MILES

Histo-Logic

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The Quality Control Dilemma in Histotechnology: A Possible Answer

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Introduction

The difficulties of developing quality controls for microscopic slides in histotechnology are well known. No doubt, we can use a specific fixative that helps us to predict some of the effects on tissue as far as speed of penetration, hardness of tissue, effects on sectioning, and staining results. It is not difficult to program tissue processing schedules and thermostatically control paraffins to prevent burned or denatured tissue. With the numerous automatic knife sharpeners and disposable knife systems on the market today, it is not difficult to learn the mechanics of knife sharpening. Nor is it difficult to learn the mechanics of microtomy, which are necessary for obtaining tissue sections. We are all familiar with numerous staining procedures available in literature to assist in producing hematoxylin and eosin (H&E) stained slides. The same can be said of stain technology (special stains), and methods for decalcification of bone specimens, etc.

The last 15 years has ushered in a good many books on histotechnology. These books can be found in many laboratory libraries where they are used by the Histotechnologist for reference and/or assistance in his/her attempts to demonstrate the highest quality and most specific tissue entity. The demonstration of these entities involves normal or pathological, human or animal tissue specimens. In addition, many Histotechnologists are familiar with the use of tissue microscopic slide controls containing a specific organism, material, or structure to control stain technology. For example: Goblet cells in the villi of the small intestine are frequently used to determine if the Mayer's or Southgate's mucicarmine procedure stains satisfactorily. Skeletal or heart muscle is used to quality control the staining properties of Mallory's phosphotungstic acid hematoxylin (PTAH). A piece of in fected appendix is often used to quality control th methods for the demonstration of gram-positive and gram negative bacteria, etc.

The efforts at quality control are commendable, but th present state of the art is not satisfactory. Thermostatically controlling paraffin pots, availability of processing schedules, and different processing agents do not ensur

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properly processed tissue. Nor do they prevent the possibility of denatured tissue. Using automatic knife sharpeners and disposable knife systems do not ensure a sharp knife. Mastering the mechanics of microtomy does not ensure high quality, artifact-free sections. Utilizing any of the many standard hematoxylin and eosin staining procedures and following the staining schedule to the letter do not result in high-quality H&E's. Using normal or well-fixed tissue and tissue containing certain materials (bacteria, etc.) as control for stain technology does not guarantee that the special stain being performed will react satisfactorily on the unknown specimen. Possessing reams of information in your laboratory or library will not make you a knowledgeable Histotechnologist.

If the art of microscopic slide quality control in histotechnology is not satisfactory as it presently exists, what then is necessary to make this most important laboratory function an effective element in the overall patient-care process?

I believe several things are a must if we are to reach this important and necessary plateau. Provided below are some suggestions and ideals for your consideration.

First, the following are necessary ingredients to making quality control operational in histotechnology.

- One must realize and admit that the need exists for improved quality control and be dissatisfied with its present status.
- There must be a willingness to acknowledge that knowing everything about histotechnology does not necessarily mean we make it work. *Remember that it is not what you know that counts so much as what you put to use.*
- One must possess an inner need and desire to know more about the profession of histotechnology and work toward attaining the set goals.
- One must be *desirous* and dedicated enough to pay the price required in time and study. The use of common sense creatively in performing every aspect of histotechnology is a must for achieving success in all aspects of this laboratory discipline.
- One must be *intently* involved in the performance of all duties in the laboratory. For example, a person walking on a sidewalk is not intently involved in where he places every step, but a person walking a thin wooden plank 200 feet above ground will be intently involved.

Achieving the above goals leads us to the second stage of developing quality control. Provided below are some suggestions for quality controlling the science of histotechnology.

Fixation

Much has been written about fixation so I will not elaborate extensively but merely mention the most troublesome area to a Histotechnologist, namely the fact that most specimens are macrosectioned (grossed) too thick for adequate fixation and subsequent processing. Most surgical services fix tissue from a minimum of 3 hours to a maximum of 8 hours. It takes 24 hours to completely fix a piece of tissue measuring 4 mm in thickness. This suggests that most surgical tissue specimens placed in fixation on the tissue processor are not properly fixed. There is nothing a Histotechnologist can do about this, since overnight fixation and processing of surgical specimens are controlled by the need to provide as rapid a diagnosis as possible. However, we can do something about the persistent problem of tissue being grossed thicker than 4 mm. We should constantly badger our Pathologist or Resident with the need for thinner specimens. In my opinion, specimens should not measure more than 3 mm in thickness if they are being fixed and processed on an overnight schedule.

Processing

There are probably as many processing schedules as there are laboratories in this country. This in itself points out the need for some means of determining if a processing schedule is adequate. A useful means to accomplish this is found in Fig. 1. The Histotechnologist can quality control the dehydration, clearing, and impregnation of tissue specimens by utilizing the following procedure: Take a highly cellular specimen (spleen, lymph node, liver, etc.) from the morning's surgical run, place it on your microtome, and rough cut (macrosection) into the paraffin block to expose the tissue surface. Next, dip a square piece of cotton in the warm (42°-45°C) flotation water bath until totally saturated. Squeeze out most of the excess water and place the cotton against the face of the specimen. If the specimen turns chalky white after a 2-minute exposure, your tissue has been excellently processed and properly impregnated (Fig. 1). Obtaining quality sections during microtomy will be possible.

If the specimen turns white with a 45-second exposure, it has been processed and well impregnated (Fig. 1). Complete surface cut sections will be possible although they will have some holes in the area that is poorly processed. However, deeper cuts will result in some incomplete sec-

MOISTURE EXPOSURE	QUALITY OF IMPREGNATION	PROCESSING REMEDIAL STEPS
5 seconds	Extremely poor (All specimens turn white.)	Requires drastic processing schedule changes in every step. (Change solutions. Increase time in all steps by 30 minutes each.)
10 seconds	Very poor (90% of the specimens turn white.)	Requires drastic processing changes in most steps. (Change solutions. Increase time in all steps by 30 minutes each.)
20 seconds	Poor (50% of the specimens turn white.)	Requires moderate processing changes in most steps. (Change solutions. Increase time in all steps by 15 minutes.)
45 seconds	Good (20% of the specimens turn white.)	Requires minor processing changes. (Usually an increase of 15 minutes in the last dehydrant, clearant and paraffin.)
2 minutes	Excellent (5% of the specimens turn white.)	Requires no changes.

Figure 1: This chart provides valuable information on how to identify processing problems and what steps to take to solve those problems.

tions. A well-processed, properly paraffin-wax impregnated specimen will withstand 5 minutes or more exposure to the warm water soak treatment without turning chalky white.

One other effect to look for in poorly processed tissue, if the chalky white color is not evident, is the swelling of the specimen beyond the surface of the paraffin. Figure 1 provides simple corrective measures that can be taken to improve improperly processed tissue specimens.

