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More Than Just a Mandatory Exercise

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"Mandatory Safety Training Sessions." Mandatory. Makes it sound like you have no choice but to attend. Well, I figured out that I actually had choices. I actually did not HAVE to go to a session. Of course, then I would be subject to suspension, thus not getting paid and potentially having to find other employment. Those were three potential options I chose not to initiate, so I went. No session = no paycheck? Now there's an incentive. It's amazing how the reading of that "deficiency memo" prompts you into finding that hour to attend a session.

I just attended my 26th mandatory annual safety session. So I've been through more exaltations of "Don't let the picric acid dry out or it could explode"; "Make sure the glassware is heat rated or it could explode"; "Don't put chemicals like acetone in the refrigerator or they could explode", than some of the session presenters. Since my anniversary date is in 2 months I guess I'll be attending two sessions in less than 4 months so statistically that should make me safer than most. John Marchese is a safety expert. He has a Master's degree in Mechanical/Environmental Engineering and currently serves as the executive director for Safety and Human Resources at Collaborative Group, Ltd., a manufacturer of personal care products. Previously he served as Assistant Director for Hospital Safety at the State University of New York at Stony Brook. He has published in *The New England Journal of Medicine*, contributed to book chapters, and is an adjunct instructor at Stony Brook Department of Health, Technology and Management. He has over 25 years of experience in the safety and chemical hygiene business. When John was in charge of safety at my former place of employment, he and his crew became notorious for things like wandering into a lab and looking in the refrigerator for flammables, and calling in the county bomb squad to remove picric acid.

Although it will never be noted on that sign-out sheet passed around at the end of the safety class, my exposure to safety dogma is actually far more intensive than the annual mandatory session. You see,

IN THIS ISSUE

This photo depicts the aftermath of what happens when approximately 50 ml of isopentane is placed in a loosely capped 1-L bottle and the bottle is placed in a freezer that is not explosion-proof. Eventually the freezer thermostat will kick on when the isopentane vapors contained in the freezer are sufficient to support ignition. It's the same basic thing that happens to gasoline vapors in a car engine cylinder ignited by the spark plug. Ignition: Blast Off.

It might suffice at this point to simply say, "See what happens? Don't do this," but the forensics behind the event are interesting. Isopentane (2-methyl butane) is commonly used to flash freeze biologic specimens. I have used the reagent in this manner for over a decade to snap freeze muscle biopsies for enzyme histochemistry and immuno workups in several work settings without incident.

The placement of the loosely capped bottle of remnant isopentane into that particular freezer was at the behest of an experienced PhD-level researcher, who had stored isopentane in freezers in a similar manner numerous times over 15 years in other settings without incident. This was not the act of an uninformed novice technician or some volunteer student helper. In fact, some thought actually went into the process. Putting the isopentane in the freezer would save the time that would otherwise be needed to cool it from room temperature all the way down to the requisite temperature (approximately -160°C). Also, by leaving the lid ajar, vapor pressure buildup sufficient to break the cap or the bottle could be avoided. And since this was done successfully numerous times before, what reason would there be to think that this particular time would lead to such an explosive outcome?

It was subsequently learned that the investigator had worked in a different facility with brand-new

Mr. Marchese and I have been fishing partners for nearly as long as we have been in our respective professions, and some of the safety-related things you learn while out on the boat or answering an emergency page are not readily available in a safety class. The one thing I've appreciated from John over the years is his frustration in trying to reach an audience that is highly skilled and highly intelligent but often not terribly interested in the message.

When a wiseguy like me asks "where's the refrigerator?" on the boat so I can "get even" for the acetone search, this prank sparks the type of dialogue not normally heard in a typical safety training session. When I postulate in John's presence that exploding picric acid might be urban legend since nobody we know has actually experienced it in the cumulative centuries of experience between us, his response typically ends with, "you know, people like you make my job more difficult . . . ."

Despite the playful banter, I've come to realize that I've learned a lot from him—a guy who does not view safety simply as a profession, but as a way of life. He's bounced lecture concepts off of me as a sounding board before they were delivered to an audience. I've critiqued the in-house videos that were produced to drive home the dangers of sharps handling. I've heard the stories and statistics supporting the dangers of household coffee makers in the workplace to the point where the last thing I do when I leave the house is make sure the coffee maker is not only turned off but unplugged. Believe me, you can learn a lot during a 30-minute run back to the dock.

So when the clock radio alarm sounded to the news on September 5, 2002, informing the listeners of Central Arkansas that there had been an overnight fire in one of the research buildings where I work, my first words to my wife were, "I bet someone left a coffee maker on overnight and caused a fire." I knew the majority of the research staff working on that floor. I knew they were well trained. I knew they all were supposed to have attended the annual mandatory safety session. So thinking back to a conversation I once had with John who said, "coffee makers should be included in every annual safety session," my money was on this as the most likely cause of the fire at our facility. Little did I know what really caused it.

equipment. The freezer in the prior facility was an explosion-proof unit. The freezer in our facility was not. A number of important features distinguish explosion-proof freezers from common household units. In explosion-proof units, the controls and electrical components that could potentially cause a spark (such as motors and condensers) are located outside the cold box or are insulated from the interior atmosphere of the storage chamber.[1] Some units have compressors and other electrical components mounted on top to avoid heavier-than-air vapors that can accumulate along the floor. Price is another distinguishing characteristic. One can purchase a household 20-cu-ft. upright freezer for under $500.00, while an explosion-proof unit of the same size will cost over $2000.00. In this instance, the investigator did not take into consideration the construction of the freezer despite the label above the door handle designating the unit as "Not Suitable For Flammable Liquids."[2]

The Material Safety Data Sheet for isopentane specifies that its flash point is -51°C,[3-5] which is by definition "the minimum temperature at which a liquid gives off vapor in a test vessel sufficient to form an ignitable mixture with air near the surface of the liquid." One might anticipate that as the temperature continues to climb past the -51°C mark, continued vaporization might cause sufficient pressure buildup to warrant leaving the bottle cap ajar. But a far more significant factor can also be gleaned from the MSDS: *"Vapor is heavier than air and may travel along ground; distant ignition is possible."*[3,4]

The compressor and motor on this freezer were located under the storage box, however, the thermostat was located inside the storage box. This compounds the possibilities, but the fact that the freezer door detached and jettisoned some 12 feet from the freezer leads one to believe that ignition was probably caused by the interior thermostat after the requisite accumulation of vapors. It was a big bang for such a small volume of liquid.

The cost accounting, including replacement of equipment, books, and reagents, is approximately $165,000.00. This does not take into account the facility cleanup and reconstruction, water damage to the floors below, loss of productivity, loss of data, and potential loss of future funding due to missed deadlines. It could be a year before the actual complete loss total is realized and the final numbers will far exceed the quarter million dollar mark.

In summary, an experienced PhD researcher, in an effort to shorten a procedure time by about 2 minutes and to reuse approximately 100 ml of reagent (value approximately $2.00), places a volume of flammable liquid into a loosely capped bottle and stores it in a regular household freezer. The liquid gases off into the confines of the freezer and at 3:50 AM when the thermostat clicks on to start the compressor motor, a spark from the thermostat ignites the collected vapors and blows the door off the freezer. The blast severs a compressed air line which in effect creates a "flamethrower across the lab,"according to the firefighters at the scene, ultimately costing the institution a huge amount of money. But, if you think about it, it could have been a lot worse. Suppose the thermostat had clicked on at 3:50 in the afternoon while people were working at the benches across from the freezer. Even worse, a spark from the freezer's interior light could have ignited the vapors when the freezer door was opened by lab personnel. The potential for loss of life was substantial.

Needless to say, this incident prompted a campuswide mobilization to check for potentially flammable materials located in non-rated refrigeration units and for good reason. Chilled acetone is commonly used for a variety of laboratory procedures. We all need to maintain vigilance in dealing with the storage of flammables. Far too often we hear the warnings regarding these reagents once a year at the mandatory safety training session and then file them in our mental recesses because we don't think we'll have immediate use for

the information. It's only after an event like ours that people are prompted to check the following:

Are your refrigeration units (refrigerator and freezer) explosion-proof or rated for the storage of flammables?

If your units are not rated for this application, is there anything in them that shouldn't be there?

Are your non-rated refrigeration units clearly labeled as not approved for the storage of flammable liquids? Are you maintaining the necessary vigilance to heed the warnings?

Have you familiarized yourself with the MSDS for the materials you are working with? Do you have the information necessary to properly and confidently work with these types of materials?

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Remembrances of Opportunities Lost

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In the early 1960s, after completing my Master's in Hematology, I went to work at the Brookhaven National Laboratories in Upton, New York. I spent over 6 hours a day cutting serial sections of Bouin's-fixed mouse spleens that displayed macroscopic nodules derived from stem cells of normal mouse bone marrow. The goal of the senior scientists was to reconstruct the spleen at the microscopic level to determine its hemodynamics (open or closed system). To keep my sanity, I offered to do cytogenetic karyotyping of bone marrow aspirates obtained from patients in the leukemia outpatient clinic. The technique, developed by Nowell and Hungerford in the 1940s, was a way to induce peripheral blood lymphocytes to dedifferentiate and enter the cell cycle. After a 3-day growth in culture, the cells were treated with colcemid to arrest them in mitosis. The cells were subsequently harvested, and conventional air-dried preparations were stained with a standard Giemsa solution obtained from the hematology laboratory. Photomicrographs taken of cells caught in metaphase were enlarged sufficiently to allow cutting out of homologous chromosomes to prepare a karyotype of a patient's blood. The goal was to find the Philadelphia chromosome, which was thought to be chromosome 21 with a deletion of its long arm, pathognomonic for chronic myeloid leukemia.

