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Histologic Techniques for Analysis of Bone Implants (A Historic and Artistic Perspective)

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"The artistic representation of history is a more scientific and serious pursuit than the exact writing of history." Perhaps Aristotle's quote can be interpreted as a bit of sarcasm, but taken at face value it reminds us of the importance of history. History serves to enrich our lives and to educate us about the mistakes of the past, so that we may avoid making the same errors. To a bone histotechnologist, whose duties at times can become repetitive, the concept of history may seem somewhat irrelevant to daily life. However, everything has a history, except maybe history itself. The same holds true for the history of bone histology, bone-tissue preparation, and bone-implant analysis.

It may seem ironic, but Aristotle can also be considered somewhat of a bone histologist himself. Of course, he was not "blessed" with the opportunity to look through a microscope, but he did ponder the question, What makes up bone? As described in one of his early writings, *De generatione animalium (On the Generation of Animals)*, Aristotle viewed bone as a "seminal residue." He believed nature extracted only the purest of materials to produce flesh (muscle), and the rest, or this so-called "residue," went into the construction of bone, sinews (tendons), nails, and hair. Needless to say, the idea of bone being created from unclean residue is not a comforting thought, not even for the bravest of histotechs, but we'll have to find it in our hearts to forgive Aristotle. Let's face it, he never knew Mr. Zeiss!

Most of the understanding concerning the true nature of bone remained a mystery or at least speculation until the early 17th century when one of Mr. Zeiss' predecessors, Antony Van Leeuwenhoek, came on the

scene. Others may claim to be the first to look at bone through a microscope; historians, however, credit Leeuwenhoek with this achievement in the mid-1600s. Leeuwenhoek's first observations of mineralized tissue came from his examination of teeth, actually the dentine of teeth, in which he observed "tiny globules." Since popular thinking of the time suggested that teeth were nothing more than bone covered with ivory, when Leeuwenhoek first observed bone under this microscope he expected to see these same globules. But this was not to be the case. His initial examinations of bone revealed small canals, which prompted him to write, "I conceived

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Bones to be constituted of globules: but finding my mistake, I retracted that opinion; for what I took for globules was the tops of tubes or cylinders whereof bone is composed..." This observation gave Leeuwenhoek the honor of being the first to describe bone as a tissue and the first to examine structures that we now know as Haversian canals.

Although Clapton Havers, an English physician for whom the Haversian canals were named, was not the first to observe these histologic structures, he had some rather unique interpretations for their function in skull bones. He published his findings in 1691 in his *Osteologia Nova (Some New Observations on Bone)*, in which he wrote the following: "As it was requisite that the Upper Region of our Bodies, where the Soul... is seated, should be clear, free from Clouds, and Vapors, so these Pores seem to be formed as one way to expel, and scatter those moist, and misty Particles, which may be apt to eclipse, or weaken the influences of the Principle which animates us. I do therefore conceive that they are Perspiracula by which the offensive Vapors, which arise, and gather within the Cranium do perspire." This could easily be the true interpretation behind the old phrase, "just blowing off steam." Whatever the case, Havers' ideas about the nature of his Haversian canals in the skull were false.

Dr. Havers had also established ideas about the nature of these canals in long bones. He believed that they functioned for the transport of medullary oil up and down the bone, which served to "mollify" (soften) the bone. Monro, who was one of Havers' contemporaries and was influenced by Havers' thinking, took this idea one step further. He stated that these canals served for transportation of medullary oil to joints for the purpose of lubrication. Both Havers' and Monro's interpretations were incorrect. But it would take almost two centuries and the observations of numerous other investigations to define the purpose of Haversian canals.

Winslow (1734) was the first to see blood vessels in the center of these canals. More than a hundred years later, Todd and Bowman (1845) described them as concentric systems—"Haversian systems." Tomes and Demorgan (1853) were the first to recognize that these systems served as a replacement unit for the turnover of bone. Von Ebner (1875) defined the concept of "cement lines" as an isolating region between separate systems, and not until 1914 was "osteone" used by Biedermann to replace the term "Haversian system."

While the debate about the function of Haversian canals was taking place, these early investigators were still trying to answer the question: What makes up bone? Monro suggested that bone was made from "phlegm, spirit, volatile salt, fetid oil, and black caput mortuum, the proportions of which vary according to age." Let's just be glad, as histotechs, that Monro was wrong. The idea of working with phlegm on a daily basis is just not that appealing!

Malpighi, whose works were published in 1743 after his death, is the first person credited with recognizing that bone has an organic matrix composed of animal matter. He described bone as being composed of fibers and filaments, filled with an intermediate "osseous juice." Although his idea about fibers was correct, the nature of this osseous juice was not defined until the work of Herissant in 1758. Using "flame and acid," Herissant showed bone to be composed of "animal" and "saline." Shortly thereafter, Gahn discovered that this "salt" of bones was a combination of phosphoric acid and lime (calcium oxide). This observation was close to the truth, although the term "hydroxyapatite" would not be used for nearly another century.

The stain technology that revealed so much to the early histologist seemed to develop hand-in-hand with some of the early observations of bone. The root of the madder plant, which grew in Europe and Asia, was known for its staining qualities as far back as the time of the early Greeks and Romans. The first staining of bone was reported by Mizaldus in 1566, but the use of madder as a stain for bone was forgotten for nearly two centuries until Belchier (1736) rediscovered its usefulness. Soon after, Duhamel (1739) observed that madder colored only the active, growing parts of bone, and concluded that much of the circumferential growth in bones occurred because of the osteogenic periosteum layer on the outside of bones. In fact, he coined the term "cambium" layer for the periosteum because of its similarities to the bark of a tree and the way that a tree grew in girth.

Surprisingly enough, Duhamel can also be considered the father of implant analysis in bone. In his quest to confirm early experiments that showed long bones grew at their ends, Duhamel placed silver rings around the shafts of growing long bones. He was puzzled by the fact that as the bones grew, these silver rings became embedded in the shaft of the bone and sometimes ended up in the marrow cavity itself. Although Duhamel was

not aware of the principle of endosteal resorption, we in this modern age must be sympathetic to Duhamel's confusion. Some of the results we now see in present-day bone-implant analysis are nearly as puzzling to us as Duhamel's rings were to him.