In the unlikelihood that tissue is still incompletely processed by following the suggestions cited in Fig. 1, look for some other factor in the tissue processing system or schedule including the type of dehydrant, clearant, or impregnation medium in use. Remember, most problems relative to poor processing are the direct result of inadequate exposure to the clearant and paraffin and, to a lesser extent, exposure time to the dehydrants. Also, remember that any aid to impregnation is beneficial. For example, the use of vacuum and pressure hastens penetration of paraffin and produces better impregnation. There are several excellent closed processing systems on the market that allow for the introduction of vacuum and pressure. Also remember that the processing schedule need not be any longer than what is required for forming a solid matrix in all tissue interstices.

Embedding

Always ensure that the surface of the block that is to be sectioned is flat and pressed gently against the bottom of the embedding mold. This will help to ensure all areas of the block are obtained during microtomy. One of the biggest concerns to the diagnostic process are those specimens that have been embedded on edge or small areas of the block that are not included on the slide. Remember, even the smallest piece missing from a section could hold the key to the final diagnosis. The problem of missing tissue from a section is particularly significant when one deals with biopsy specimens (Fig. 2). As indicated in the introduction, be intently involved in what you are doing, especially during the embedding phase of histotechnology.

Microtomy

Before we discuss microtomy, it is essential that we understand what constitutes a sharp knife. First, there is no such thing as a sharp knife in the literal sense. The cutting effectiveness (efficiency) of a microtome blade is determined not by sharpness alone (the convergence of the beveled facet to a mathematically exact angle) but by the molecular status of the knife facet. Specifically, is the surface of the facet in an amorphous or crystalline state? The quality of tissue sections obtained during microtomy is determined to a great degree by the molecular status of

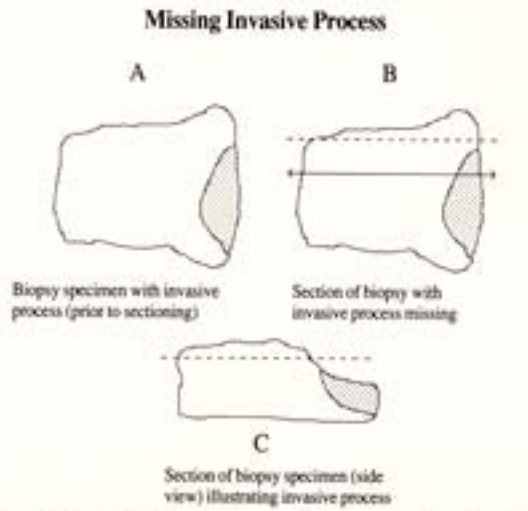

Figure 2a, b, and c: This schematic attempts to demonstrate the value and importance of proper embedding and subsequent microtomy of tissue specimens. Schematic (a) illustrates an invasive process (dark area) prior to embedding and microtomy. Schematic (b) illustrates two possible scenarios: 1. a section taken through an area that would miss the invasive process (dashes) and 2. a deeper cut and/or better embedding procedure (arrows) would have solved this potential problem. Schematic (c) illustrates a side view of the same specimen to show the missed invasive process (dashes).

the microtome blade facet surface. It is essential to develop (produce) an amorphous surface on a microtome knife facet, or to purchase disposable blades that contain an amorphous surface. The latter are generally those disposable blades that have a noncrystalline surface coating of some sort. Regardless of which kind of blades one uses or where one obtains them, it is impossible to produce high quality tissue sections without a blade facet with an amorphous surface. You are encouraged to review the article in *Microviews* cited in reference one. Any further reference to "knife sharpness" will encompass the amorphous knife bevel surface theory. That is when the term *sharp* is used to automatically mean a knife bevel with an amorphous surface.

I sincerely believe that the failure to obtain a maximally sharp knife produces 60% of the problems in obtaining good sections in histotechnology. There is a difference between a sharp knife and one that is maximally sharp. One can obtain a "sharp knife" by using any one of the knife sharpeners on the market. However, that knife may not be sharp enough to produce thin, wrinkle-free sections. The degree of sharpness of a microtome cannot be gauged. That is, no mechanical or scientific way exists to quality control knife sharpening. This being the case, alternate means for quality controlling knife sharpness must be utilized. The following suggestions are not the ultimate, but they will assist in determining whether a microtome knife will cut satisfactorily: A sharp knife should section a large (2.0 cm), well-processed lymph node at 4 microns with little difficulty. Sections should come off the knife with just a slight downward curvature (like paper coming off a printing press) and without compression. There should be no difficulty in sections sticking together to form the ribbon. One should be able to lay the ribbon on a flotation water bath and not have to eliminate wrinkles by pricking or excessive pulling on the ribbon. The need for using alternate procedures (for removing wrinkles), such as adding wetting agents to the flotation bath water or exposing sections to alcohol before placing them in the water bath, are not necessary with a sharp knife. Microtomy becomes a pleasure with the use of a sharp knife, but a difficult, frustrating, and sometimes impossible task with a dull one.

The other aspect that deals with a sharp knife (presence of an amorphous surface) is the method and sharpener used. Is one sharpener better than another? This may be true but I tend to lean toward the concept that all sharpeners on the market today differ only in the way the knife is attached, the mechanical motion, and the variable times required to sharpen. I believe all are capable of producing optimally sharp knives. Why then do we face the constant inconsistency in reproducing a good degree of sharpness? I believe the major part of the problem lies with the operator and, to a much lesser extent, the instruments. The one fault in knife sharpening is that most of us have taken the term "automatic knife sharpener" literally. Since the instrument is designated as automatic, we think all we have to do is sharpen the knife for a given number of minutes or strokes and our knife is sharp. This is not true. In my opening comments, I stated that one of the ingredients for success was to be intently involved in the performance of each task. This requirement is an absolute must in knife sharpening. You must make every attempt to produce an amorphous surface. An amorphous surface can be produced by facet surface polishing by hand or by mechanical means. Polishing the knife facet alters the crystalline structure of a metal surface and causes a molecular flow, whereby the surface becomes covered with an amorphous layer that may be likened to a highly viscous liquid. This development of an amorphous surface at the molecular level on a knife facet can best be explained by the following analogy: The continual polishing of the surface of a crystalline structure (microtome metal blade facet surface) is seen to fade slowly as if one were observing through a powerful microscope the solution of crystal supernatant on