After many months of karyotyping I discovered a problem with the Giemsa staining. Instead of obtaining the uniform deep blue-black staining that facilitated photomicrography, I was getting dark and light ribbon-like staining. When I checked the pH of the Giemsa, I determined that the original batch of stain obtained from the hematology lab had become alkaline. I promptly discarded that batch, obtained fresh Giemsa, and restained the preparations to obtain the expected result. All future batches of Giemsa were routinely pH checked to avoid this aberrant result.

Approximately 6 months later, an article appeared in the prestigious research journal *Nature* that described the use of an "alkaline Giemsa" method to produce band staining of metaphase chromosomes. These investigators determined that homologous chromosomes display identical banding patterns, which greatly enhanced the ability to discern translocations and deletions of portions of chromosomes. In fact, later researchers using the alkaline Giemsa technique determined that the Philadelphia chromosome was not chromosome 21, as was originally thought, but chromosome 22 that had lost portions of the long arm! I was annoyed with myself for not having the insight to take what looked like a lab error and explore what a fortuitous change in pH could do to improve chromosome analysis.

A decade later while employed at Stony Brook Health Sciences Center, I routinely scored paraffin sections of breast tissues immunostained for estrogen/progesterone receptors (ER/PgR-ICA). Since Long Island is considered to be one of four regions where the incidence of breast cancer exceeds the national average, the opportunity to observe the rare cases displaying cytoplasmic receptor staining instead of the expected nuclear staining presented itself. Because this pattern of staining had not been described as being biologically

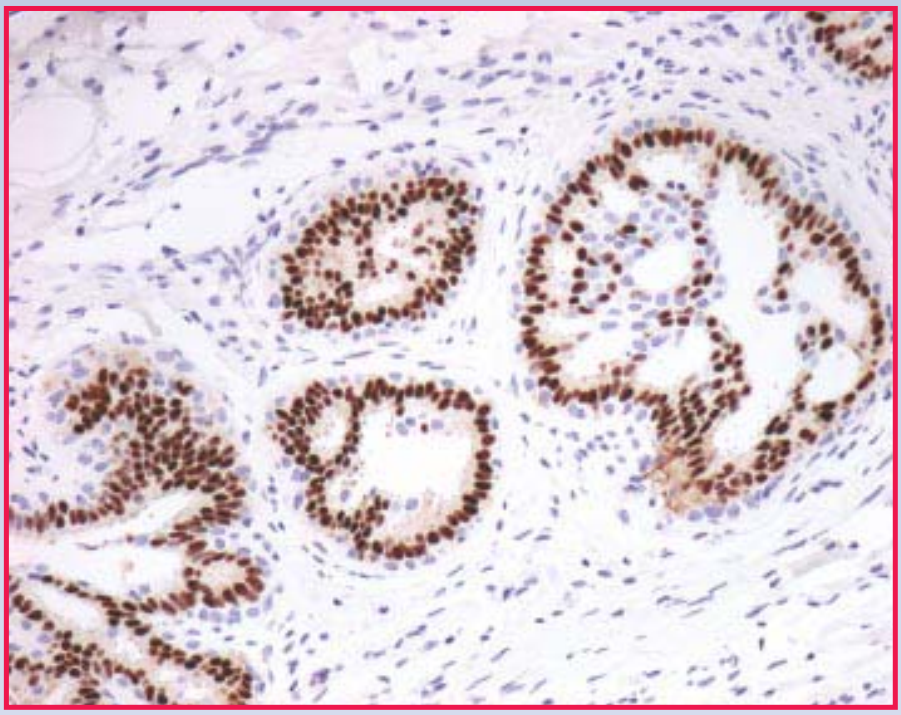

Fig. 1. Estrogen receptor staining in human breast by immunohistochemistry most commonly results in a nuclear staining pattern. 200X. Photo courtesy of John Metcalf, MD, Medical University of South Carolina.

significant and did not meet the criteria for being scored as a positive result, these cases were scored as negative. Moreover, cases with cytoplasmic staining were not followed to determine if this variation had any clinical value. Parenthetically, a major criticism of the ER/PgR-ICA was that it did not measure biochemical functionality of the receptor and, therefore, did not unequivocally select the patients who would benefit from some form of endocrine therapy. In essence, the ER/PgR by IHC was a static assay. To overcome this limitation, the immunohistochemical demonstration of pS2, a protein product that was intimately associated with steroid receptor function, was performed. We subsequently published a report that validated the value of pS2 with regard to ER/PgR functionality; those patients with positive cytoplasmic staining were more likely to score a high positive for both ER and PgR.

Decades later a report entitled, "Cytoplasmic localization of wild-type p53 in glioblastomas correlates with expression of vimentin and glial fibrillary acidic protein" (*Neuro-Oncology*, July 2002), recalled my experience with cases that were scored ER-ICA negative because of cytoplasmic staining. These investigators determined that the cytoplasmic accumulation of wild-type p53 in tumor cells indicates that the tumor suppressor gene is inactive with regard to growth-repressive functions. Their study suggested that vimentin expression is a strong indicator for the cytoplasmic expression of p53 in tumors that also express GFAP. Again, my biological frustration level was tweaked as there were isolated reports that vimentin expression in breast tumors was indicative of a poor prognosis.

These experiences are a reminder of how often we choose to ignore those laboratory results that do not meet the established standard and thus, missing potentially important observations. James Watson and Francis Crick, who discovered the duplex structure of DNA, personify the ultimate seekers of phenomena that others chose to ignore, consciously or otherwise. They visited the laboratories of several key DNA researchers and analyzed their data. From this heterogeneous database, Watson and Crick were able to synthesize a model of DNA structure that still stands. By being receptive to the potential in the work of other equally brilliant scientists who did not see the big picture and consequently missed the opportunity to make a significant scientific contribution, Watson and Crick have gained a permanent place in science history.

The scientific literature is replete with experiments that did not produce expected results, and those sensitive enough not to discard data that do not produce the expected outcome seem to make the leaps that contribute to the advancement of science and medicine. Sherlock Holmes knew this in his early career as a research chemist when he remarked that a fellow scientist would never succeed in his experiments because he saw only what he expected to see. Progress comes from those who see beyond what we expect to see.

Having said all that, if I were still active in the field, I would take the interesting opportunity to determine if cytoplasmic ER-ICA staining is indeed indicative of a nonfunctioning estrogen receptor. A call for all ER-ICA cases displaying this unexpected pattern would provide enough material for a retrospective study of immunostaining for pS2. Defying the great Sherlock, I predict that the majority of cases will lack pS2 or have only weak cytoplasmic staining. Thus, we could deduce the functional status of the estrogen receptor without having to perform molecular analysis. I can't do it, so I offer the suggestion to some future researcher looking to make a significant contribution to the advancement of knowledge.

Beware—Some Things You Don't Want to Share

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Laboratory personnel working in human or veterinary medicine are at risk of exposure to a variety of infectious agents. Workers must protect themselves from age-old infectious diseases like tuberculosis, emerging diseases such as West Nile virus, and agents used for bioterrorism. Safe handling of infectious agents requires early risk assessment. Knowledge of the number of laboratory infections, route of transmission, infectious dose, incubation time prior to the onset of symptoms, and severity of the disease provides the information needed to develop appropriate techniques and equipment safeguards.

In 1949, Sulkin and Pike published the first in a series of surveys about laboratory-associated infections summarizing 222 viral infections, 21 of which were fatal.[1] In at least one-third of the cases, the source of infection was considered to be associated with the handling of infected animals and tissues. Accidents were recorded in only 12% of the reported cases.[2] By 1976, data indicated that, of the nearly 4000 cases of laboratory-associated infections, fewer than 20% were associated with a known accident.[3]

Tuberculosis (TB), one of the most widespread infectious diseases, may be encountered when working with human and animal samples. It remains the most common cause of death among adults worldwide, killing 2 to 3 million people each year. All known mycobacterial pathogens of animals, *Mycobacterium bovis* being the most common, are transmissible to humans. Laboratory-acquired mycobacterial infection has been documented and there is direct correlation between the frequency of infection in laboratory workers and the number of positive samples handled. Medical personnel are at risk of acquiring TB in the course of their work through the inhalation of contaminated droplet nuclei. Infected tissue specimens or cultures have the potential to create aerosols in the laboratory and at autopsy or necropsy. Persons in environments in which the inhalation of infected aerosol is possible should wear appropriate respiratory protective devices. In 1978, two pathologists in California and a histotechnologist from another hospital seroconverted positive for TB after being present when frozen sections were taken from a lung infected with TB. The tissue block was cooled in the cryostat with a compressed gas coolant, which created a heavy aerosol.[4,5]

As stated in the December 2002 issue of *HistoLogic*, there have been reports that *Mycobacterium tuberculosis* has been cultured from surgical and autopsy tissues after weeks of formalin fixation.[6] Until there are firm data that unequivocally prove that the organism cannot retain viability in tissues after formalin fixation, added precautions are warranted when working with tissues containing this agent.

Zoonotic diseases and infections are naturally transmissible from vertebrates to humans. Up to 200 diseases have been identified as being shared between man and animals. These diseases or infections may be asymptomatic and trivial in animals but have serious or deadly results in humans. The death of Elizabeth Griffin, a 22-year-old animal caretaker at Emory University, is a distressing example of the potential danger of a zoonotic disease. Ms. Griffin developed ocular inflammation 2 weeks after a splash of an unknown substance to her eye while working with macaques. She sought treatment at a university facility and was discharged. Four weeks later she succumbed to the B-virus infection.[7] OSHA cited the primate research center with a willful, serious violation and proposed penalties of over $100,000 for the death of an employee.[8] Herpesvirus simiae (B-virus) is a member of the herpes group of viruses that is common in old-world monkeys of the macaque genus. The disease, which is similar to cold sores in humans, causes mild or inapparent infection in monkeys; however, infections in humans can be fatal. B-virus disease in humans is characterized by a variety of symptoms that include vesicular skin lesions at or near the site of exposure, localized neurological symptoms, and encephalitis. Laboratory personnel having direct contact with macaques are at the greatest risk of infection. Transmission to humans usually occurs via exposure to contaminated monkey saliva through bites, scratches, and splashing.