Obviously many scientific advances have been applied to the understanding of bone and bone-implant systems since the time of Duhamel. Advances in light microscopy and inventions such as the electron microscope in the early 1930s have provided us with a bird's-eye view of the transitions and transformations occurring in and around bone. To help educate our fellow histotechnologists and spread the know-how around, we would like to add to the "History" of bone and bone-implant tissue preparation by describing some of the techniques used in our laboratory. This we offer as a gift to our colleagues, with the hope that they may avoid some of the mistakes that plagued our lab in the early stages of its development.

Many of the accessioning principles established over the past 12 years of clinical laboratory experience are fundamental to research laboratory operations. We have a variety of species to accession, and therefore use an accession code that alphabetically distinguishes species as well as numerical sequences of our specimens. For example, P236-89L distinguishes that the species was a primate.

Unlike a clinical lab where the technician needs only to be concerned with locating blocks, slides, and gross tissue specimens, a research lab such as ours uses an extensive filing system to store all clinical and research materials relating to the specimen. We must also be able to track both photographic and radiographic material, including 35-mm slides, x-rays, microradiographs, backscattered electron micrographs and film negatives. Ground section slides, paraffin blocks and slides, polymethyl methacrylate (PMMA), glycol methacrylate (GMA), and Spurr block are also catalogued. The accession number makes it easy to locate any of these items within the information system.

Once the specimen is accessioned, the next important consideration is tissue fixation. Fixation is the first critical step for bone-implant specimens. Most of our specimens are fixed in 70% ethanol. When rapid fixation is required, 10% neutral buffered formalin (NBF) is ideal. If our specimens are fluorochrome labeled, the bone can remain in 10% NBF for no more than 48 hours. If labeled bones are left in 10% NBF for longer than 48 hours, labels may be lost.

A research tech, being involved in many aspects of a research facility, must be versatile—"a jack of all trades." One responsibility of a tech may be photographing all implant specimens that are submitted to the laboratory for analysis. In our lab, 35-mm slide film is used to document the specimen. Histotechs must also be detectives when photographing specimens and looking for unusual markings or wear patterns on an implant. This may include burnishment on the metal portion of the component and noting loose beads from the porous coating of the implant that may have been dislodged and embedded in the plastic components. Gross photography is an effective way to document these events in implants.

The next step in analyzing the bone-implant specimen is contact x-rays. Research labs usually use a cabinet x-ray machine such as the Faxitron.[®] Contact radiograph and gross observation are used to determine whether bone is in apposition with the porous coating of the implant or if there is the presence of a thick fibrous tissue layer that is radiolucent. Fibrous tissue against the porous coating indicated that the implant was not well attached to the skeleton. Since we are primarily concerned with mineralized tissue ingrowth in our laboratory, specimens with fibrous tissue surrounding the porous coating are not processed any further.

Bone-implant specimens can be dehydrated and cleared manually or on an automatic processor. We processed our large bone specimens manually for about a year



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before purchasing an automatic processor. With the purchase of a VIP 2000, a two-week manual process can now be done in 26 hours. The longest program written for our processor is 72 hours, which is for dehydration and clearing of human proximal femora with implants *in situ*.

If your lab is as busy as ours, a schedule board is a necessity. Last year we had nine PMMA schedules running at the same time. What a nightmare trying to manage manual dehydration, clearing and infiltration of specimens with PMMA. Not only was the schedule board full, but there were paper towels hanging on the wall with schedules written on them as well!

Infiltrating specimens with plastics in our lab is a manual process. Our experience to date has shown that there are no dependable automatic plastic processors for PMMA available for large specimens containing implants. We accomplish vacuum infiltration by drilling a hole in the side of a refrigerator and placing a vacuum desiccator inside, making vacuum infiltration at 40°C possible. This low temperature is necessary to prevent polymerization of PMMA curing infiltration of specimens.

Infiltration rates vary with the size and shape of the specimen. Iliac crest biopsies can be quickly infiltrated with PMMA and embedded on the third day. At the other extreme, a proximal third of a human femur with the femoral component *in situ* can take up to 22 days to infiltrate. There are many protocols in the literature to guide the technician with processing schedules; however, embedding techniques are much like cooking. Just as chefs with many years of experience know how long it takes to prepare a fine meal, the experienced histotechnologist with years of practical training will know how long it takes to process small or large specimens.

Once the specimen is embedded and polymerized, it is removed from the mold and trimmed of excess methacrylate on a bandsaw. In order to cut a specimen that contains a metal implant, a special saw is required. Our lab uses a customized high-speed saw and a cubic boron nitride (CBN) cut-off blade. Depending on the size of the implant and metal type, cutting time may vary from 20 minutes to 1½ hrs.

When sectioning an implant such as a tibial component, "serial" sections 2-3-mm thick are cut on a high-speed water-cooler saw. The cut specimen block is removed

from the saw and subsequently is ground and polished on one face to an optical finish using a variable-speed grinder. This polished face is then again removed as a 2-3-mm wafer before grinding and polishing the next serial section. In this way, specimen block is used to grip the specimen while grinding and polishing. The objective to have in mind when learning to grind and polish specimens is that you are trying to create a flat surface. Although histotechnologists are multifaceted people, the attempts they make at removing facets from a specimen block should not result in still other facets. It is said that histology is an art as well as a science; the same holds true for the art of grinding.

On the other hand, when sectioning a femoral hip component for light microscopy, we have found it easier to take 2 mm serial sections along the implant stem and then grind and polish these sections to an optical finish. It is virtually impossible to hold a specimen that is 2 mm in thickness while grinding on the variable-speed grinder. But how do you avoid grinding away your fingertips while preparing the specimen? One of our medical students, Dave Rhodes, came up with an ingenious idea after sacrificing his fingertips to the grinding wheel. He placed a latex glove on his bandaged stump of a hand and sprayed 3M 77 spray adhesive on the fingertips of the glove. He then let it dry for about 20 seconds and pressed the specimen to the gloved fingertips. This gave him full control over the specimen and, subsequently, his fingertips have grown back.