a solvent. Stropping (polishing) of a microtome blade on a linen or leather strap can be accomplished in 30 to 60 seconds. This degree of polishing is sufficient to develop the amorphous layer on the knife facet. During the polishing process, it is important that the knife facet be completely flat on the stropping system since too much facet angle will result in a dull knife. This is because excessive polishing will take place on or close to the intersection of the two facet planes. On the other hand, insufficient bevel angle will result in the polishing of the heel of the facet and therefore make polishing ineffective. The development of an amorphous layer is also necessary if one uses an automatic knife sharpener. Therefore, the purchase of such a system must be dictated by how the final polishing of the facet is performed. Is the final action sufficient to provide a good maximum polish of the knife facet? Most knife sharpeners have a polishing system incorporated into the knife sharpening scheme. This is performed by polishing with a very fine abrasive or the use of glass plates and a semiviscous liquid. Other systems incorporate special materials, such as leather or pseudo-leather, while others may incorporate specially treated paper for final polishing. Regardless of the polishing system used, the most important thing to remember is that metal microtome blades must possess an amorphous surface in order to achieve improved coefficiency as the knife bevel travels through the paraffin/tissue block. As indicated, all section cutting facet surfaces must possess an amorphous layer, including disposable blades. Most, if not all, disposable blades are sharpened (and polished) in the same way by the manufacturer. This includes blades that are used for shaving. One must remember that regardless of which blade one considers, the coefficiency of the blade facet is determined by whether the facet contains an amorphous surface. The reason some disposable blades, from the same container, cut better than others is because of the high quality of polishing of that specific run of blades by the manufacturer. I'm certain that every Histologist has experienced varied cutting efficiency with different blades from the same distributor. There are, however, some disposable blades that circumvent the development of an amorphous layer by polishing. This is done by the blade facet being coated by the manufacturer with a noncrystalline material. This coating can be equated to the formation of an amorphous layer since at the molecular level it possesses the same qualities.

Moisture in Tissue During Microtomy

Other factors are also involved in the production of good sections. Namely, the proper knife rake angle, facet clearance angle, and the quality of the paraffin. In addition, the moisture content of tissue constituents during microtomy is perhaps more important. The latter is the second most important step in the production of high quality sections for microscopic evaluation. Failure to ensure proper re-introduction of moisture into the specimen during microtomy produces a wide variety of tissue-cutting artifacts.

Hematoxylin and Eosin Staining

Many things could be said regarding the approach to quality controlling the H&E stain. Suffice it to say that the final result of a stained slide should contain the following tinctorial qualities:

Nuclear Chromatin: the nuclear chromatin should stain blue to bluish purple and be very distinct.

Nucleoli: should appear reddish purple if the hematoxylin is well decolorized. If too much hematoxylin remains in the nucleoli, there no doubt is excess hematoxylin in the section. The same can be said of the eosin counterstain. Excess eosin will produce a purple monochromatic nuclear chromatin and ill-defined nucleoli.

Collagen and Muscle: should stain well but in a delicate fashion. One should be able to see some form of fibrillar pattern by moving the microscope focal plain up and down. The same pattern is observed by reviewing muscle except that muscle should have a slightly redder color than collagen.

Eosinophils: Eosinophilic granules should be well defined and appear orange-red. Lack of well-defined eosinophilic granules suggests overstaining by either hematoxylin or eosin, or both.

Tissue Section: a tissue section that appears purple and monochromatic under the microscope has not been blued sufficiently or the eosin was not differentiated sufficiently. Proper blueing of the H&E stained slide is essential. The biggest problem with blueing is generally related to cold running water used to blue slides or used after slides have been exposed to a blueing agent. This problem can be corrected by the use of lukewarm (37°-40°) water for blueing and/or for any steps that require working in running tap water.

These suggestions for quality controlling H&E staining are not all-inclusive but should be sufficient to help remedy staining problems. A further suggestion is the use of a good microscope for daily review of H&E stained slides. Slides are reviewed to determine what may be a staining problem or tendency. A mental note of the problem or tendency is made for use the next day to institute necessary staining changes.

Special Stains

(1) The first things to consider are the use of a good microscope to review finished slides and the use of lukewarm (37°-40°C) water whenever a given method calls for a running water wash. Tap-water washes at this temperature, in my opinion, are essential in any staining procedure where washing is required, including silver reactions. (2) The movement of staining solution molecules is extremely important if not essential. Many poor staining results are directly related to the lack of good molecular movement. Inadequate molecular movement may be the result of a laminar flow effect (a flow of various fluids in which neighboring "layers" are not mixed). In staining solutions, a laminar flow-type effect is produced by the rough surface of the staining containers. In other words, molecules move slower the closer they are to the surface of the inner wall of the staining containers (Fig. 3). This slower movement of molecules eventually affects the staining results. It is for this reason that one often obtains different staining results on identical slides that have been stained in the same container. (3) One must also remember that in microwave staining results can vary considerably between the top and lower portions of a slide. This is because solutions that are heated with microwave *radiation* can vary up to a 15°C difference in temperature between the top and bottom levels of the solution (Fig. 4). Therefore, in order to equalize the temperature of the solution, the slides must be dipped up and down in the staining step(s) of the staining procedure. (4) Staining slides in a convection slide drying-type oven can also pose some staining problems. Slides situated near the outer walls of the stain container will stain darker than those in the middle. This is because there will be more heat on the outer walls than in the middle of the staining container (Fig. 5). One should be aware of this possibility when using a convection oven for staining. The second potential problem has to do with a solution reaching a stated temperature. For example, if a stain requires 30 minutes exposure in a 60°C oven, one should place the staining solution in the oven prior to the introduction of the slides. This will ensure exposure to 60°C heat for the entire 30 minutes, thus eliminating uneven staining.

Silver Reactions

The key to silver staining revolves around the fact that a reducing site must be available to reduce silver ions. An example of what may go wrong in one form of silver staining is provided.

Oxidizers

The various methods of silver impregnation of basement membranes and reticular fibers include an oxidative step following fixation and prior to the action of the silver salts themselves. The aim of this oxidative step is to increase the number of argyrophilic sites so significantly that a selective blackening of these structures can be obtained. Concomitantly, a considerable diminution in the number of argyrophilic sites takes place in neighboring structures, leading to a colorless background. There are many reasons for assuming that, following oxidation, some potentially stainable groups are transformed to stainable groups, and numerous argyrophilic radicals are converted into nonargyrophilic ones (Fig. 6).

Remarks

Cited herein are a few suggestions that may be helpful in performing quality control in a histology laboratory. It should be recognized, however, that much more needs to be said. Everyone of us in histotechnology should utilize our knowledge of the field and attempt better quality control in the laboratory.

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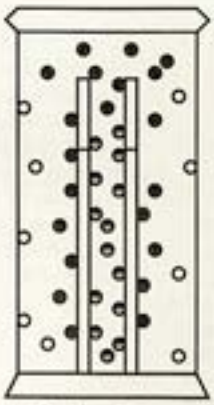
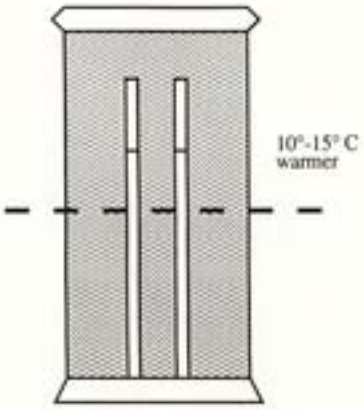


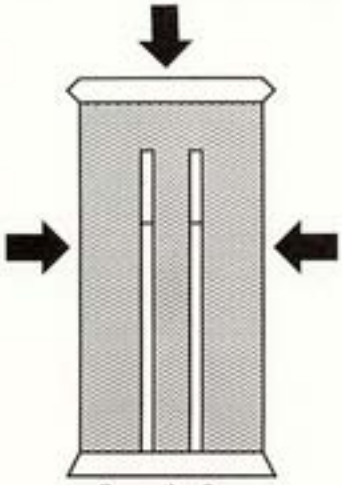
Laminar Flow

Figure 3: Illustrates the effect of laminar flow on staining reactions. The stain molecules (clear circles) move considerably slower near the walls of the staining dish due to friction of the wall surface. Conversely, the molecules in or near the middle of the staining dish (dark circles) move faster.