Between 1950 and 1960, two-thirds of occupational infections with B-virus were related to polio vaccine testing on macaques. Recent cases often coincide with use of macaques in retroviral research. One case of B-virus infection occurred following a technician's exposure to contaminated cell cultures of simian origin.[9] Laboratory workers who have the potential for exposure to macaques and macaque tissues should be properly trained and wear personal protective equipment (PPE) as recommended. Ms. Griffin's death would have been prevented if she had simply been wearing goggles.

Emerging diseases pose a particular risk because of the

unknown course of the new disease. West Nile virus (WNV) is a disease relatively new to the United States. The first reported outbreak occurred in New York in late 1999. By 2002, only 3 years later, West Nile virus was reported in 44 states. In Illinois alone, it was reported that 778 people became ill and 52 died from the disease. There were 4007 laboratory-positive human cases reported nationwide to the CDC/Arbonet as of January 29, 2003.[10]

Most West Nile virus infections in humans are subclinical. Overt disease is estimated to occur in approximately 1 in every 100 infections. Mild cases of West Nile virus infections may cause a slight fever or headache. Those with more severe infections may exhibit a rapid onset of a high fever with head and body aches, disorientation, tremors, convulsions, and possibly paralysis and death. Symptoms occur 3 to 14 days after a bite from an infected mosquito. Persons 50 years of age or older are at greatest risk for developing complications from natural infection. In animals, the greatest number of deaths has occurred in birds and horses. There is a preventative vaccine now available for horses.

There is documentation that WNV was passed from one person to another through blood transfusion and organ donation.[11] The youngest person infected with WNV was a baby reportedly infected by drinking breast milk from the mother who became ill and tested positive for WNV after receiving a blood transfusion. Although the baby has remained healthy, a blood sample from the infant demonstrated IgM antibodies to the WNV, an indication that the infant had been infected.[12]

The degree of risk to postmortem workers from ill or dead animals and humans is undetermined at this time. West Nile virus is grouped with most of the arboviruses known to have caused lab-acquired infections. There are two documented cases of laboratory-acquired WNV from a scalpel cut and a contaminated needle puncture and one case of a person working with WNV who developed neutralizing antibodies without clinical symptoms.[13] The CDC considers WNV a Biosafety Level (BSL) 3 agent. However, because many labs are not equipped for BSL 3 practices, BSL 2 procedures are acceptable if modifications are approved by the laboratory director. Modifications are as follows: exhaust air is discharged outdoors, access is restricted when work is in progress, ventilation is balanced to provide directional airflow into the room, and recommended standard microbiological practices and safety equipment regulations for BSL 3 are rigorously followed.[14]

Just as emerging diseases are of concern to healthcare and laboratory workers, so are diseases caused by agents that have no known effective treatment and are resistant to conventional inactivation procedures. Transmissible Spongiform Encephalopathies (TSEs) are caused by prions, proteinaceous particles smaller than the smallest virus. TSEs are fatal, degenerative diseases affecting the central nervous system. Prion diseases, or TSEs, include Creutzfeldt-Jakob Disease (CJD) and variant CJD, BSE, scrapie, transmissible mink encephalopathy, feline spongiform encephalopathy, chronic wasting disease (CWD) in deer and elk, kuru, Gerstmann-Straussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI).[15] Animal prion diseases, except BSE, are not regarded as a public health threat, but all TSEs should be handled with care.

The highest concentration of prions is in CNS tissues and their coverings. High concentrations are also suspected in the spleen, thymus, lymph nodes, and lung.[15] Formalin fixation inactivates most disease agents, however with prions, tissues that are formalin fixed and paraffin embedded remain infectious.

The efficiency of disinfectants in inactivating prions is unclear. Some experts recommend that equipment and surfaces may be decontaminated with 2N sodium hydroxide over a 1-hour period.[15] The following procedure substantially reduced the infectivity levels in brain tissue of rodents infected with CJD, BSE, and scrapie agents. Tissues, well-fixed in formalin, were placed in 98% formic acid for 1 hour then rinsed well in H_2O and returned to formalin before processing.[16]

Prosectors should wear cut-resistant gloves to avoid puncture of the skin. If accidental contamination of skin occurs, swab the area with 1N sodium hydroxide for 5 minutes, then wash with copious amounts of water.[15] Gloves should be worn when handling and sectioning tissues. Dispose gloves, paraffin shavings, and wipers used to clean the surface of the water bath into a biohazard bag for incineration. All residues, liquid and solid, from processing, embedding, and staining in the laboratory should be discarded into a leak-proof biohazard bag for incineration.[17-19] Liquid waste may be collected in a 4-L waste bottle containing 600 ml of 6N sodium hydroxide.[15]

It is believed that ingestion is the most common route of exposure for prion disease, but CJD has been spread by way of surgical procedures (grafts) and even by using a surgical suite that was not properly decontaminated after a procedure on a CJD patient.[20,21]

Until recently, laboratory workers have given little thought to agents used in bioterrorism. After the anthrax scare of 2001, there is greater chance that laboratories may encounter the organism. A Texas laboratory worker who was testing specimens from the 2001 attack contracted the cutaneous form of the disease, and last year

a worker in the anthrax research lab at the United States Army Medical Research Institute of Infectious Disease (USAMRID) tested positive for anthrax exposure. The CDC states that these cases demonstrate the importance of safety procedures and vaccination for lab workers who routinely handle anthrax.[22, 23]

There are three forms of the disease: cutaneous, gastrointestinal, and inhalation. If untreated, all can lead to septicemia and death. Of the three, the inhalation route is the most severe. The organism is most likely to reach the bloodstream and disseminate toxin throughout the body. If untreated, the fatality rate by the inhalation route is 90%-100%, as opposed to the cutaneous route, which is 5%-20%. Antibiotic therapy (penicillin) is often effective if administered early or used as prophylaxis.[24, 25]

Naturally occurring anthrax endospores remain viable in soil for decades. In 1996 a cow in Ontario died from anthrax. The owner reported that cows had died of anthrax on the same farm 65 years previously. Recent excavations in that area unearthed the viable organism. Similarly, anthrax occurred in cattle in South Dakota and New Mexico following oil company drilling and earth work in 1997 and 1998.[26]

Postmortem dissection should not be performed on animals with suspected anthrax. Exposure to air causes the vegetative form in the carcass to form stable endospores. If a veterinarian suspects anthrax, he should submit the tip of an ear to minimize environmental contamination and exposure to the public and laboratory personnel. Blood should be collected with as little contact as possible. All instruments and materials should be autoclaved, incinerated, or chemically disinfected after contamination with anthrax.[27,28]

Workers who are motivated to achieve a safe working environment and who are provided with accurate information can establish adequate biosafety controls. Diligence in the application of safe standard operational procedures and preventative measures is required to decrease the incidence and severity of laboratory-acquired infections. The hurried, sloppy, absent-minded, unconcerned technician will create the maximum hazard. The cautious, skilled, conscientious, and attentive technician will create minimal hazard. The knowledge, the techniques, and the safety equipment necessary to prevent most laboratory-acquired infections are available. It is the responsibility of the laboratory professional to accept the importance of a safe working environment.

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A Method to Repair Freeze Artifact in Skeletal Muscle Biopsies

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Abstract
Enzyme histochemistry is an essential component of the diagnostic evaluation of skeletal muscle biopsies.[1] Muscle enzymes are demonstrated in fresh frozen muscle that is ideally free of artifact to facilitate microscopic evaluation. Muscle specimens should be received in the laboratory fresh for rapid freezing, but far too frequently we receive specimens from outside hospitals that are submitted incorrectly, either floating in saline or having already been frozen in OCT™ Compound at the point of origin prior to shipment. In these instances we often find ourselves working with samples that are obscured with freeze artifact. We explored techniques to improve samples that would otherwise be unreadable.

Introduction
Many small hospitals are ill-equipped for snap freezing which requires access to liquid nitrogen or dry ice. For this reason, we instruct our outside clients to send two samples of muscle, one that is clamped in a rayport biopsy clamp and immersed in 4% paraformaldehyde for light and electron microscopy studies. The second piece is requested fresh, to be placed onto a saline-dampened piece of gauze and put in a biohazard bag that is shipped on ice either the same day or overnight. Inevitably, some clients fail to follow our instructions, leaving us with a compromised sample (see Fig. 1).

Slow freezing, which may include freezing samples inside of a cryostat or other low-temperature freezer will typically yield a sample that is riddled with freeze artifact, causing gross distortion of the tissue.