From this point on, specimens may be prepared for Backscattered Electron Imaging (BSE) analysis or sent directly on for further grinding and polishing necessary for light microscopy. BSE provides more detailed and refined analysis of the bone-implant interface. It also

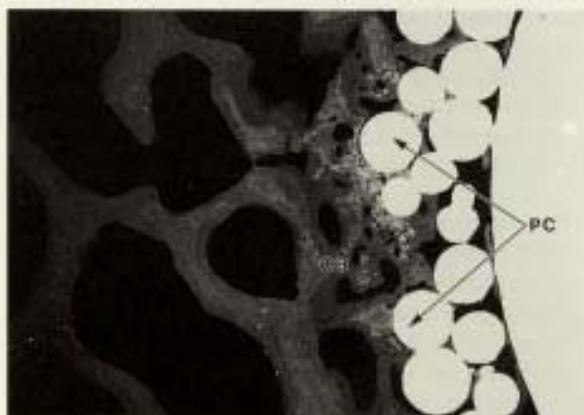


Figure 1: BSE photomicrograph demonstrating porous-coated (PC) implant with cancellous bone (CB) ingrowth.

Under light microscopy, the cell detail we observed was almost identical to that of fluorescence microscopy, except that we were unable to reproduce the "light to prominent jade green" stain of the bone matrix reported by Villanueva (Fig. 5). This does not pose a problem, because the new information that we've been able to observe with the MIBS stain has been a tremendous asset to our laboratory.

As you can imagine, it is a winding road that the research histotechnician must travel. But armed with the knowledge of history, the inspiration of art, and the patience of Job, the histotech can enjoy the fascinating journey to discovering the reason for implant success and failure in bones. We invite fellow histotechnicians to explore the opportunities offered by areas outside the realm of clinical histology. Aside from the field of orthopedic research, these may include veterinary, marine, herpetologic, avian, botanical, and dental areas of research. Our experiences have proven to be very rewarding and we hope fellow histotechs will test their adventurous natures as well.

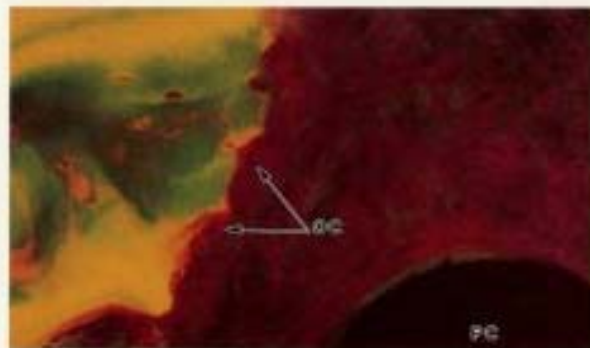


Figure 4: Osteoclasts (OC) in resorption pits on cancellous bone. Porous coating (PC). $\times 400$.

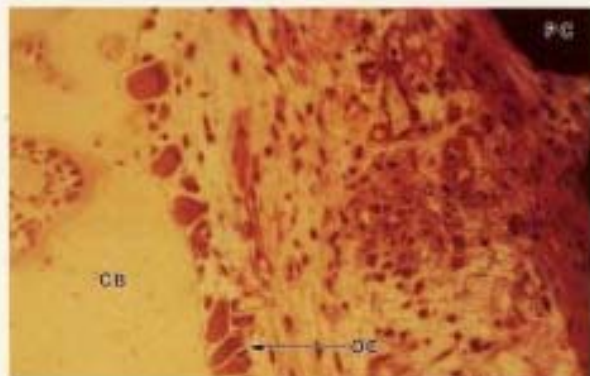


Figure 5: Light microscopy using Villanueva (MIBS) stain. Note the pale appearance of the cancellous bone (CB) and obvious layer of osteoclasts (OC). Porous coating (PC). $\times 400$.

Acknowledgments

This paper has been supported by the Department of Veterans Affairs Medical Center, Salt Lake City, Utah. We also acknowledge Dr. Roy Bloebaum, Ph.D., and Todd Boyce for their assistance in editing.

Swiss Speaker Exchange Program Marks Second Year

Brent Riley
Managing Editor

When Leonard Noble, HT, HTL, arrived in Geneva, Switzerland, with his wife and two children, there was nobody there to meet them. It wasn't that the Swiss Society for Histotechnology was inhospitable, but a communications mix-up caused the Swiss society welcoming committee to be at the airport at the wrong time.

Fortunately, Noble speaks "just enough French to get by." He and his family made their way to the railway station, which was at the airport, and boarded a train for Neuchâtel where their hotel room was waiting.

A welcome banquet the following evening more than made up for the missed greeting at the airport. Dinner was served on an old lake steamer on Lake Neuchâtel. During the banquet, the president of the Swiss society warmly greeted all the guests in three languages.

Noble was in Switzerland to represent the National Society for Histotechnology in a speaker exchange program sponsored by Miles Inc. This is the second year for the program.

Noble, of Winston-Salem, North Carolina, is the vice president of the NSH. A histotechnologist for 21 years, he was chosen by the NSH board of directors to speak at the national symposium of the Swiss society. He decided to spend an extra week in Switzerland to vacation with his family.

The meeting was held April 27 at the city university in Neuchâtel. Noble was the first speaker at the 1-day meeting. "I can look at it two ways," Noble said, "you get it over with, or you ruin the whole meeting for everybody."

But Noble was a big hit. "The only person I put to sleep was my 6-year-old daughter," he said. Speaking on "The Correlation of Histological, Histochemical, and Electron Microscopical Procedures in the Diagnosis of Disease," Noble discussed three case histories concerning three known diseases, rhabdomyosarcoma of the uterus, malignant melanoma of the breast, and membranous glomerulopathy. He showed how the different techniques used in a main histology lab correlate with the electron microscopy lab and histochemical functions such as enzyme histochemistry and immuno histochemistry.

He chose that topic because the Swiss society had requested that he discuss "what you do to make your diagnosis." "Many of the Swiss society members are in research, working for large pharmaceutical companies," Noble explained. "So I left it up to them, and this is what they chose."

"My talk could have been as long or as short as I needed," he continued. "The hard part was determining how long it would take for me to make a statement and them to translate." He ended up talking for about one hour, although at least 20 minutes of that time was spent waiting for the translations.

According to Noble, the entire meeting was devoted to lectures. All the lectures were translated into three languages—English, French, and German. The audience wore headsets so they could tune into whatever language they understood.

"It was very different," Noble said. "I usually talk very fast, but they asked me to slow down so they could translate. I tried to enunciate very carefully. I was asked to watch for the signals from the translators, but there were no signals. I was, however, able to watch their mouths, and when they quit talking, I started talking again."

At first, Noble was disappointed in the response to his talk. "There weren't a lot of questions immediately," he recalled. "But after the break, I was bombarded with questions. I guess they were afraid that it would be hard

to translate questions during the meeting. So whoever wanted to ask me a question would find someone who could speak English, then walk up to me and ask." Noble estimates attendance at about 150 people. There were also 20 exhibitors. "It was very well organized," he observed.