Microwave Heat Dispersion

Figure 4: This schematic illustrates that microwave heating results in a 10°-15°C difference in temperature between top and bottom levels of a staining container.



Convection Oven Heat Dispersion

Figure 5: Convection heat radiates from all sides of a staining container when a conventional type oven is used. This results in variable staining between slides in the middle vs. those close to the outer walls of the staining dish.

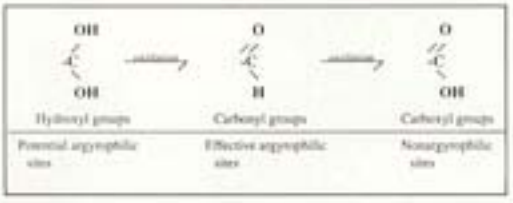


Figure 6: This formula demonstrates an example of the importance of proper utilization of oxidizers.

1991 NSH Convention Party — YES!

"This time, Scarlett, I do give a...."

...because at the 17th Annual NSH Convention in Orlando all attendees are invited to the Miles STAR NITE CAST PARTY. It's a Tuesday night (October 1) gala event destined to make the glitterati Hollywood press writhe with envy.

So think about dressing "up" like Scarlett O'Hara or Pretty Woman... or dress "down" like Sam Spade or Indiana Jones ... or get crazy and dress like Jason from Elm Street ... or better yet, get a group of friends and come as the cast of "Planet of the Apes" or "Star Wars" aliens. It really doesn't matter what movie, but "dressed" you must be to attend.

So "cast about a bit" for just the right costume for (but not limited to) the following:

- "Wizard of Oz" • "Lawrence of Arabia" • "Casablanca"
- "The Dirty Dozen" • "True Grit" • "Mutiny on the Bounty" • "Mary Poppins" • "West Side Story"
- or any Disney Character

More about it all in the next issue.

"Here's looking at you, kid."

Modern Trends in Histotechnology IV

Ottawa Academy of O.S.M.T.
Sponsored by MILES CANADA INC.

Saturday, October 26th
9:00 a.m. — 4:00 p.m.

Oxford/Cambridge Room
Park Lane Hotel
Cooper and Driveway
Ottawa, Ontario

Registration contact person:
Barbara Kosabek-Williams
(613) 761-4845

Snap Freezing Tissue Using Dry Ice and Cryomolds

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Snap freezing tissues for cryosectioning and immunoperoxidase staining is simplified by using dry ice blocks and Tissue-Tek II cryomolds. Our laboratory uses a freezing method that may be used less extensively in other laboratories. The primary objective is to study adhesion molecules in mouse, rat, goat, bovine, and human surgical tissues. Antigenicity has been consistently well preserved as seen in immunohistological stains and functional binding assays.

Materials Needed

Solid block of dry ice, approximately 5 pounds for multiple samples.
*Tissue-Tek II cryomolds, size needed
*Tissue-Tek O.C.T. compound

Procedure

1. Place cryomold on dry ice block.
2. Add a small amount of O.C.T. to bottom of mold, remove bubbles with a curved forceps, and allow O.C.T. to begin freezing.
3. Embed tissue in O.C.T.
4. Cover embedded tissue with O.C.T., remove bubbles, and freeze block totally.

If you allow the block to completely freeze (Step 4) and then add O.C.T. to the block surface, an interface forms between the two frozen layers that may cause separation during sectioning. Frozen blocks are stored in the cryomolds at -70°C until needed.

Discussion

The advantages for using this snap freezing method include the elimination of (1) expensive, two solvent snap freezing systems, (2) having to cool down isopentane (2-methylbutane) with each specimen for optimal cold temperature, and (3) mixing acetone or alcohol and dry ice with subsequent disposal of chemical solvents. Multiple samples are handled faster since we can freeze several tissue blocks at once, preventing lengthy time delays between specimen collection and freezing. Cracked O.C.T. blocks caused by colder liquid nitrogen temperatures do not occur with dry ice. Liquid nitrogen is not always easily accessible to smaller institutions or community hospitals. It also is more expensive and requires special storage due to its rapid evaporation rate. Dry ice lasts longer, is available at many local grocery or convenience stores, is safe to use, and is disposed of simply by evaporation. If necessary, we store large dry ice blocks for next-day use by wrapping in newspaper and placing in a -70°C freezer. We prefer Tissue-Tek II cryomolds since the bottom sits directly on the dry ice surface (Fig. 1). The thickness of the vinyl is not excessive and allows for rapid freezing.

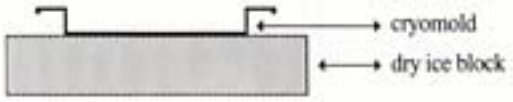

Figure 1.

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*Miles Inc., Diagnostics Division.

NSH Health and Safety Committee Deals with Hazardous Chemicals

Brent Riley
Managing Editor

Histotechnology has been practiced as a distinct profession for more than 60 years. But for much of that time, it was a profession fraught with danger as exposure to hazardous chemicals created health problems for many early Histotechnologists. These problems included chronic neurological dysfunction, respiratory impairment, and hypersensitivity after long-term exposure.

Today, things are very different because there are ways to virtually eliminate the dangerous effects of hazardous chemicals in the histology laboratory. Relatively safe conditions are the result of increased awareness through communication and education, as well as new technology and federal, state, and local regulations. The potential for danger is still there but, according to Lynn Montgomery, Chairman of the NSH Health and Safety Committee, with modern processing equipment and proper precautions, there is no reason for Histotechnologists to compromise their health in order to practice their profession.

Even with the changes, however, there will always be a need for caution in the handling, storage, and disposal of chemicals used in the histology laboratory.

Communication has played a critical role in diminishing the dangers of hazardous chemicals. Until the early 1970s, Histotechnologists had no way of knowing how their counterparts in other labs worked. "There were books available, but information was certainly not consolidated," Montgomery explained. "With the advent of professional histology societies, the interchange of knowledge and ideas began."

Another contributing factor was the increase in the number of articles published in histology and associated medical journals regarding chemicals used in histotechnology. This increase has been dramatic in recent years.