Rapid freezing at ultracold temperature is imperative to yield an optimal sample for diagnosis.[2] Underlying disease processes can either be masked by ice crystal artifact or the artifact itself can mimic a disease process, confusing the diagnosis, e.g., lipid storage myopathies. Freezing artifact also makes it very difficult for the pathologist to assess fiber types. If a muscle is full of vacuoles, the pathologist may order stains for lipid and glycogen in order to determine if the vacuoles are a part of the pathology or artifact. Some

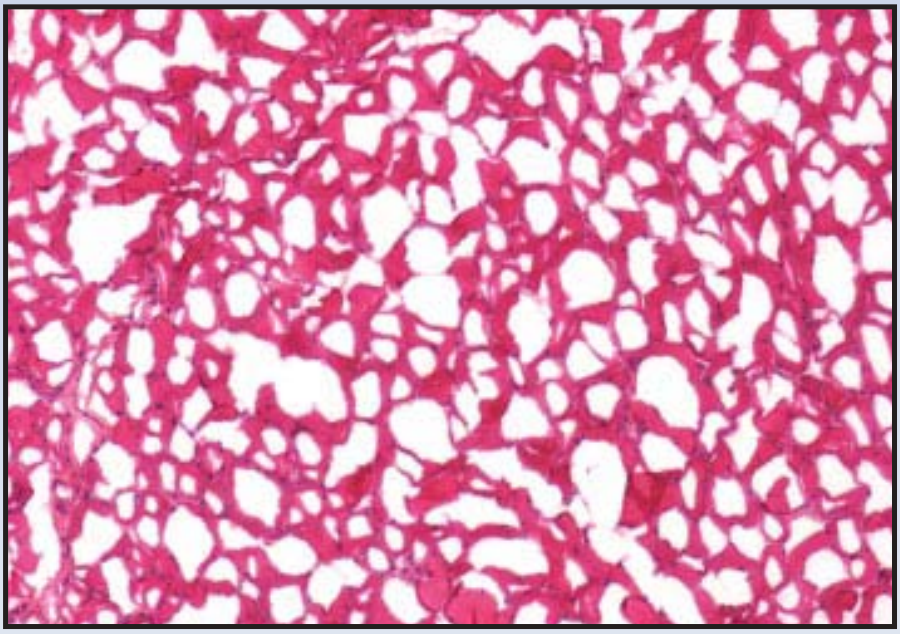

Fig. 1. Skeletal muscle sample riddled with ice crystal artifact resulting from inadequate freezing technique. H&E stain. 100X

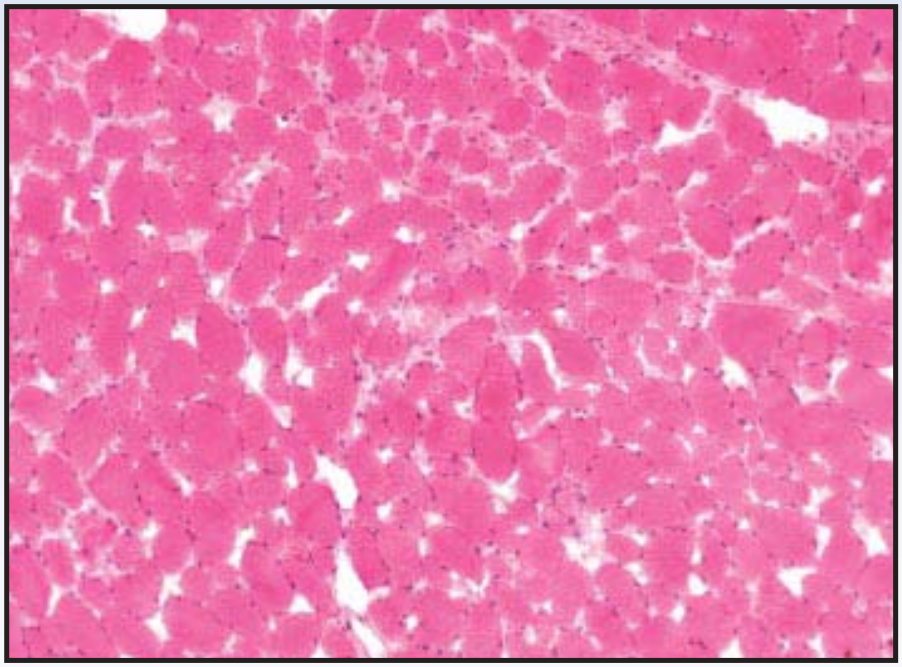

Fig. 2. Skeletal muscle sample thawed and refrozen to correct for prior ice crystal artifact. H&E stain. 100X

instances of freeze artifact may be so severe that a definitive diagnosis cannot be made from the available material. For those cases it is best to thaw the tissue in cold normal saline and refreeze. Often this will lead to a vast improvement in morphology and little if any loss of enzyme reactivity (see Fig. 2).

Method
We prefer to mount the muscle specimen protruding out of a gum tragacanth-supporting medium which provides a firmer hold than OCT™ and will effectively hold the muscle in the correct orientation during freezing. To prepare the muscle for this technique it is necessary to remove as much of the gum tragacanth as possible from the initially frozen block by cutting it away from the specimen with a sharp blade. Once this is achieved, place the muscle in cold (5-7°C) normal saline for a period of 30 minutes. We believe that the salt content of the saline aids in drawing away the excess water molecules from the muscle fibers that formed the ice crystals during the initial freezing of the sample. It is important to blot the specimen thoroughly after removing it from the saline. You can use any absorbent pad, or several layers of paper towels. This will effectively eliminate the excess moisture on the exterior of the sample which is now ready to be refrozen.

Method for Snap Freezing Tissues
You will need the following materials and equipment:

- 10% gum tragacanth
- 2-methyl butane (isopentane)
- Liquid nitrogen
- Cork square approximately 2 x 2 cm
- 300 ml beaker
- Small styrofoam container
- Long forceps
- -70°C freezer
- Ultralow-temperature thermometer

SAFETY PRECAUTIONS:

2-methyl butane is highly flammable and harmful if swallowed or inhaled. It affects the central nervous system, causes irritation to skin, eyes, and respiratory tract.

Contact with liquid nitrogen can cause frostbite. Avoid inhalation, wear cold-insulating gloves, face shield or eye protection.

1. Using a tongue blade or flat spatula, place a mound of gum tragacanth approximately 1 cm high on the cork.

2. With a small spatula, open a small hole in the center of the mound about 3 mm deep.

3. Carefully place the muscle specimen on top of the gum tragacanth, orienting the tissue so that a cross section of muscle fibers can be obtained. Avoid getting the gum material onto the surfaces that will be sectioned as it will not cut. Very gently push the base of the specimen into the gum tragacanth about 1-2 mm.

4. A marking pen may be used to write the specimen number on a small strip cut from a file card. Slip one end of the strip into the gum tragacanth against the cork, taking care to leave the number exposed from the gum.

5. Immerse a beaker containing 2-methyl butane into an insulated container of liquid nitrogen. Care must be exercised to establish the correct amount of liquid nitrogen, taking into consideration that the beaker of 2-methyl butane will displace the liquid nitrogen. Ideally, the nitrogen will rise sufficiently to surround the beaker which will accelerate cooling. However, do not allow any liquid nitrogen to overflow into the beaker and contaminate the 2-methyl butane. If this occurs, you need to get a fresh beaker of 2-methyl butane and start again. Cool the 2-methyl butane to at least -150°C, verifying with a low-temperature thermometer. If liquid nitrogen is not available, dry ice may be substituted to cool the 2-methyl butane if a thermometer is used to monitor the temperature.

6. Pick up the block with long forceps by the edge of the cork; turn block upside down and plunge it into the beaker of 2-methyl butane for 30 seconds (longer if the specimen is thick), swirling the block while freezing. Transfer the block to a -70°C freezer until you are ready to section.

7. Affix the cork base to a cryostat chuck with OCT™ for sectioning.

8. Cut enough sections to perform the required stains and enzymes.

Conclusion
This simple method has been reliable in our hands and is useful to remove or reduce ice crystal artifact that has rendered a muscle specimen uninterpretable. It is reproducible and relatively easy to perform if sufficient care is exercised to conduct refreezing at ultralow temperature.

Acknowledgment
The authors wish to thank Robert A. Skinner, BS, HTL(ASCP), University of Arkansas for Medical Sciences, for his advice and insights into the use of this technique.

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Mercury's Rising

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The shipping of pathology cases, notably slides and paraffin blocks via the postal service, is a common task for many of us. Even in an age when computer electronics make the rapid transmittal of microscopic images over the internet simple and convenient, most consulting pathologists prefer to receive the slides themselves, wishing not to be limited to only those microscopic fields offered electronically by the referring physician. We also encourage referring facilities to include the paraffin blocks with any cases they send for consultation to give us maximum flexibility to render the diagnosis in the shortest time possible. The need for additional or repeat staining of outside cases that have been fixed or processed at their point of origin with protocols unfamiliar to us cannot be anticipated in advance, and at times, are critical to the completion of the case.

Those of us who are charged with the responsibility of packaging patient materials for shipment probably exercise greater care with the glass slides which we know to be fragile. This is understandable as experience has taught us that even an intact parcel on arrival is no guarantee that the contents haven't been reduced to multiple shards of colored glass.

I will admit to you that even I have given less thought to the packaging of paraffin blocks leaving my facility. After all, they are, for the most part, shock resistant; and until recently I would have expected them to resist any of the rigors of transportation that I could imagine.

As you can see in Fig. 1, the sender took extra care to wrap this block in bubble wrap packaging material which we know protects even the most fragile of shipments. It is clear that this block was subjected to elevated temperatures, and given the insulating properties of the air-filled plastic wrap, the temperature of the shipping environment must have been extreme.

In the past I've not considered sending cold packs along with paraffin blocks, but this experience suggests that it would serve us well to consider the climates we ship to and take appropriate precautions to prevent this unfortunate outcome. Despite our best efforts in this particular instance, we were unable to recover the specimen from the packaging material.

