional journals.

A direct mail campaign will also be initiated. The computer at the NSH headquarters in Bowie, MD, has the names of about 4,000 nonmembers who have had some past connection with the Society, either through former membership or by earning CEUs at NSH meetings.

The Membership Committee is also investigating the role of career counselors in promoting Histotechnology and the NSH. "We want to find out how to get in touch with high school and college counselors to put NSH information in their hands. We would like to get those who are interested in Histotechnology as a profession to become familiar with the Society from the very beginning."

Freida Carson, NSH president, explained that the Society is making every effort to make membership more appealing. "We've enhanced the benefits of membership," she said. "We're also working to increase the differential between members and nonmembers." An example of that differential is that all of the literature, including the popular self-assessment series, will be offered to members either free or at a substantial discount.

Massey believes that, for most Histotechnologists, the annual NSH dues will be more than paid for by the savings they will receive on NSH materials and services. "Anyone going to our national Symposium/ Convention will save enough on reduced workshop rates to pay for their membership," she said.

Carson and Massey agree that when Histotechnologists realize the many member benefits, the value of NSH membership will be very obvious. Those benefits include:

- A free subscription to the Journal of Histotechnology
- A free subscription to NSH in Action
- · A free subscription to Advance
- Reduced registration fees at the national NSH Symposium/Convention
- Access to many free NSH publications
- Access to the popular self-assessment series and other publications at reduced prices
- A record of CEUs earned
- A voice in the goals and policies of the NSH.
- Eligibility for NSH awards and scholarships
- · An opportunity to participate in teleconferences
- An opportunity to apply for an NSH VISA card and loans
- Access to low-cost malpractice insurance
- Access to long-term nursing and home healthcare insurance
- · Access to the control tissue network
- Representation on the American Society of Clinical Pathologists Board of Registry, the National Accrediting Agency for Clinical Laboratory Sciences, the National Commission for Clinical Laboratory Standards, and the Biological Stain Commission

For more information about NSH membership, call the NSH National Office at 301-262-6221.

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Magelay Rojas Helps Advance the State of Histotechnology in Egypt

Imagine yourself in an unfamiliar country, 5500 miles from home, working with people who don't speak or understand English. That is exactly the position in which Magelay Rojas, HT, HTL, found herself when she traveled to Egypt to help set up a histology laboratory at Alexandria University.

Rojas is technical manager of surgical pathology at George Washington University in Washington, DC. In 1992, she met Nabila Metwali, MD, a Pathologist from the University of Alexandria who was doing a fellowship at George Washington University. Metwali was very impressed with the quality of the sections that were processed in the laboratory. "We helped her do some immuno studies and got her involved in some of the work we were doing here," Rojas recalled.

When Metwali went back to Alexandria, Egypt, she applied for a grant with US Aid to set up a new laboratory at the University of Alexandria. She also requested technical assistance and specifically asked that Rojas be the one to provide it.

The company that eventually was awarded the contract for the laboratory contacted Rojas and included her CV in their proposal. When the proposal was approved, she began to play a significant role in the project.

Her first task was to make recommendations about what equipment and supplies to buy. After that was determined, the orders were placed and Rojas waited for the equipment and supplies to be delivered to Alexandria. By September 1992, the equipment had passed customs and was waiting for her in Alexandria. When she arrived at the laboratory, the real work began.

During the month she was in Alexandria, Rojas stayed in a hotel across the street from the university. There was a lot of curiosity among local residents about why a woman was staying in a hotel alone, a very unusual situation in a country where women don't have the same status as men. Rojas was totally involved in setting up every aspect of the laboratory. She talked with plumbers and electricians to make sure they were putting in the appropriate connections. She set up many of the instruments herself. But Rojas wasn't familiar with all of the equipment, so she spent a lot of time reading installation manuals.

One of her most important tasks was to teach the local Histotechnologists how to use the equipment. The problem was that none of them spoke or understood English. "The doctors spoke English, but the techs did not," Rojas explained. "I had to use my hands and show them with gestures and actions," she continued. But whenever she wanted to communicate something that she could not demonstrate, she had to call one of the doctors. Eventually, however, Rojas learned a few words that were key to the functioning of the laboratory.

In trying to train the local Histotechnologists, Rojas was challenged in many other ways as well. "There was such a lack of basic training of laboratory practices that I was really amazed," she said. The instruments they were using were very old and poorly maintained. "But they were doing miracles with the kind of instruments they had," Rojas explained, "They were cutting very thick sections and were placing huge pleces of tissue on the slides because they were using the old L-bars to make the blocks."

Rojas was surprised to find that most of their chemicals were in unlabeled bottles. "One day I asked for some hydrochloric acid," she said. "They brought me a bottle without a label, and it looked just like the bottle they kept alcohol in. When I asked how they knew that it was hydrochloric acid, they said they just smelled it."

In Egypt, there are few government regulations concerning laboratory health and safety. Rojas even had to convince the local Histotechnologists that they should not smoke in the laboratory because of the fire hazard. "There were eigarette stains on the microtome," she said.

The laboratory in Alexandria processed tissue for the private sector as well as for the university. "Pathologists are all in private practice," Rojas said. "Doctors refer specimens and Pathologists process them in their own facilities up to the xylene, and then take them to the university lab for the Techs to impregnate with paraffin and cut." As the new instruments were installed, Rojas tried to talk them into throwing out their old equipment. She was concerned that their unfamiliarity with the new equipment would cause them to revert back to their old ways. She wasn't successful, however; they kept the old equipment.