"Today, Histotechnologists are a highly educated group of people," Montgomery continued. "For years they suspected that there was something very dangerous about what they were doing. Now they have the educational background to know and understand the dangers.

"As I see it, there are several important issues," Montgomery said. "One is the immediate physical health and well-being of personnel. And another is the long-term effect of some of these hazardous chemicals."

Hundreds of the chemicals used in the practice of histotechnology are irritants or carcinogens. And many of those that aren't specifically designated as carcinogenic are highly suspected. "In order for a chemical to be classified as carcinogenic, an official study must be conducted," Montgomery explained. "But these studies are expensive and time consuming, so there are only a few chemicals on the official OSHA carcinogen list. There are, however, several hundred on the 'suspected' list.

"We can't be concerned only with those chemicals that are 'officially' carcinogenic," she said. "I look at it the other way around. I consider a chemical to be carcinogenic unless it has been proven otherwise. All chemicals should be handled as though they are potentially dangerous."

The concern for hazardous chemicals doesn't end with their handling and storage. As Americans become more aware of environmental issues, the disposal of laboratory chemicals becomes an important concern. "By the early '80s," Montgomery said, "everyone was beginning to become concerned not only about the hazardous effects of handling chemicals, but also about the disposal of these chemicals so they wouldn't destroy the environment."

These concerns have resulted in numerous federal, state, and local regulations regarding hazardous waste disposal. While no one denies the benefit of these regulations, most Histotechnologists realize the burdens they place on laboratories. "With the tremendous amount of regulatory guidelines that have been put on labs," Montgomery explained, "not only is the storage and disposal of chemicals a logistical problem, but the actual cost of disposal has absolutely skyrocketed. So we're looking at not only the physical health problem but also a tremendous economic factor that has been imposed on the lab."

Some chemicals can be deactivated in the lab by chemical treatment. But most labs pay a licensed hauler to dispose of their hazardous waste. Depending on the chemical, these haulers might incinerate it, recycle it, or dilute it and dispose of it in a landfill. The specifics depend on state and local ordinances. But whatever the method, it is a very expensive process.

Federal regulations are controlled by OSHA and tend to be more general. They cover the more extreme chemicals such as formaldehyde. State and local hazardous waste laws are often more specific, but they differ dramatically across the country. Those states that are more sensitive to environmental issues in general tend to have stricter regulations relative to histology labs. If a state or local law is more strict than a federal law, it takes precedence over the federal law.

The NSH Health and Safety Committee has played a very important role in educating NSH members about hazardous chemicals. One goal of the Committee is to achieve more standardization in the way information is disseminated about chemicals. Federal regulations require manufacturers to provide a manufacturer's safety data sheet, for hazardous products, which gives all the pertinent information about a hazardous chemical. Although a standardized form is used, the way the information is presented varies considerably from one manufacturer to another. And if you're not a Chemist or a Toxicologist, the information can be very confusing.

The Health and Safety Committee works with manufacturers to help them provide more standardized information. The Committee also provides assistance to NSH members to help them read and interpret the information.

Last year the Committee collected more than one thousand safety data sheets on a variety of chemicals. These sheets are currently on file at the NSH headquarters. If a member is having trouble finding information about a chemical, he or she can call the NSH office.

"This effort has been very successful," Montgomery said. "We have received a number of calls and letters from members requesting more information about a chemical. We don't make it a practice to make recommendations regarding chemicals; we simply pass along information and help our members understand it. Any information we give must be certified by the manufacturer or referenced by a regulatory agency, a professional journal, a federal bulletin, the Centers for Disease Control, or some other verifying agency."

The NSH Health and Safety Committee was established in 1981. The immediate goal was to open the lines of communication among Histotechnologists about health and safety practices. The Committee consists of 12 members who are very involved in state and local activities. By writing articles, presenting workshops and lectures, and corresponding with NSH members in their areas, these Committee members provide the majority of health- and safety-related information to Histotechnologists nationwide.

Lynn Montgomery has been on the Health and Safety Committee for the past 9 years and is in her second 2-year term as chairman. "I take this very seriously," Montgomery said. "I think it's the most important responsibility I've ever had in my career. I have been sensitized to a chemical myself, so I know what it's like. It's frightening."

Montgomery has been a Histotechnologist since 1959. She is also a certified Cytotechnologist, and she is certified as a safety technician by the World Safety Organization. She is currently supervisor of Cytopathology at Ochsner Clinic in Baton Rouge, Louisiana.

"What we're trying to do now is to establish a 'less is best' philosophy," Montgomery said. "This requires that all Histotechs, and all labs, re-evaluate their procedures one by one and find those areas where there has been excessive use of a chemical for which a substitute chemical could be used. You should also determine if the amount of the chemical used is actually necessary or could the procedure be altered so that smaller amounts could be used." Learning to use chemicals more efficiently would not only lessen the dangers of exposure but also reduce the amount of waste.

Recycling is also a viable alternative. "I'm a great champion of developing recycling or reclaiming procedures," Montgomery said. "I promote the use of some of the new distillation systems to reclaim some of the solvents we use. It's a tremendous economical and environmentally safe technique." Although such technology is in its infancy, the payback for these systems is very fast. According to Montgomery, many labs are using this technology, and the number is steadily increasing.

"Histotechnology is a potentially hazardous profession," Montgomery summarized. "So it is up to the professional to learn how to handle the instruments (chemicals) of their trade. The bottom line is that the Histologist must be well trained in the storing, handling, and use of these chemicals. With careful and prudent use of these chemicals, the Histotechnologist is quite safe."

Did You Know

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- that one can add one of the following salts if it is determined that 10% buffered neutral formalin does not have the correct pH after mixing or when received from a vendor. Add potassium phosphate, monobasic if the pH is too high (above 7.3) or add sodium phosphate, dibasic if pH is too low (below 6.7).

- that well-stained, reticulum fibers should exhibit black, well-defined fibers without beading and have a relatively clear background. If beading and a dark background occur during staining, replace the ammonical silver and the reducing solutions.

- that Ammonical Silver Solution problems can be resolved if one follows the suggestion provided below. To 10 mL of 10% silver nitrate solution add 2.5 mL of a 10% aqueous solution of potassium hydroxide, add 28% ammonium hydroxide,* drop by drop, while shaking the container continuously until the precipitate is completely dissolved. Add 4 drops of silver nitrate solution for every 10 mL of silver nitrate used. Make the solution with distilled water to twice its volume.

- that the ideal counterstain adds color contrast to give additional information and orientation without obscuring the intensity variation of the principal stain. Pink or red counterstains contrast slightly better with the bluish color of ammonium alum hematoxylin, and greenish and bluish counterstains with potassium alum hematoxylin. But, in any case, the counterstain should be relatively pale.