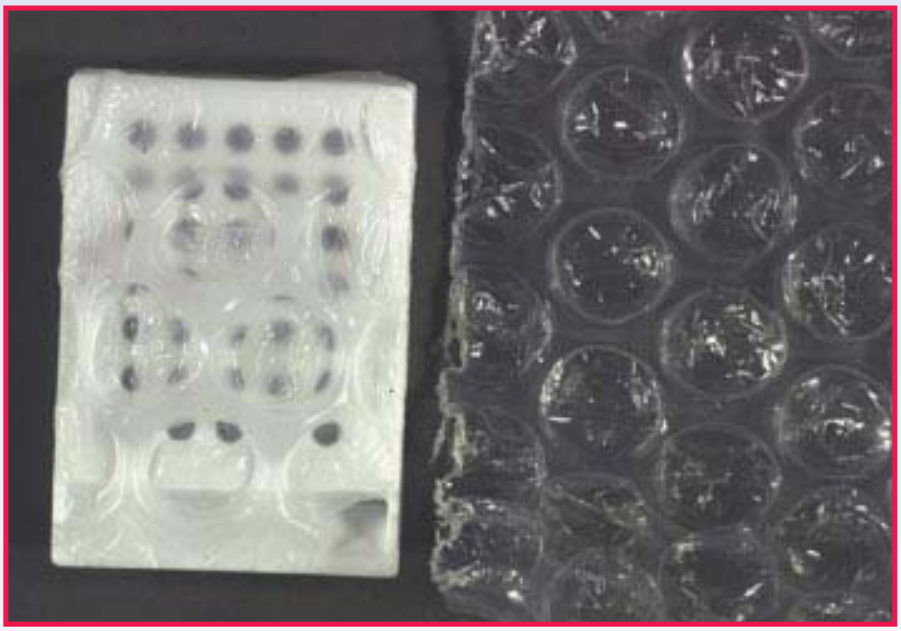

Fig. 1. The bubble wrap melted into the paraffin block, contributing to the loss of the specimen.

Enhanced Detection of Microorganisms in Tissue

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Abstract
In the field of histopathology, two key challenges present themselves as pivotal in achieving diagnosis: for the pathologist, the ability to microscopically identify and distinguish individual pathogens and/or cellular changes within a tissue host; for the histotechnologist, the ability to reliably target suspected agents and/or cellular disruptions, and highlight them in such a way as to enhance the pathologist's ability to selectively view them apart from all other tissue elements.

The term microorganism by definition denotes the extreme minuteness or microscopic/ ultramicroscopic size and nature of an organism. Our ability to observe them oftentimes relies on the fact

that they generally exist in aggregate communities as interdependent elements that function as a unit. Given their ability to grow and reproduce within a host independent of other like organisms, pathologists must be able to identify even a single organism. In a survey of pathologists who were asked to comment on the factors most affecting their ability to discern the presence of infectious particles in tissues, the overwhelming response leaned toward three reasons: 1) minimal populace of organisms in tissue; 2) inadequate dye intensity of stain; and 3) high degree of background noise (counterstain) masking out the organisms. While the availability of high-powered microscopes has largely facilitated the detection of scant organisms, the greatest challenges remain with the histotechnologist to demonstrate these pathogens when present, in a clear and distinct manner. If organisms are not able to be viewed clearly enough to distinguish their morphology, they may go undetected or be misidentified. In some instances stain artifact may be mistaken for the presence of microorganisms, when in fact none are present.

While a number of microorganisms are pathogenic to humans, this article will focus on acid-fast bacteria to illustrate three methods of enhancing the detection of these organisms. The first two are very similar in nature, both using similar reagents and heat inducement. The third method utilizes a non-antibody fluorescent dye marker that provides optimal contrast resolution even for single non-aggregated organisms.

Contrast Sensitivity

Optimal organism detection requires a higher sensitivity and affinity of a stain toward the target agent and an optimal balance of background color that will delicately contrast and therefore highlight the organism. Contrast sensitivity may be defined as the

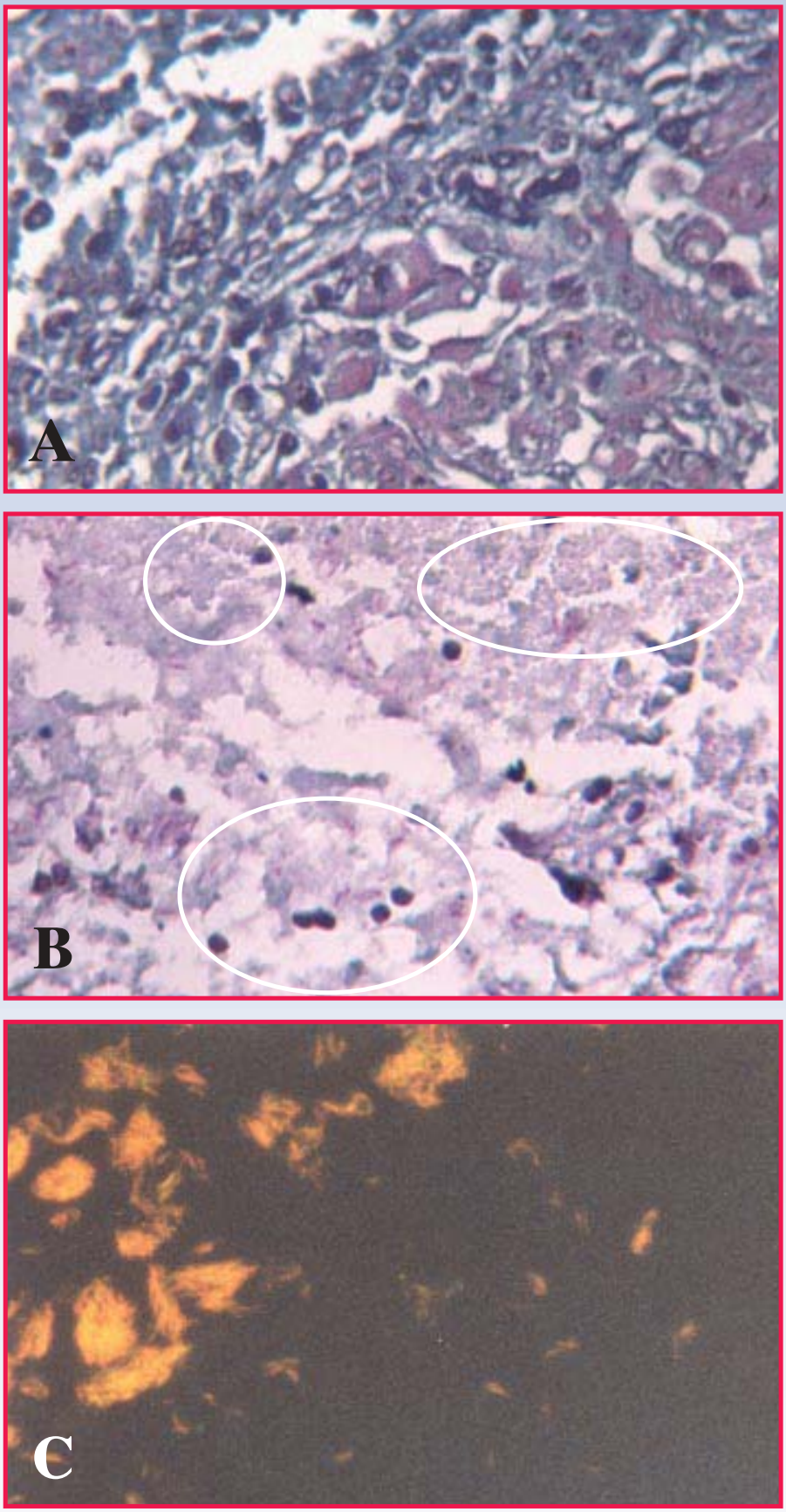

Fig. 1. *Mycobacterium tuberculosis* is illustrated with three varying degrees of contrast. A. Overstaining of methylene blue counterstain masking the bacilli. B. Optimal counterstain (m. blue) with distinguishable red organisms. C. Aggregate and individual organisms using Acri-Fluor fluorescent stain.

Fig. 2. Sigma H2100 microwave oven.

ability of the visual system to distinguish between an object and its background color. Various cells of the human eye participate in the visualization of an object, distinguishing such characteristics as color, size, shape, contrast, etc. These cells process the information into neural codes and transmit them to the brain, which in turn decodes and formats all of the information into visual perception.[1] Most persons have normal contrast sensitivity and can readily perceive an object in a color environment. If, however, that environment has a low contrast, such as a pink object with a red background, or a brown object with a dark tan background, the sensitivity can be sufficiently diminished as to render an object difficult to distinguish morphologically, if not totally impossible to see. This concept is illustrated by the images in Fig. 1. Histotechnologists must be attentive to a stain procedure that will present a sharp visual image to their pathologist, and make it easy to see outline and detail of the organism through contrast.

Principle/Primary Stain

By nature, acid-fast bacilli possess a semipermeable membrane surrounded by a lipid layer. Once the primary stain is applied (commonly carbol fuchsin), this lipid layer enables the bacillus to resist the decolorizing effects in the steps that follow. When using this stain, all cells, including acid-fast bacilli, are stained a deep characteristic red with carbol fuchsin, then the tissue is decolorized with a mild acid-alcohol to differentiate the bacteria from other tissue elements. Non-acid-fast bacteria will not retain the stain after decolorizing. The tissue is counterstained with a softly contrasting stain of light blue or aqua, usually with methylene blue.[2]

The most commonly used primary dye solution in acid-fast staining is carbol fuchsin, which contains a phenylmethane dye (new fuchsin, basic fuchsin, or pararosaniline) in an alcoholic-phenol solution. The phenol in the solution helps enhance the basic fuchsin dye and promotes a stronger binding of dye to the organism. Two common formulas of carbol fuchsin are found in the Ziehl-Neelsen and Kinyoun techniques. Both stains impart a rich red color to acid-fast bacilli. Kinyoun's, because of its higher concentration of dye, is often preferable for smaller, more filamentous bacteria, or for procedures requiring a rapid staining time. Heating of the primary stain with a microwave or convection oven has been shown to promote enhanced staining and also decrease staining time.