"Histology procedures are pretty much universal," Noble remarked. "They may use some different reagents than we do, but their techniques are the same. One thing they did find fascinating was that everything—the routine histology, the immuno, the enzyme histochemistry—could all be done under one roof."

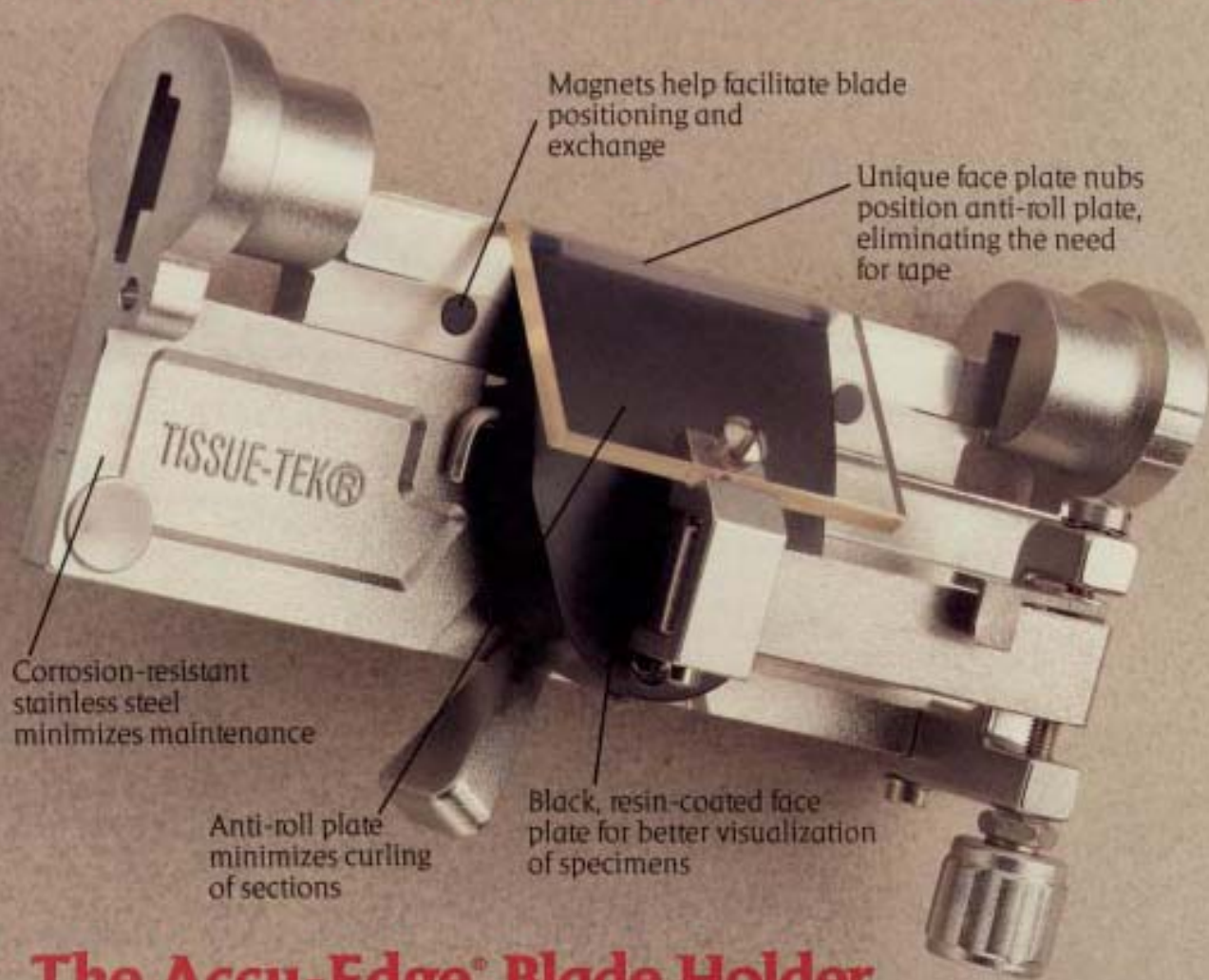
Three other histotechnologists from the NSH also attended the meeting. They were Donna Simmons, Regina Hermann, and Ben Schelkowski, all from California. They did not officially represent the NSH but were part of a private tour group that happened to be in the area at the time. "They provided moral support," Noble said.

The Swiss society will send two representatives to the 1990 NSH Symposium/Convention in San Antonio. They are Bert Jaspers, president of the Swiss society, and Salome Kiefer-Raez. Jaspers will speak on "Practical and Technical Comments in the PAS Reaction and Worthwhile Information Concerning Hematoxylin." Kiefer-Raez will speak on "The Preparation of Undecalcified Bone for Histologic Evaluation." Their trip will be sponsored by Bayer AG (Miles).

"We were able to interact and talk a lot with the histotechs who are coming here in September," Noble said. "As this will be their first trip to the United States, we told them what to expect. I even took over one of our programs so they could duplicate it and send it out to all their members. We'd like to have even more come over. I think they'd get a kick out of seeing what a really big convention is like."

"Our whole trip was very nice," Noble said. "Everyone was very hospitable. It was more like a big family reunion. You wouldn't think it was a big national meeting. Everyone knew everyone else. But it was a very serious meeting," he concluded. "They were there to learn and to discuss techniques."

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Modified Kluver-Barrera Method for Myelin and Nerve Cells

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This modification was developed to shorten staining time and to have consistent staining on serial sections while batch staining. Microwave techniques we used did not give our lab consistent staining, making it necessary to decrease the number of slides processed at one time. With the standard method of placing the slides in Luxol Fast Blue in a 56°-60°C oven overnight, some of our serial sections fell off the slides. This modification provides a shorter staining time with consistent staining.

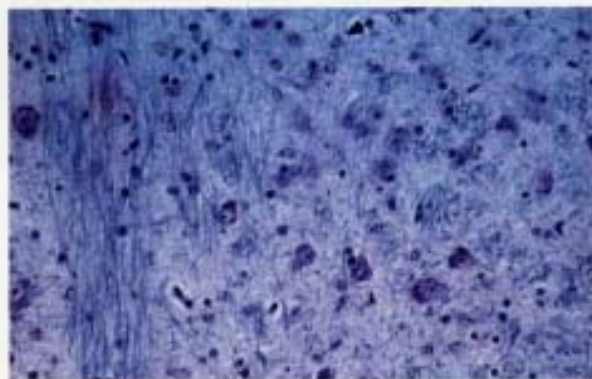


Figure 1: AFIP, Kluver-Barrera Method for staining myelin and nerve cells.