- that when solutions are heated with microwave irradiation, there can be up to a 15°C difference in temperature between the top and bottom layers of the staining container. Therefore, in order to equalize the temperature of the solutions, the slides should be dipped up and down in the required steps of the staining procedure to ensure proper and even staining of the tissue section — Charles J. Churukian, University of Rochester Medical Center, Rochester, NY 14642.

- that shelf life of many solutions, including those utilizing silver nitrate and hydroquinone, can be greatly increased by storing the reagents in a refrigerator at 3°-6°C. — Charles J. Churukian, University of Rochester Medical Center, Rochester, NY 14642.

- that cholesterol and cholesterol esters in tissue reactions can be demonstrated only on frozen cut sections. Various fixatives can be used but formalin works quite well. Although the Schultz reaction does not provide permanent slides, it is the most specific method for cholesterol.

- a section of skin is the best control for demonstrating ELASTIC FIBERS since skin contains coarse fibers in the dermis and fine fibers immediately below the epidermis. In my opinion, the demonstration of these fine fibers indicates the staining solution is working at its optimum.

- Histoplasma capsulatum is one of the most difficult fungi to demonstrate with the use of Grocott's method. For this reason Histoplasma capsulatum serves as the best control for paraffin-embedded tissue sections. Generally speaking, if this fungus stains well, most likely all other types of fungi are going to stain well also.

- that using tap water to dilute nitric acid may cause it to explode because of contaminants in the water. A gallon of 5% solution was made one day and exploded the next morning. The technical service person at Mallinckrodt told me to use only deionized or distilled water. — Fran Allison, HCA Palmyra Medical Center, Albany, GA 31703.

*The 28% ammonium hydroxide used in making this solution must be fresh. That is, a solution that has not been on the shelf too long or opened frequently. Use of old ammonium hydroxide results in excessive use to completely dissolve the precipitate mentioned above. The use of excessive ammonium produces silver precipitate on the fibers and dark uneven staining of the cytoplasm.

Gomori Aldehyde Fuchsin/Trichrome: A Useful Method

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Gomori's aldehyde fuchsin has been used extensively for the demonstration of many tissue entities such as elastica, Beta cell granules in the islet for Langerhans, fungal capsules in the Gridley method, mast cells in Luna's method, and highly acetic sulfated mucosubstances. On the other hand, Gomori's one-step trichrome has been used for demonstrating muscle fibers, collagen, fibin, and nemoline rod. We have used the combination of Gomori aldehyde fuchsin and Gomori one-step trichrome for a multitude of useful purposes. Presented below (Figs. 1-5) is an example of the combined methods.

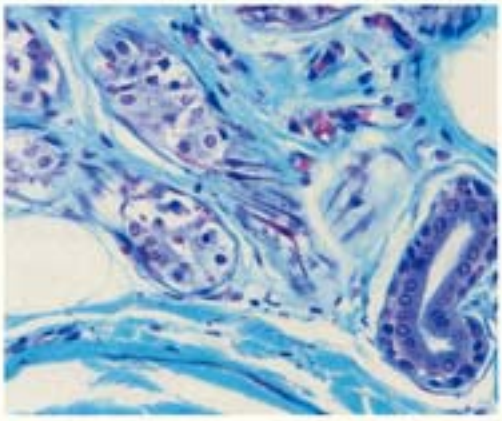

Figure 1: Section of sweat gland that demonstrates red staining myoepithelial cells (× 100 skin).

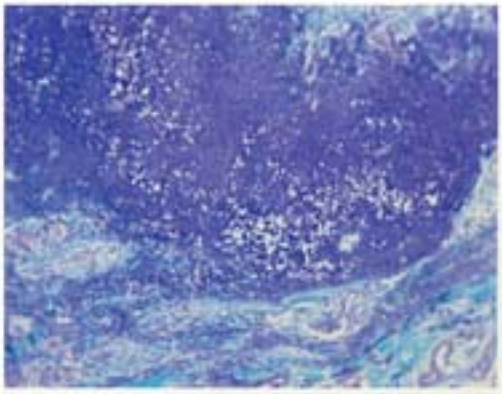

Figure 2: Solar elastosis (× 40 skin).

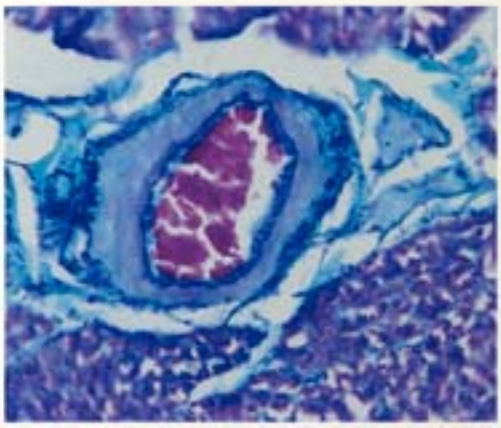

Figure 3: Section of cardiac muscle demonstrating red muscle, green collagen, and purple elastic fibers surrounding blood vessel (× 100).

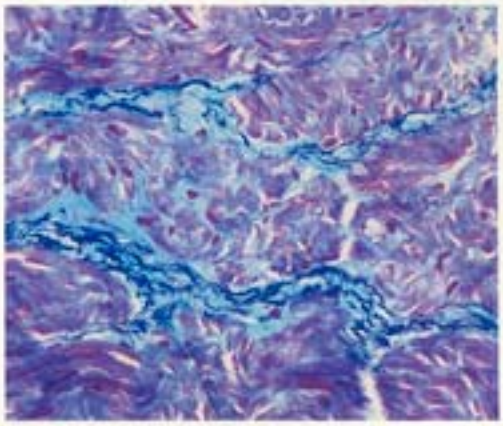

Figure 4: Section of cardiac muscle demonstrating red muscle, green collagen, and purple elastic fibers (× 100).

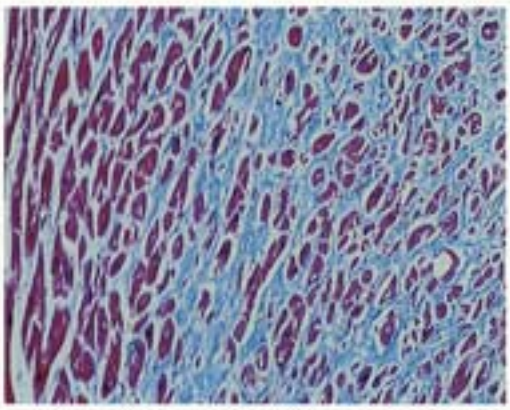

Figure 5: Section of heart muscle showing myocardial infarction right side. It should be pointed out that higher magnifications demonstrate excellent delicate staining of muscle, elastica, and collagen (× 40).