The training process involved orienting residents and doctors. "I talked with residents to give them guidelines on how to send pieces of tissue for processing in the VLP.TM tissue processor," she said. "I showed them how to use cassettes and told them not to send specimens that were too big. I tried to give them specific rules to follow to ensure tissue would be processed properly." She also taught the residents and doctors how to use the Miles Tissue-Tek® Microtome/ Cryostat.

When the laboratory was fully operational, the Pathologists were very pleased with the results. "They were very, very happy with the sections because they could see things that they had never seen before," Rojas said.

Although the Histotechnologists were very skilled with their obsolete equipment, Rojas had to teach them as though they were new to the field. "I showed them how to set up portable fume hoods," she explained, "how to use forceps, and how to clean slide covers without touching them. I showed them how to put all the slides on the rack and follow it through. Before, they were holding the slides in the bottles with their fingers. I showed them how to stain sections without putting their hands in xylene.

"There was one lady who was doing special stains and her fingers were exposed to all the stains and chemicals. She developed such a rash that she had to see a doctor."

Rojas is from Chile and has a medical technology degree from the University of Chile in Santiago. "I look very Mediterranean," she said. "That helped in some ways, but many people addressed me in Arabic because they thought I was Egyptian."

When her work was complete, Rojas took advantage of the opportunity to tour Egypt. On a tour set up by a travel agency, she traveled to Aswan, Luxor, Abu Sunbul, and other cities along the Nile River. Someday, she would like to return to Egypt to see how the new laboratory is progressing. "I have stayed in contact with Dr. Metwali," Rojas said, "but I worry about the Techs and helpers at the lab. There really wasn't enough time to teach them everything they should know. I would like to spend more time with them because they have so much potential. They are very talented people."

If Rojas does return, one thing is certain — she'll nolonger be a stranger.

Correction

In the December 1993 Issue of *Histo-Logic*, Vol. XXIII, No. 4, there was an incorrect formula for the preparation of Ammoniacal Silver in the article entitled "Combination Iron, Collagen, and Reticulin Stain for Liver Biopsies." The formula in the article listed 20.0 mL of Sodium Hydroxide solution instead of 2.0 mL (20 drops) to be added to the Silver Nitrate. The correct formula for the solution is:

Ammoniacal Silver Solution

Add 2.0 mL (20 drops) sodium hydroxide solution to 20.0 mL of 5% silver nitrate solution. Then, add ammonium hydroxide, drop by drop, until only a few granules of the resulting precipitate remain on the bottom of the cylinder. Add distilled water to make a total of 50.0 mL of solution and use at once.

NOTE: Ammonium hydroxide must be fresh.

Our apologies to the author and our readers for this transcription error.



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Calendar of Events for Histotechnologists

April	
7-9	 North Carolina Society of Histopathology Technologists
9	 Zoo Pathology – Connecticut Society of Histotechnology
9-14	 American Society of Clinical Pathologists/ College of American Pathologists Annual Spring Meeting
13-15	-Illinois Society of Histotechnologists Annual Symposium
15-18	-NSH Region 6 & Texas Society for Histotechnology
20-22	 Indiana and Illinois Societies for Clinical Laboratory Science
22-23	 New York State Histotechnological Society Annual Meeting & Seminar
28-30	- Wisconsin Histotechnology Society Symposium
28-30	 Minnesota Society for Histotechnology and NSH Region 5 Conference
28-30	- NSH Region 2 Meeting
	- Florida Society for Histotechnology
29-30	- NSH Region 8 Meeting
May	
5-7	 Georgia Society of Histotechnology 22nd Annual Symposium
10	- Maryland Society of Histotechnologists
11-15	 California Society for Histotechnology Annual Convention/Symposium
14-15	- Mississippi Society for Histotechnology
18-20	 Hawaii Society for Clinical Laboratory Science
19-21	- Michigan Society of Histotechnologists
20-21	 Missouri Society for Histotechnology 17th Annual Symposium
20-22	 Colorado Society of Histotechnology State Meeting
23-27	- American Society of Microbiology General Meeting

June

- 2-4 - NSH Region 1 Symposium
- 3-4 - Louisiana Society for Histotechnology Symposium
- 3-4 - Ohio Histology Society
- -NSH Region 7 & Arizona Society of 9-12 Histotechnology
- 17-23 - American Society of Clinical Laboratory Sciences Annual Meeting and Exposition

July

17-21 American Society of Clinical Laboratory Scientists

August

September

- Maryland Society of Histotechnologists 13
- 15-17 - Indiana Society for Histotechnology State Meeting
- 19-23 20th Annual Education Conference of the American Association of Pathologists' Assistants (AAPA)

October

1-7 - NSH Symposium/Convention

22-28 - American Society of Clinical Pathologists/ College of American Pathologists Annual Fall Meeting

November

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- Connecticut Society of Histotechnology
- Maryland Society of Histotechnology General Meeting
- 13 - Mississippi Society of Histotechnology

For additional information, please contact the NSH National Office at 301-262-6221.

⁸⁻¹³ -International Society for Clinical Laboratory Technology Annual Convention

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