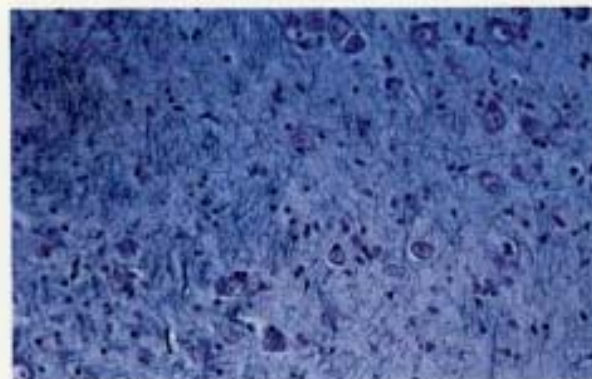


Figure 2: Modification of Kluver-Barrera Method for staining myelin and nerve cells.

Fixation 10% Neutral Buffered Formalin.
Process Paraffin embedded specimens.
Micrometry Section cut at 10 micrometers.

Solutions

0.1% Luxol Fast Blue

Luxol fast blue, MBS 0.1 gm
95% alcohol 100.0 mL
Dissolve dye in alcohol. Add 0.5 mL of 10% glacial acetic acid to each mL. Solution is stable.

1% Cresyl Violet Acetate

Cresyl violet acetate 0.1 gm
Distilled water 100.0 mL
Just before using, add 15 drops of 10% glacial acetic acid and stir. Allow solution to sit a few minutes after stirring before using.

0.05% Lithium Carbonate

Lithium carbonate 0.05 gm
Distilled water 100.0 mL

70% Alcohol

100% alcohol 70.0 mL
Distilled water 30.0 mL

Staining Procedures

1. Preheat Luxol fast blue in 60°C oven until the Luxol fast blue reaches 60°C.
2. Deparaffinize and hydrate slides to 95% alcohol.
3. Place slides in preheated Luxol fast blue for 3 hours in 60°C oven.
4. Rinse in 95% alcohol to remove excess stains.
5. Rinse in distilled water.
6. Begin differentiation by a quick dip in lithium carbonate.
7. Continue differentiation in 70% alcohol until gray and white matter can be distinguished. (This is to be done very quickly and watched carefully so that you do not overdifferentiate.)
8. Rinse thoroughly in distilled water.
9. Cresyl violet acetate solution for 1 minute.
10. Differentiate in several changes of 95% alcohol with quick dips. (Be careful not to wash the cresyl violet acetate completely out of sections.)
11. Dehydrate in absolute and clear in xylene, two changes each.
12. Mount coverglass with resinous media.

Results

Myelin blue
Cell products pink to violet

(continued on page 178)

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Carcinoma

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A carcinoma is a malignant tumor of epithelial origin. A light microscopic criterion of carcinoma is invasion of malignant epithelial cells into the surrounding stroma. In the process, they destroy normal tissue and may give rise to hemorrhage, necrosis, fibrous reaction and loss of function of normal tissue. These tumors vary widely in rate of growth, degree of anaplasia (differentiation), and gross and microscopic appearance. Some resemble their tissue of origin very closely (well differentiated), while others may be so undifferentiated (anaplastic) that they resemble embryonic tissue. In the latter type of tumor, even its epithelial nature may be in question. Carcinomas usually spread by metastasis (the spreading of the tumor to distant sites, away from its primary site of localization). In contrast, a nonmalignant (benign) tumor is generally well circumscribed, with no peglike invasions, and does not spread but continues growing and destroys only surrounding tissue by virtue of displacement. Carcinoma-in-situ (intraepithelial carcinoma) is the state of malignancy just before it has spread

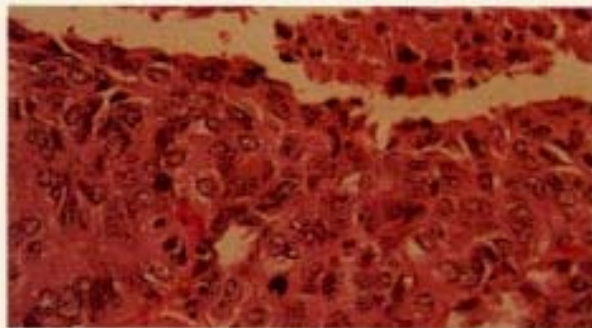


Figure 1: Squamous cell carcinoma-in-situ from bronchial mucosa. Cells with marked atypia, abnormal configuration, crowding, and mitotic activity are evident. Invasion into the adjacent stroma has not yet occurred. H&E \times 250.

into the stroma; the cells are exhibiting malignant features, just ready to invade the surrounding tissue but not yet having done so (Fig. 1). Histologically, malignant cells exhibit inadequate maturation; the degree of anaplasia correlates directly with the degree of malignancy (or rate of growth). Other features of malignant cells are pleomorphism of cells and nuclei (variation in size and shape), enlarged nuclei with increased nuclear-cytoplasmic ratio, clumping of chromatin, and hyperchromatic nuclei with prominent nucleoli. Mitotic figures are more numerous. The two most common forms of carcinoma are the squamous cell and the adenocarcinoma.