In a previous study we demonstrated that there is a direct relationship between staining time in carbol fuchsin and depth of color achieved in the bacteria. Slide sections of tissue containing tuberculosis in the form of lesions of aggregate colonies were stained with carbol fuchsin. Microwave irradiation was used with the slides left to incubate in the warmed solution for increments of 1 minute, 5 minutes, 10 minutes, and 15 minutes. The slides stained for 1 minute expressed only a faint tinge of pink color and had a very low contrast resolution.

Progressively we could see a marked increase in intensity in staining from 5 minutes through 15 minutes. A distinct staining pattern associated with the duration of staining time became apparent. With the staining times of 1, 5, and 10 minutes, the difference in color was more noticeable in the center of the tubercular lesion than on the outer periphery. Bacilli that were in the more central portion of the lesion showed a deeper stain than those on the outer areas. When stained for 1 minute, organisms on the outer edge were almost translucent, demonstrating very little stain retention, while the central area of the lesion was a slightly more intense pink. The bacteria at the periphery of the lesion stained very well when incubated in carbol fuchsin for 10 minutes but remained slightly weaker than those in the central area. Since we know that all tissue elements will generally stain with carbol fuchsin, it was apparent that the older bacilli (those on the periphery of the lesion) appeared to lose their ability to retain the dye. One theory is that with the aging of bacilli there is a progressive loss of lipid content in the surrounding capsule layer, thus making it more susceptible to acid-alcohol rinses.

PROCEDURE A
(Microwave Technique)

This method utilized a modification of the Ziehl-Neelsen technique with microwave heating of the carbol fuchsin solution. An 800-watt laboratory microwave instrument (Fig. 2) was used (H2100, Sigma Diagnostics).[3] Sections of infected lung were taken at 4 microns, mounted, and dried on slides. The slides were deparaffinized, hydrated to water, and placed in 40 ml of carbol fuchsin solution contained in a plastic coplin jar. With a vented lid placed on top, the slides were irradiated at 50% power for 30 seconds, then gently agitated with a plastic pipette (3-4 draws), and incubated for 10 minutes. Slides were then rinsed in distilled water to remove excess stain. After rinsing, the slides were placed in malachite green solution (Sigma) for 1 minute at room temperature. Malachite green solution serves as a differentiator and counterstain.[4] It simultaneously removes excess carbol fuchsin while leaving

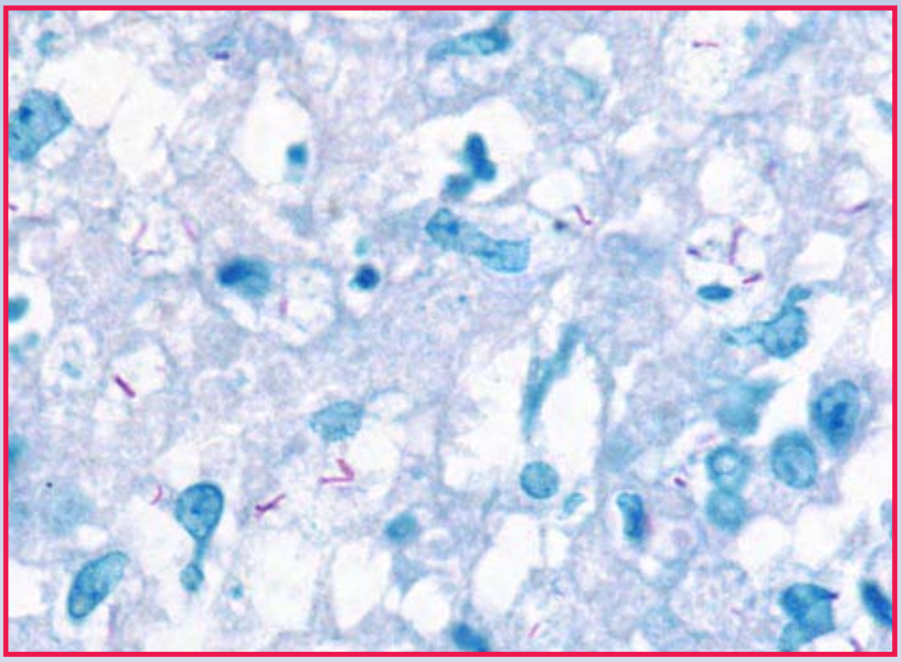

Fig. 3. *Mycobacterium tuberculosis* stained with heat-induced Ziehl-Neelsen technique for acid-fast bacteria. (Oil immersion)

a contrasting turquoise-green background. This step added to the quality control by consistently applying the same level of optimized differentiation and counterstain to the procedure, thus eliminating variability from slide to slide for those done in the same special stain run. While the turquoise-green background appeared to offer a higher contrast sensitivity to the bacilli than methylene blue, both were excellent counterstains when used properly (see Fig. 3). Slides were rinsed in tap water after counterstaining, air dried, then dipped in xylene and mounted with a permanent mounting medium. Results demonstrated bacteria in a deep red with a green to turquoise background.

PROCEDURE B
(Convection Transfer Heating)
The second procedure is a proprietary variation of the Fite method for acid-fast bacilli where slides are sectioned, dried, then placed in a xylene-peanut oil mixture for deparaffinization, blotted, and stained. The Fite method was developed primarily to demonstrate more thinly filamentous types of acid-fast bacteria such as *Nocardia* and lepra bacilli. Morphologically, *Mycobacterium leprae* are thinner organisms than *M tuberculosis* with a reduced lipid layer content. This difference accounts for less dye uptake by the organisms due to a decreased diameter and diminished resistance to alcohol and acid decolorization. The xylene-peanut oil mixture is believed to enhance the lipid layer and the alcohols typically used in the slide deparaffinization scheme are eliminated from this stain method. Heat was applied through the use of the StainQuick™ System (Cel-Tek, Inc.) in which six staining jars are housed in a stain rack with each container holding its respective dye or reagent for the procedure (see Fig. 4). The container reserved for the carbol fuchsin has an outer metal sleeve, which heats the solution to 53°C. Four-micron paraffin sections were dried and placed in Paraclear™. The slides were transferred into warmed Enhancing Fluid™ (Cel-Tek, Inc.), which is a xylene-peanut oil mixture, for 1 minute. Slides were washed in warm running water for 3 minutes and placed in the prewarmed jar of carbol fuchsin for 1 minute. After primary staining they were washed again in warm running water for 1-2 minutes then decolorized in 2 changes of decolorizer (5% sulfuric acid in 25% ethanol) for 1 minute each. Slides were washed in warm running tap water for 1 minute, counterstained in methylene blue for 30 seconds, and rinsed again in warm water. They were then blotted, allowed to air dry, dipped in Paraclear™, and coverslipped. The organized rack system makes the procedure flow very quickly and with ease. Results demonstrated acid-fast bacilli in a deep red color with a light blue contrasting background.

PROCEDURE C (Fluorochrome)
The last method of acid-fast detection discussed here provides the highest degree of visual contrast and gives the microscopist the best opportunity to identify individual organisms within a tissue matrix. Acri-Fluor Fluorescent Stain™ (Scientific Device Laboratory, Inc.)[6] utilizes a non-antibody fluorochrome dye that can be visualized under the microscope after excitation with ultraviolet light (see Fig. 5). The electrons of the fluorochrome dye, in their excited state, emit energy in the form of visible light (yellow, orange, red), thus causing the organism to visibly fluoresce against a dark green to black background.

Four-micron paraffin sections were placed on a slide, dried, deparaffinized, and hydrated to water. Care must be taken to avoid having the tissue dry out during staining. Slides were laid out horizontally on a staining rack or countertop for staining. Drops of the Acri-Fluor dye were placed on the slide just liberally enough to cover the tissue; slides were stained for 15 minutes at room temperature, then rinsed in deionized water and allowed to drain. Drops of Destainer Solution™ (Scientific Device Laboratory, Inc.) were placed on tissue sections and allowed to stand

Fig. 4. StainQuick™ System (Cel-Tek, Inc.).

Fig. 5. Acri-Fluor Fluorescent Stain Kit™ (Scientific Device Laboratory, Inc.).

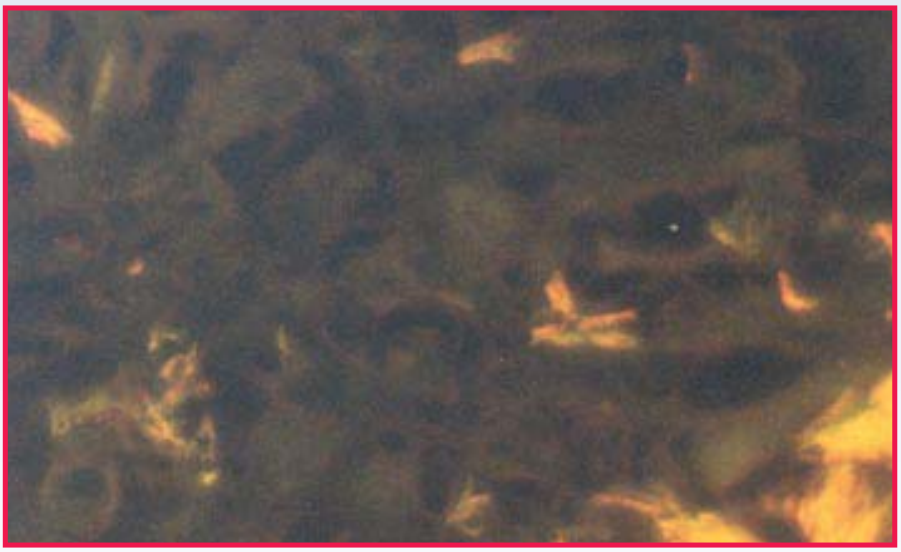

Fig. 6. Enhanced visualization of acid-fast bacteria is shown using fluorescent stain technique. *Mycobacterium tuberculosis* bacilli are illustrated in bright yellow.

for 2 minutes. As in procedure A, the Destainer Solution acts as a simultaneous decolorizer and counterstain. Slides were rinsed in deionized water, drained, air dried, and coverslipped with an aqueous mounting medium. After staining, the slides were viewed with a fluorescence microscope. Acid-fast bacteria appeared bright yellow to orange against a dark background of dark green to black (see Fig. 6). This procedure is often used as the final confirmation to rule out the presence of acid-fast bacteria when other attempts have failed to produce the suspected organisms, as contrast sensitivity is enhanced. When optimally performed, it allows one to more easily discern the morphology of individual bacilli.