Fixation 10% buffered neutral formalin
Process Paraffin, frozen or celloidin
Microtomy Cut sections at 6 micrometers

Solutions

0.5% Glacial Acetic Acid

Glacial acetic acid ..... 0.5 mL
Distilled water ..... 100.0 mL

Weigert's Hematoxylin
Solution A

Hematoxylin crystals ..... 1.0 gm
Alcohol, 95% ..... 100.0 mL

Solution B

Ferric chloride, 29% aqueous ..... 4.0 mL
Distilled water ..... 95.0 mL
Hydrochloric acid, concentrated ..... 1.0 mL

Working Solution

Equal parts of Solutions A and B.

Bouin's Solution

Picric acid, saturated aqueous solution ..... 750.0 mL
37% to 40% formalin ..... 250.0 mL
Glacial acetic acid ..... 50.0 mL

Aldehyde Fuchsin Solution

Basic fuchsin ..... 1.0 gm
Alcohol, 70% ..... 200.0 mL
Hydrochloric acid, concentrated ..... 2.0 mL
Paraldehyde ..... 2.0 mL
Let stand at room temperature for 2 to 3 days or until stain is deep purple in color.

Gomori Chromotrope-Light Green

Chromotrope 2R ..... 0.6 gm
Light green, SF yellowish ..... 0.3 gm
Glacial acetic acid ..... 1.0 gm
Phosphotungstic acid ..... 0.8 gm
Distilled water ..... 100.0 mL
Aniline blue may be substituted for light green if it is more desirable to have collagen stained blue.

Staining Procedure

1. Decerate slides in xylene, 2 changes, 2 minutes each. Place in absolute alcohol, 2 changes, 2 minutes each; 95% alcohol, 2 changes, 2 minute each; then rinse in distilled water.
2. Mordant in Bouin's in an oven at 56°C for 1 hour.
3. Wash well in running water or until yellow color disappears.
4. Stain nuclear chromatin with Weigert's or Gomori's chromium-hematoxylin solution for 10 minutes.
5. Wash in running water for 10 minutes.
6. Stain in aldehyde fuchsin for 30 minutes.
7. Dip in 95% alcohol.
8. Stain in Gomori chromotrope-light green for 15 minutes.
9. Place in 0.5% glacial acetic acid for 2 minutes.
10. Dehydrate slides in 95% alcohol and absolute alcohol 3 changes each. Clear in xylene, 3 changes.
11. Mount coverglass with appropriate media (refractive index 1.48-1.56).

Results

Elastica ..... purple
Muscle fibers ..... red
Fibrin ..... red
Collagen ..... green (or blue if aniline blue is used)
Nuclear chromatin ..... blue to black (if hematoxylin is used)

Remarks

If sections are too dark, differentiate in 1% glacial acetic water to which 0.7 gm of phosphotungstic acid has been added. Rinse in distilled water. The use of hematoxylin is optional; however, one should be aware that it may obscure some of the muscle staining.

Dedication to Histotechnology Proves to Be Global Trait at Swiss Society Convention

Brent Riley
Managing Editor

Although 1991 is only the third year for the Swiss Speaker Exchange Program, it demonstrates a long-standing bond among Histotechnologists throughout the world. "There obviously is a commitment to the profession globally," explained Marilyn Gamble, HT, (ASCP), HTL, President of the NSH and this year's representative to the Swiss society's ninth annual national meeting. Gamble recently returned from the 1-day meeting that was held on April 26 in Basel, Switzerland. Patricia Turner, another NSH member, also attended the meeting.

The Swiss Speaker Exchange Program is sponsored by Miles Inc., Diagnostics Division. Each year, the NSH sends a representative to speak at the Swiss society's 1-day April meeting. The Swiss society then sends a speaker to the national NSH symposium/convention.

"Their meeting was very impressive," Gamble said. "I think they had about 130 people in attendance." The Swiss meeting consists primarily of lectures that are presented in either French or German. The Swiss society provided a translator for Gamble and Turner. They were also given written translations for some lectures. "Mine was the only lecture in English," Gamble recalled, "but they translated it into both French and German."

At the request of Bert Jaspers, President of the Swiss society, Gamble's lecture covered large volume laboratory management and the HMO concept. The first HMO in Switzerland is currently being developed. "Bert Jaspers had visited our facility and was very interested in how we handled the large volume of work," Gamble said. She is supervisor of the Histopathology Department at Southern California Permanente Medical Group's regional laboratory, which serves 10 hospitals.

Gamble wasn't sure how well her talk was received until after the meeting. "I didn't get a lot of questions during my lecture or at the end, but when I met privately with people there were plenty of questions," Gamble explained. "That's when I realized how well they had grasped my lecture. I think some people were just shy, especially about speaking a foreign language."

A poster session and technical exhibits were part of the Swiss meeting. Gamble estimates that there were 10 posters and 30 exhibits. A self-assessment exercise was also conducted. Several slides were shown to the participants, for which they were to identify the tissue and the stain. The member with the most correct answers won a weekend train trip to Paris.

The evening before the meeting, the Swiss society held a welcome reception and dinner in Gamble's honor. There, she presented the society with a set of NSH Self Assessment Books and the *Carbohydrate Stain Manual*.

This was Gamble's first trip to Europe. While enjoying the beautiful scenery afforded by German castles, the Rhine River region, and the Swiss Alps, she took time out to visit a number of labs including the Pathology Institute in Giessen, the Institute of Pathology at the University of Jena, and the Pathology Institute at the University of Munster. In Switzerland, Gamble toured the labs at Ciba-Geigy and Sandoz.

"Just to have the opportunity to participate and share technical knowledge with foreign colleagues is a thrill and an honor," Gamble said. "I think we always have the capability to learn from each other."

The Swiss society will send two speakers to the NSH symposium/convention in Orlando, Florida, September 28 through October 4. Brigette Greiner, of Sandoz AZ in

Pratteln, will discuss in-situ hybridization, and Esther Busser, of the Institute of Pathology in Winterthur, will discuss dyes.

"I think this exchange program is helping to provide worldwide recognition to the NSH," Gamble said. "It's very encouraging that people from other countries are wanting to come and present here and are submitting abstracts to us. That's very complimentary to our society."

Science and Magic Join Forces at 17th NSH Symposium/Convention

Brent Riley
Managing Editor

It will be a week of contrasts. A week of work. A week of fun. A time to learn the intricacies of a disciplined science against a background of magical entertainment. The 17th Annual Symposium/Convention of the National Society for Histotechnology will take place September 28 through October 4 at the fabulous Stouffer Resort in Orlando, Florida.

More than 1,000 histology professionals are expected to attend. That represents an increase of more than 100 percent since 1984.

The society has declared the nineties to be the "Decade of Progress." This meeting will not only reflect the most recent progress in histotechnology, but it will also anticipate the progress to come in the next 9 years.

The program, which is open to all Histotechnologists, will follow the successful format of last year. Four days of workshops will begin on Saturday, September 28. There will be 72 full- and half-day workshops, 18 more than last year.

"The hotel is quite large so we took advantage of the space to increase our workshop offering," explained Phyllis Boris, cochairperson with Kerry Crabb of the NSH Convention Committee. Many, especially those that involve hands-on participation, will have limited attendance. These workshops fill quickly so it is important to register as soon as possible.