The well-differentiated adenocarcinomatous cells tend to be arranged in a glandular fashion, often producing mucin (Fig. 2). The well-differentiated squamous cell

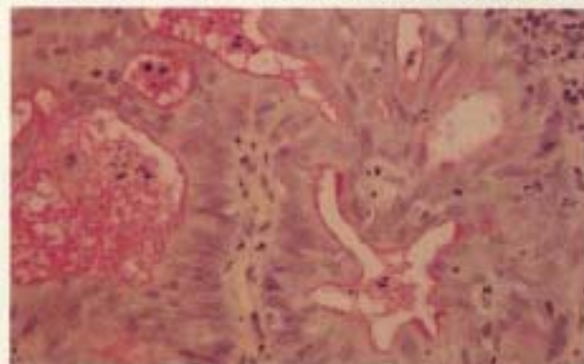


Figure 2: Well-differentiated adenocarcinoma from endometrial curettings. Note the glandular configuration, mucinous deposits, and malignant features. Mucicarmine stain. \times 250.

carcinoma, on the other hand, may present keratinization with keratin "pearl" formation (a swirling configuration of keratin mixed with malignant squamous

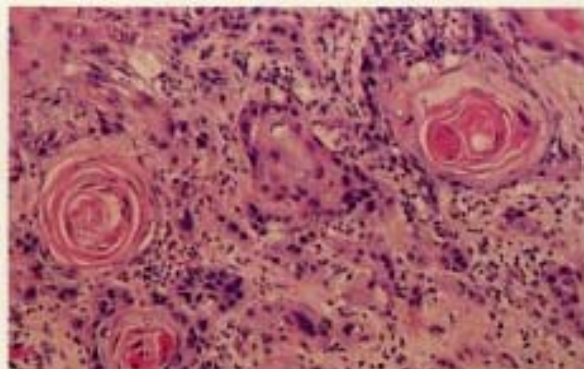


Figure 3: Well-differentiated squamous cell carcinoma from tongue mass. Note the keratinization with keratin "pearl" formation, the sheetlike arrangement, and malignant features of neoplastic cells. H&E \times 100.

epithelial cells) (Fig. 3). The cells in this type of tumor are generally arranged in a sheetlike fashion. Very fine cytoplasmic bridges between individual cells are seen under high power. They are especially well demonstrated in glycol-methacrylate sections.

Mucin deposits are usually prominent in a well-differentiated adenocarcinoma and are more discrete in anaplastic forms. The sensitivity of the mucin stain depends on the amount of mucin present, and the specificity depends on the site of origin. Although mucins are heterogenous compounds and differ chemically depending on the cells from which they are derived, many react similarly to the various mucin stains. Mucins are usually PAS-positive, metachromatic, and basophilic. The mucicarmine stain usually reacts with mucin from epithelial origin. Mucins with acid groups also stain with alcian blue and colloidal iron methods. Not only are these stains useful in determining the adenocarcinomatous nature of the tumor (as opposed to squamous cell or other carcinomatous types), they can also be useful in determining the site of a primary tumor since finding mucin-positive tumor cells in a tumor from a part of the body that lacks mucin-positive cells would indicate that this is a metastatic lesion, not the primary tumor. The Kreyberg technique combines staining for keratin (prominent in squamous cell carcinoma) and mucin (in adenocarcinoma).

Immunohistochemistry

It is frequently necessary for the pathologist to determine whether an anaplastic tumor is or is not of epithelial origin. A broad range of polyclonal and monoclonal antibodies have been reported to demonstrate for individual or combinations of antigens for the 19 or more keratin determinants known. Keratin demonstration by immunohistochemistry is best accomplished by utilization of a polyclonal pan keratin "soup" as well as epithelial membrane antigen isolated from human milk fat globule membranes. Positive keratin with one or both of these antibodies indicates the epithelial nature of the lesion, especially if leukocyte common antigen is negative. Optimal detection of keratin in formalin-fixed paraffin section requires trypsinization prior to staining. It is now possible to subclassify specific groups of carcinomas, distinguish adenocarcinoma from squamous cell carcinoma, and distinguish gastrointestinal adenocarcinomas from urogenital, respiratory, or mammary tumors by utilization of specific keratin antibodies.

Electron microscopy

Ultrastructural analysis may be necessary to ascertain the epithelial lineage of anaplastic tumors. In most

instances, the presence of well-defined desmosomes at the cell membrane of adjoining tumor cells reveals the probable epithelial nature of the lesion (Fig. 4). However, desmosomes are frequently atypical in form and reduced in number in malignancies. A squamous cell carcinoma will show bands of cytoplasmic tonofibrils (Fig. 5). On the other hand, adenocarcinomas will frequently contain cytoplasmic mucinous vacuoles and intercellular pseudopodia in addition to desmosomes (Fig. 6).



Figure 4: Electron micrograph of a portion of normal human epidermis showing multiple well-developed desmosomes at sites of intercellular bridging. $\times 16,000$.



Figure 5: Electron micrograph of a portion of a tumor cell from a thymoma showing a paranuclear bundle of tonofibrils. $\times 20,000$.

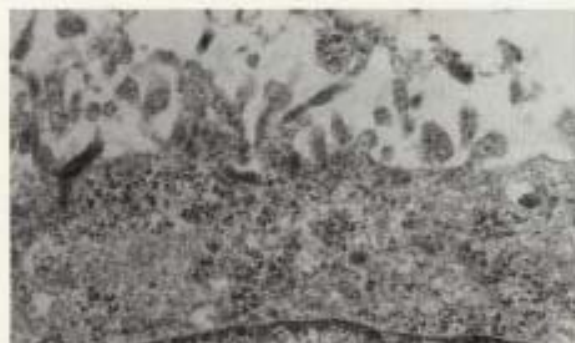


Figure 6: Electron micrograph of a portion of an adenocarcinoma of the lung showing the apical part of a tumor cell to contain numerous pseudopodia projecting into the lumen between two cells. $\times 32,000$.

NSH Prepares to Invade San Antonio

Brent Riley
Managing Editor

September will bring one of the most anticipated events of the year for the hundreds of histotechnologists who will converge on San Antonio, Texas, for the 16th Annual NSH Symposium/Convention. It promises to be one of the best ever. If you've already made plans to attend, it might be one of the wisest decisions you've ever made. If you haven't yet made plans, time is running out.

Attendance is expected to be about 1,000, according to Sue Judge, convention coordinator. The meeting will be held at the new Marriott Rivercenter Hotel located on the famous River Walk near the Alamo. It will cover a full 7 days, from Saturday, September 8, through Friday, September 14. Workshops will be held for the first 4 days. Most workshops have limited enrollment, so it is important that you sign up early.

There will be 54 workshops this year, including a number of new workshops. Many of the most popular workshops from previous years will be repeated. In order to meet demand, some will even be held twice at this year's convention.

"We went to four days of workshops thinking that, by starting on Saturday rather than Sunday, it would give small labs a better opportunity to attend two days of workshops and get back to work," Judge explained.

The workshops will cover a variety of basic, intermediate, and advanced topics. "We try to fill the needs at all levels," Judge said. Workshops will be held on immunocytochemistry, AIDS, formaldehyde standards, laboratory management, preparation for the HT and HTL registry, plant histology, microwave techniques, undecalcified bone processing, hematopathology, starting a school of histotechnology, and many other subjects. Most of the workshops are 3 to 4 hours in length, but there will also be five all-day workshops.