Summary

Each of the procedures presented above offers an enhanced targeting ability of the primary dye through its specific chemistry and/or the inducement of heat. With each stain, bacteria were presented as richly colored, well-defined entities, whether they were in aggregate colonies or as single organisms. Additionally, optimal background color provided the ability to further enhance identification of organisms through improved contrast.

Conclusion

In histological staining, success in the detection of microorganisms is as much a part of contrast sensitivity as it is primary stain effectiveness. Histotechnologists must find ways of optimizing their ability to target and visualize these microscopic structures. Contrast sensitivity of the eye must be considered with attempts made to optimize the crispness of perceived structures, making it easier for the eye to locate and identify. While the pathologist and histotechnologist may rarely interact while performing their respective duties, their roles are intimately linked and must be in alignment to ensure optimal diagnostic success. The pathologist must be able to trust that all steps were taken to maintain the integrity of methods

and procedures, and that the picture being presented to them is an accurate and clear representation of tissue elements within. The histotechnologist must strive not only to perform analyses, but also to create a perfect picture, demonstrating and highlighting, or eliminating, structures that might be misidentified as pathogenic organisms.

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Tools to Facilitate and Standardize Grossing

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Grossing, a term that refers to examination and dissection of surgical specimens, along with preparation of sections from those tissues requiring processing, is the initial step in the practice of surgical pathology. While textbooks and manuals of surgical pathology teach about the sampling of specimens, they are silent regarding the dimensions of individual tissue slices, other than to suggest a thickness of 2 to 4 mm. Unfortunately, it is not an uncommon practice to place as much tissue as will fit into a specific size cassette. Although this liberal approach to grossing

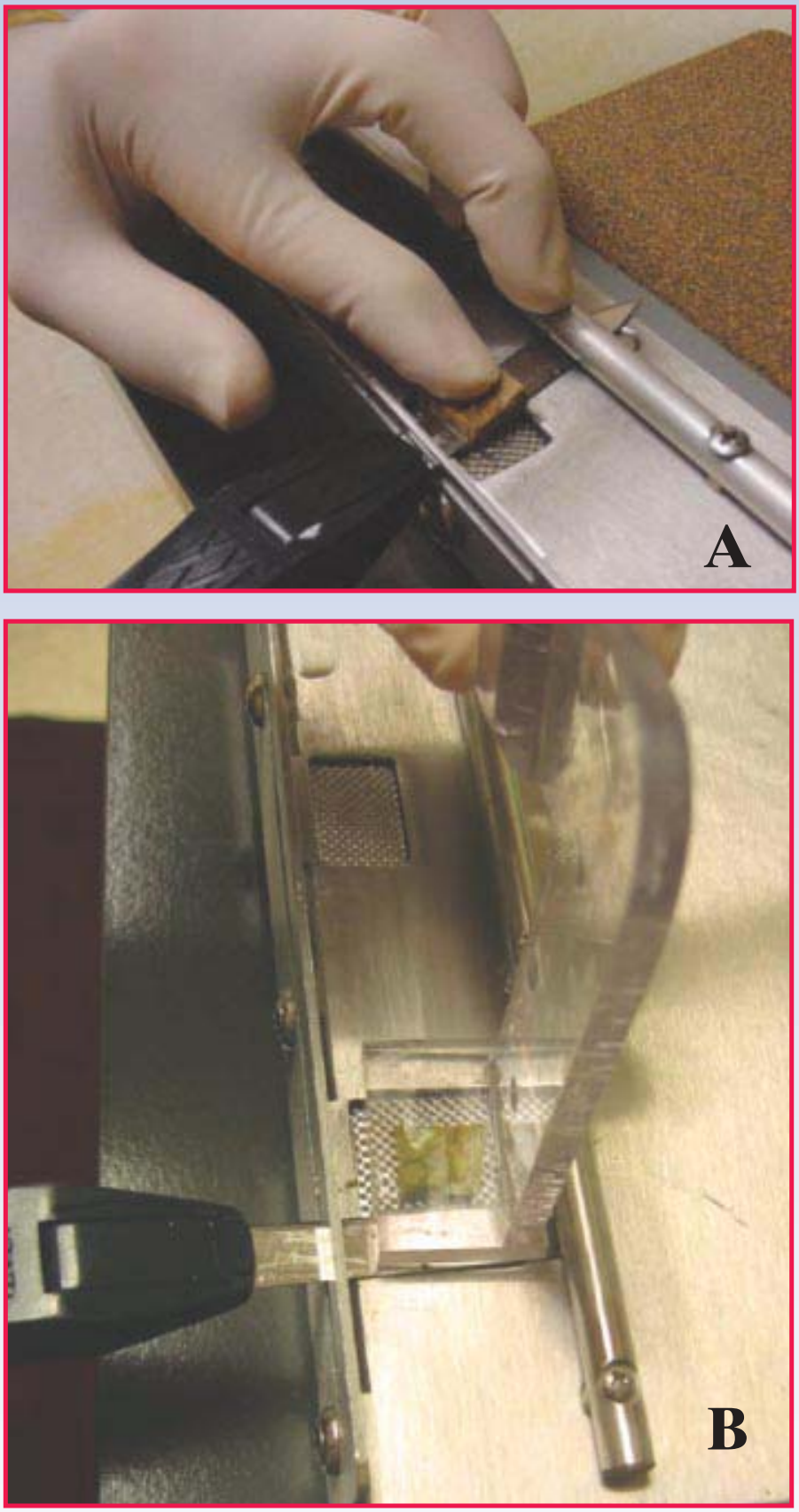

Fig. 1. These photos show slicing of tissue that is held in place by either A) index finger, or B) plastic holder.

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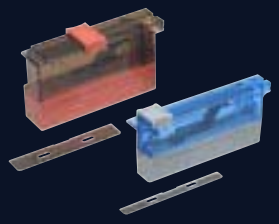

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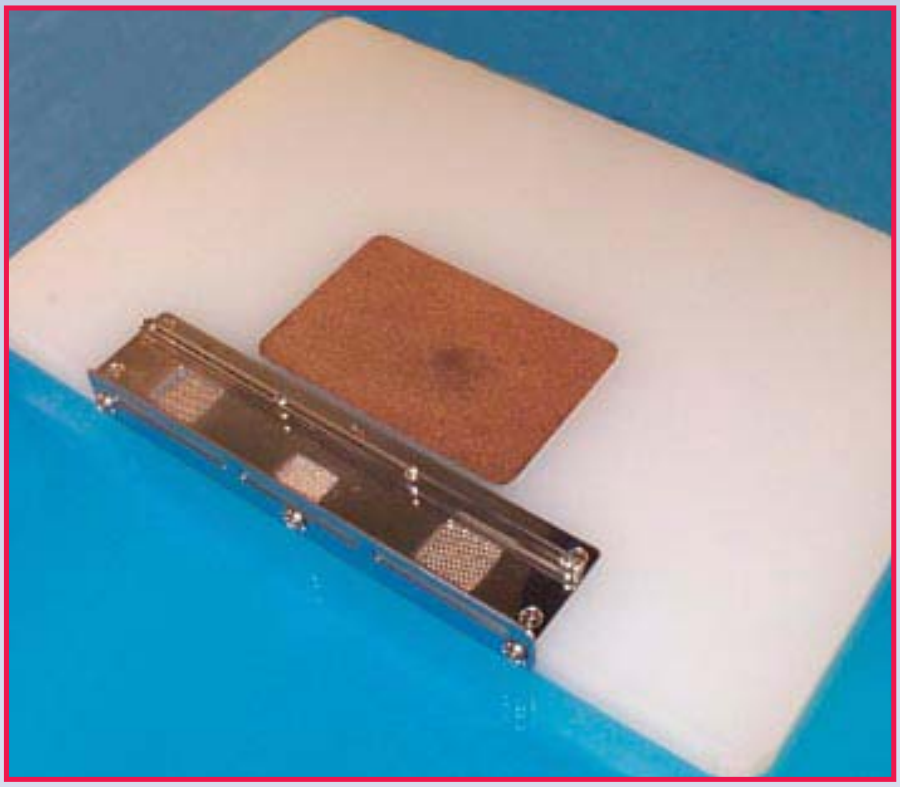

Fig. 2. Grossing board with slotted metal plate and knife-guiding assembly.