The workshops will cover basic, intermediate, and advanced topics. Among the new workshops this year will be one designed to give participants a working knowledge of how to handle and dispose of hazardous chemicals. Another workshop, presented by a group of physicians from the United Kingdom, will acquaint participants with the diverse methods by which microscopic preparations of smaller tissue samples are made.

In addition, a workshop called "Controlling Procedures" will send unstained slides to participants, giving them an opportunity to stain and return them in advance. The slides will then be evaluated during the workshop.

"We're getting more and more into the V.I.R. (Veterinary, Industrial, Research)," explained Boris. "We have quite a few workshops and lectures aimed at them this year as they are becoming a large part of the society."

Many of the most popular workshops from past years will be offered again this year. "One thing we tried to do this year was to offer several workshops on what seemed to be the same topic," Boris said. "We found that too many of the registrants just can't get to all the workshops they would like to attend. So we're offering workshops that approach similar topics from different perspectives."

Dr. James McCormick will conduct a workshop on the study and preparation of antique prepared microslides. Participants will have an opportunity to create their own "antique" slides.

Scientific Sessions will be held Wednesday and Thursday, October 2 and 3, and will culminate in a panel discussion on Friday, October 4. These sessions will cover both clinical and V.I.R. topics. The session will begin with the Professor C.F.A. Culling Memorial Lecture. This year's lecture, "Evolution and Present Status of Methods Utilizing Alcian Blue 8qx (and related dyes) for the Detection of Complex Carbohydrates Containing High Concentrations of Acidic Groups Whether Mainly Sulfates or Mainly Carboxyls or Both," will be presented by Dr. Robert W. Mowry of the University of Alabama, Birmingham.

This lecture will be followed by two Swiss Society Exchange Lectures. Brigette Greiner, of Sandoz AZ in Pratteln, Switzerland, will discuss "In-Situ Hybridization," a method used to study RNA in individual cells.

Esther Busser, of the Institute of Pathology in Winterthur, Switzerland, will discuss "Dyes." In addition, the NSH International Lecture will be presented by Bettina Duhm, of the University of Munster, Gerhard-Domagh Institute for Pathology in Munster, Germany. Duhm will discuss the "Presentation of Intermedia Filaments Using the APAAP Method."

In addition to the panel discussion scheduled for Friday morning, another panel discussion directed primarily to V.I.R. topics will be held Wednesday afternoon. The panels will consist of experts with a combined knowledge of basic histology and staining, immunology, veterinary, industry, research, management, safety, and other appropriate areas. You are encouraged to send your questions for the panels to the NSH office. However, questions can also be submitted during the sessions.

Scientific Exhibits will open Tuesday afternoon and remain open through Thursday. More than 100 suppliers will be on hand to display and demonstrate their laboratory equipment and supplies. This is an excellent opportunity to see the latest technological advancements and to ask questions about products.

Poster Sessions and Technical Exhibits will run from October 1 to October 3. These special displays give Histotechnologists an opportunity to share their ideas and accomplishments with their peers.

A number of social events are planned for the evenings. A welcome reception for first-time attendees will take place on Saturday evening, September 28. This will give first-timers a chance to get acquainted with NSH officials and veteran attendees.

The NSH Awards Banquet will be held Thursday evening. The banquet will be preceded by a cocktail party hosted by the Diagnostics Division of Miles Inc. In addition, Miles Inc. will sponsor a party on Tuesday evening. It will be a "star nite cast party," and everyone is encouraged to dress up as a member of the cast from their favorite movie.

Those who want to become more involved in the NSH will have an opportunity to attend any of several official meetings. All of the NSH committees will be meeting during the week. NSH Region meetings will be held from 5:30 to 6:30 on Monday. In addition the NSH Board of Directors will meet all day on Tuesday, and the House of Delegates will meet Friday afternoon.

The symposium/convention will present many other opportunities as well. Attendees can receive information about job opportunities, and review and purchase histotechnology books, aids, and other educational material. Nonmembers can find out more about the NSH. And all Histotechnologists will have an opportunity to meet, talk, and compare notes with their peers from other parts of the country.

When the day is done, it will be time for the magic of Orlando, the number one vacation destination in the world. Theme parks abound in Orlando. You can visit Walt Disney World, Sea World, Universal Studios, Cypress Gardens, Busch Gardens, and Gatorland Zoo. There are also plenty of unique shopping opportunities, including Church Street Station, a block of restaurants, shops, and entertainment. Cultural activities are also available. There are plenty of museums, galleries, and gardens. You can even visit the Kennedy Space Center, NASA's site for the shuttle launches.

Travel arrangements should be made through The Travel Concern, the official agent for the NSH. Reservations made through this agency provide credits that are used to reduce the total cost of the symposium/convention. You can call The Travel Concern at 1-800-373-4100. In Canada, call 1-800-3905-2359.

If you've attended this exciting event before, you know why you should attend again. If you've never attended before, don't miss this opportunity for a week of learning fun, and, of course, contrast.

Ask anyone who has been there before. It is well worth it.

Management Corner: Effective Written Communications

Brent Riley
Managing Editor

Let's be honest. Most of us *do not* like to write reports or any other type of written communications to upper management. But, like it or not, writing is an important responsibility for lab managers and supervisors.

Writing doesn't have to be difficult or time consuming. It simply has to be clear. Here are a few things to remember the next time you have to communicate in writing.

1. Get right to the point.
   Don't beat around the bush with elaborate introductory statements. Say what you have to say and be done with it. Chances are, whoever is reading your words doesn't have a lot of time either. Journalists learn to organize their articles in an inverted pyramid style. The most important point is made in the first sentence. Other information is then provided in a declining order of importance. If the reader doesn't have time to read the entire article, he knows that he has read the most important part.

2. Be concise.
   Make your sentences short and to the point. And don't give the reader information he already knows.

3. Choose the right word.
   Be sure you know the meaning of the words you use. Some words sound alike but have very different meanings. If you are not absolutely sure about a word, look it up.

4. Shorter words are better.
   As a general rule, use the simplest and shortest word that expresses the idea you want to convey. Nobody is impressed with big words if it makes your writing hard to read. If you can think of a one-syllable word that means the same as a three-syllable word, use the one-syllable word.

5. Watch your grammar and punctuation.
   There isn't room here for a course in English grammar, but it's a good idea to have a good reference book handy. One such book is *The Elements of Style* by William Strunk and E. B. White (MacMillan Publishing Co., Inc.).

6. Check your spelling.
   And when you're finished, check it again.

Your writing communicates in two very different ways. First, there is the literal message found in the words themselves. Second, there is an impression revealed about the writer. This impression is made by the style, look, and overall effectiveness of the written word. It ultimately reflects on you.

The most important thing to remember about writing is to be comfortable and natural. Try this: say what you want to say out loud, then simply write it down.

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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