Lectures will start on Wednesday morning and continue for two days. This year's lectures will include both clinical sessions and V.I.R. (veterinary, industrial, research) sessions. V.I.R. sessions will cover a variety of nonclinical topics.

In addition, a panel discussion will be held Friday morning. This is a new feature of the symposium/convention and will follow an open-forum format. The panel will have a combined knowledge of basic histology and staining, immunology, veterinary, industry, research, education, certification, management, safety, and other important areas of histotechnology. "We have a panel that is very diverse," Judge said. "We are asking participants to send in questions prior to the meeting or to submit questions during the meeting. We will also take questions from the audience."

The panel will consist of Frieda Carson, Ph.D., Baylor Medical Center; Lee G. Luna, editor of *Histo-Logic*; John Ryan, immediate past president of the NSH; Craig Allred, M.D., Medical Director of the laboratory at the University of Texas Health Science Center; Ada Feldman, NSH Safety Committee; and Gene Hubbard, D.V.M., Southwest Foundation for Biomedical Research.

"I think a panel discussion is a good learning experience," Judge observed. "It provides an opportunity for histotechnologists to interact with their peers and experts on specific topics."

Scientific exhibits will open Tuesday evening and continue through Thursday afternoon. This will provide an opportunity to see the latest products and equipment available and to talk with the manufacturers.

Poster sessions and technical exhibits will also be presented Wednesday and Thursday. This provides everyone with an opportunity to share ideas. Subjects can be from any discipline.

A number of social events are also scheduled. A special reception will be held on Saturday evening for first-time attendees. This will give first-timers a chance to get acquainted with NSH officials and veteran attendees. The annual Awards Banquet will be held Thursday evening. A cocktail hour, hosted by Miles Inc. will be held before the banquet. Miles will also host their annual party. This year's party will follow a "Saturday Night Live" theme.

NSH Committee meetings are scheduled for Sunday through Tuesday. Region meetings will be Monday evening. And the Board of Directors meeting is Tuesday. These meetings are open to all attendees.

Travel arrangements can be made through The Travel Concern, the official agent for the NSH. They can make

(continued on page 182)



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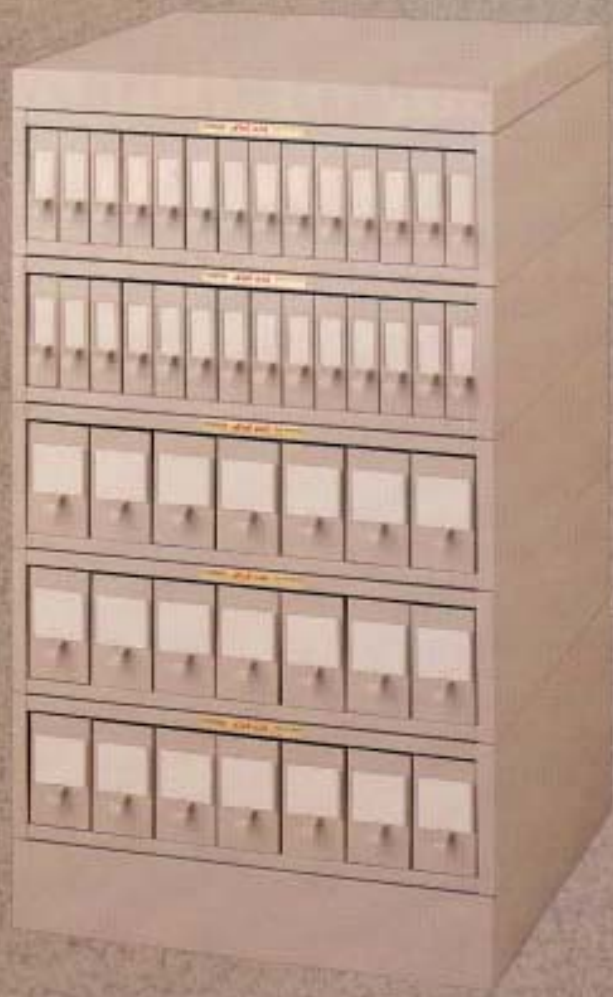
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both air and ground reservations for your trip to San Antonio. Special discount fares are available when you make your reservations early. In addition, representatives from The Travel Concern will be on hand at the San Antonio Airport to greet you and help you get to the hotel. They will also have a hospitality desk in the hotel during the convention week. You can reach The Travel Concern at 1-800-373-4100.

If you have any free time at the symposium/convention, be sure to see the sights of San Antonio. The River Walk offers virtually limitless shopping, dining, and entertainment. For those interested in history, the Alamo is within walking distance from the hotel. In addition, many other historic missions are open for tours.

"San Antonio is a very hospitable town," Judge said. "We're looking forward to everybody being here and we want them to have a really good meeting," she continued. "We're proud of the program we've put together, and we're proud of our city. It's a fun place to be and a fun place to live. We want to share that with everybody."

And, in September, she can guarantee warm weather.

NAACLS to Present Workshop

The National Accrediting Agency for Clinical Laboratory Sciences will present The Accreditation Process Workshop on September 6-7, 1990, in San Antonio, Texas, in concurrence with the National Society for Histotechnology's Symposium/Convention.

This workshop familiarizes the participant with aspects of the NAACLS accreditation process. The first day covers the purposes of accreditation and how the process is carried out. The participant learns how to perform a self-evaluation of a program, prepare a self-study document, and do a paper review of a self-study document. The second day covers the site visit. The participant learns how to prepare for a site visit both as a site visitor and as an official of a program being visited. The participant also experiences the process review committee's use in making accreditation recommendations.

The workshop will be held at the Marriott Rivercenter in San Antonio and will be presented by Mike Laman, NAACLS Review Board member, and Barbara Fricke,

NAACLS Medical Technology Programs Review Committee Member.

To receive a brochure describing the workshop, please contact Megan Hennessy-Eggert at NAACLS (312) 461-0333.