Fig. 3. Tissue sample obtained as illustrated in Fig. 1.

may have been permitted by conventional processing methods lasting 12 hours or longer, it fails with rapid tissue processing now done by microwave-based methods and by shortened conventional assays. The requirement to adjust the length of the processing cycle according to the thickness of tissue sections is discussed in publications of microwave-based methods such as Visinoni et al,[1] and Willis and Hinshew.[2] Conversely, Morales and associates advanced the notion of standardizing tissue sections to accommodate the processing cycle in microwave methods.[3]

Appropriate tools are required to obtain proper slices of tissue for processing and are essential to standardization of tissue sections, but grossing tools created specifically for pathology are rare. A review of the literature and MEDLINE searches for "grossing tools" or "pathology tools" failed to yield a single reference. There are, however, several publications about the birth and evolution of surgical instruments that have evolved with the advances of surgical ingenuity and its demands. It has been customary to use the same instruments developed for the practice of surgery for grossing. It is a tradition, passed on from one generation to the next, with poor results and no standardization in grossing the tissue.

During the course of developing and implementing an automated microwave-based rapid tissue processing system at the University of Miami/Jackson Memorial Hospital, the need to standardize the dimensions, and particularly the thickness of tissue sections, became readily apparent. To that end, we created two tools that greatly facilitate grossing, not only for microwave-based methods, but also in conventional tissue processing. These tools are illustrated in Figs. 1-5.

Fig. 4. These photos show slicing of bowel with the assistance of the grossing tool: A) sliding the blade against the outer surface of the tines; B) slice of tissue occupying gap between tines.

As previously reported[3] and also described in detail elsewhere,[4] the grossing board permits preparation of uniform tissue slices of the desired thickness. It consists of a board with a slotted metal plate and a track for the knife or surgical blade (Figs. 1, 2). The slots serve as wells for the placement of the tissue and the knife-guiding assembly keeps the cutting blade in place. As the bottom surface of the slots are parallel to the cutting surface, sliding the cutting blade in the track along the surface of the cutting board yields tissue pieces of uniform thickness, which facilitates processing, as well as microtomy. Because the depths of the slots are easily adjusted, sections of desired thickness are obtained. Moreover, the slots can be built large enough to facilitate serial slicing of organs, such as prostate and breast.

While the grossing board greatly facilitates sampling of solid organs and tumors, flat structures such as skin and small tubular organs like the appendix, fallopian tubes, and umbilical cord, are not amenable for its use. For those, we developed another tool that holds the tissue in place to permit sampling of uniform desired thickness. This tool is particularly helpful in grossing organs composed of layers of different structures that slide over each other during sectioning, such as the bowel and gallbladder. Additionally, tissues that are soft and slippery, such as adipose tissue, or fatty tissues, such as breast, are easily handled with this tool. The tool is composed of a handle and a head supporting a double array of four tines (Fig. 5). The gap between the double array of tines is predetermined to accommodate the desired thickness of tissue sections (Fig. 5 inset). As illustrated in Fig. 4, the tissue is held in place while sliding the blade against the outer surfaces of the tool, thus yielding a section of the desired thickness in the gap provided between the double array of tines.

Fig. 5. Grossing tool consisting of handle, tine-supporting head, and double array of tines (inset).

This contrasts with the common practice of holding such tissues with forceps during sectioning, a procedure which produces irregular slices of tissue, varying considerably in dimensions and thickness.

In summary, we describe two tools that allow standardization and greatly facilitate grossing by providing tissue sections consistently uniform in thickness.

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A Technique for Correcting Poorly Processed Paraffin Blocks

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Every histotechnologist is familiar with the frustrations of trying to cut a block that was underprocessed and "mushy" because it was grossed in too thick. In some cases, adjusting the microtome to a thicker setting (6 or 8, or even 10 microns), re-embedding the specimen after squeezing the tissue to remove residual xylene, or freezing with freeze spray, may make it possible to obtain a section. Some of these blocks are so poorly processed, however, that they cannot be cut at all no matter what tricks we attempt in order to get that one section the pathologist needs. When nothing works to obtain a satisfactory section, the tissue must be reprocessed. This is typically a time-consuming, hands-on process.

Histotechs are always looking for a way to automate the process in order to save time and energy. One of the most common techniques is to first melt the block down to remove the paraffin. The tissue is then put back in the cassette, placed in a tissue processor, and run through a purge or cleaning cycle. The tissue emerges with the paraffin and xylene removed and wet with 100% alcohol. Then the tissue is manually run through 95% and then 70% alcohol before it is placed back in formalin and reprocessed with that night's surgical tissues. This works satisfactorily in most cases. However, it does subject the unprotected tissue to hot xylene and hot alcohol which is quite harsh and can cause distortion of the cellular morphology.

Several years ago, the histotechnologists at Sacred Heart Medical Center (Spokane, WA) returned from an NSH symposium with a novel technique for reprocessing tissues that involved far less technical time, and it spared the tissue from the harsh treatment of a purge cycle (see Fig. 1). This procedure involves melting the block down and blotting off the excess paraffin before putting the tissue back in the cassette and placing the cassette directly into formalin for reprocessing with the regular run of tissues for that night. The results are remarkably good with less handling time.

The rationale for reprocessing blocks this way is simple. The portions of the block that were adequately processed initially are spared further dehydration as the remaining paraffin insulates the tissue from the effects of alcohol, which in excess can make some tissues very hard and brittle. The paraffin remaining in this previously processed block is eventually removed in xylene on the processor and the entire sample is then reinfiltrated with new paraffin. The portion of the block that was previously underprocessed is reexposed to the effects of fixative, dehydrant, clearing agent, and then infiltrated with paraffin. When complete, all parts of the block are properly processed and infiltrated, and the block cuts very well. Undoubtedly, part of the reason the tissue processes better the second time around is that it is thinner because some of it was cut away during initial attempts to cut the block.

Although the originator of this method is unknown to me, I thought it would be a useful technique to share with the histotechnology community.

Perhaps someone among us knows who first reported this method so that he or she can receive credit for such a novel and useful technique.

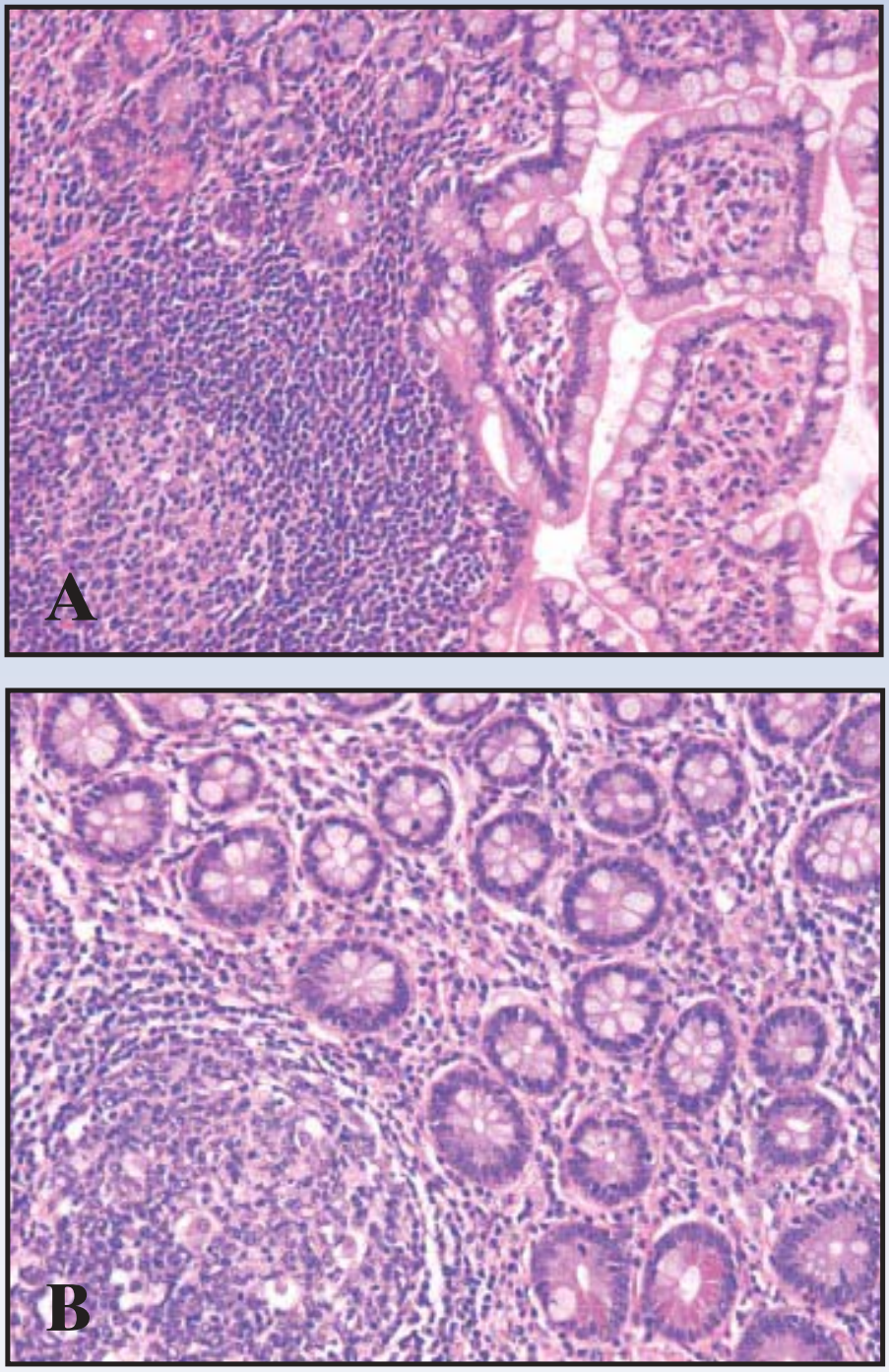

Fig. 1. Above are photos of tissue blocks reprocessed using A) the new technique described, in which the melted-down block is placed back in formalin and reprocessed, and B) the purge technique. Nuclear detail is quite good in both cases, but nuclei seem to show somewhat more distortion using the purge technique.

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