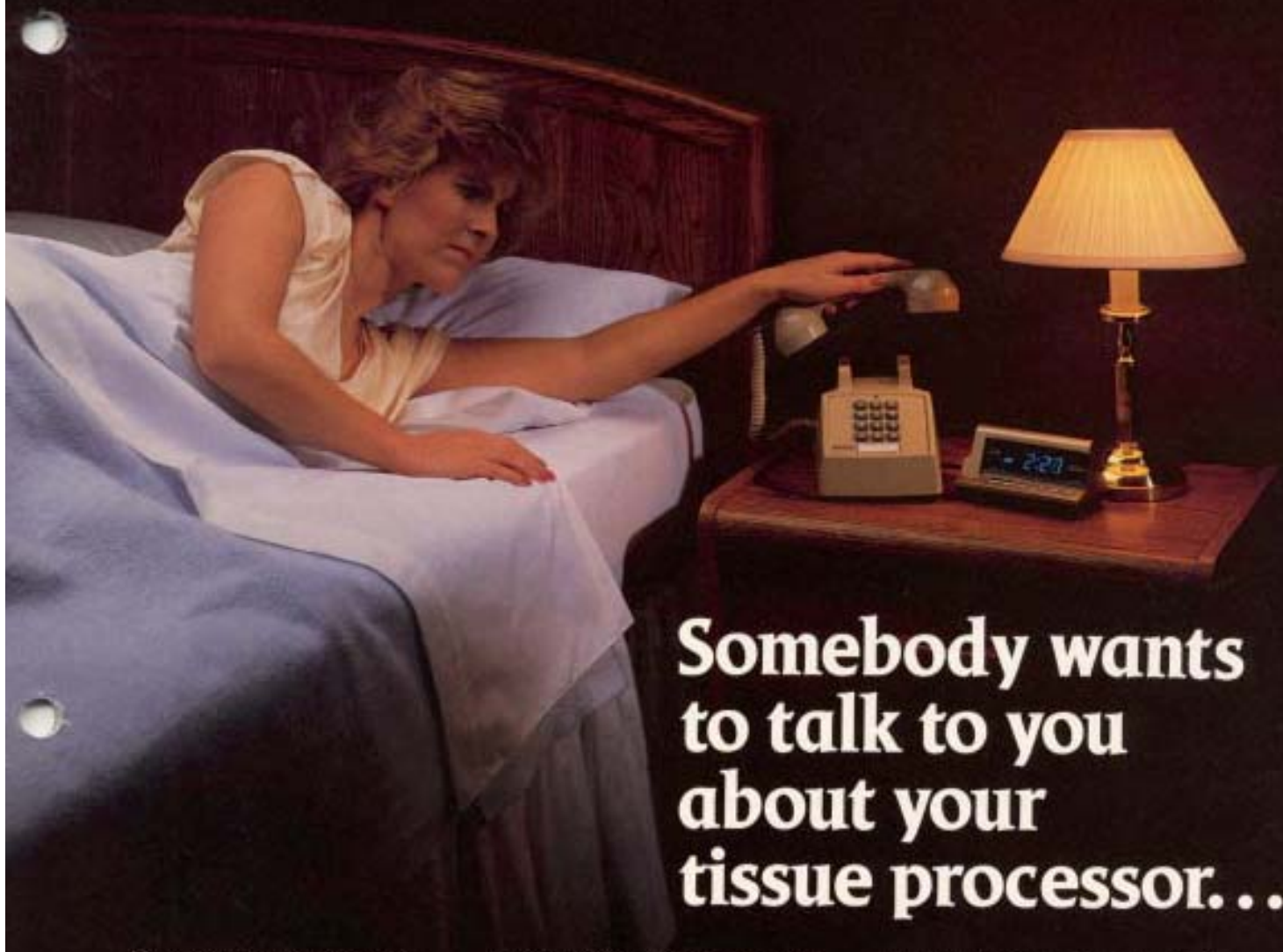
Helpful Hints Flotation Water-Bath Contamination

Louise Burrell, HT(ASCP)
Washington University School of Medicine
St. Louis, MO 63110

Glass water flotation baths should be cleaned daily with the use of a mild solution of soap (teaspoon of Septisol) and hot water, then dried thoroughly. Place bath upside down on top of the water-bath base or in a 60°C oven overnight. This is done to eliminate dust particles from collecting in the water bath overnight. If this is not done, extraneous material will collect and appear on the tissue slides. When using gelatin in the water bath as a section adhesive, be sure to wash water bath thoroughly. Bacteria can grow in gelatin and produce false-positive results when stains for fungi or bacteria are being performed. When using silver nitrate techniques, the use of chrome alum subbed slides produces the cleanest finished product.



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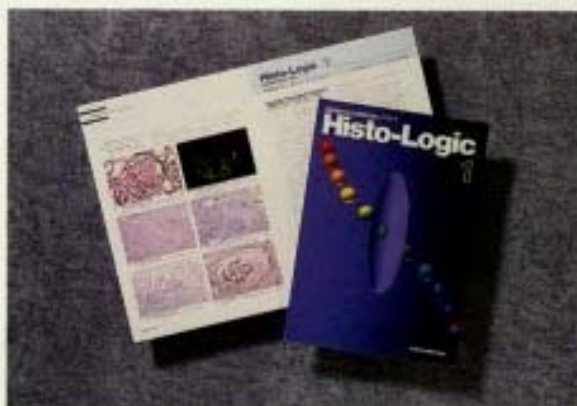
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Histo-Logic Gets New Look in Japan

Brent Riley
Managing Editor

Histo-Logic is translated into several languages and published in many countries throughout the world. A few years ago, however, the Japanese-translated version of *Histo-Logic*, which had been published for about 10 years, was discontinued. Although the English version was substituted, the Japanese histologist greatly missed the translated version.

The new version includes a core of scientific articles translated into Japanese from the English version of *Histo-Logic* published in the United States. The rest of the articles, about two-thirds, are written in Japan. Many are contributed by Japanese histotechnologists, while others are printed versions of technical lectures. Presently, plans include publishing two issues per year.



Initial reader response is very encouraging. The favorable response is attributed not only to the informative nature of the articles but also to the quality of the printing. The new version is printed in four color and includes many four-color photomicrographs.

The objectives of the Japanese version of *Histo-Logic* are the same as those in the United States, according to T. Sato of Miles-Sankyo in Japan. "*Histo-Logic* is the only technical source of timely information a Japanese histologist obtains," he said, "so it is very well accepted."

To receive your own copy of *Histo-Logic*® or to have someone added to the mailing list, submit home address to: Miles Inc., Diagnostics Division, P.O. Box 70, Elkhart, IN 46515.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, *Histo-Logic* Editor, 7605-F, Airpark Rd., Gaithersburg, MD 20879. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.



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