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As the Century Draws to a Close…

Vinnie Della Speranza, Scientific Editor

Years before the first person ever coined the phrase "Y2K," I used to wonder where I would be when the clock struck 12:01 AM on January 1, 2000. After all, changing centuries seems like a pretty big deal. My thoughts always strayed to imaginings of what life was like at the last turn of the century, and about the incredible technological changes that my grandparents had witnessed in their lifetime. The automobile, the airplane, computers, and all of the rest (see Countdown to the 21st Century, *Histo-Logic,* Spring, 1999) have left their indelible marks on society so that today, none of us can imagine living without such modern conveniences.

While everyone else is fretting over Y2K and the millennium bug that threatens to cripple civilized society as we know it, we at ***Histo-Logic*** have decided instead to celebrate the arrival of 2000 with a whole new look for our publication that we are excited about! This is in keeping with our rich tradition since 1971 of serving you and the discipline of histotechnology with up-to-date, relevant and useful technical information that so often finds application in your work.

Over those many years, ***Histo-Logic*** has had a tremendous impact on the histotechnology profession, serving for many years as the sole technical bulletin in the discipline. Many authors have, in fact, enjoyed seeing their very first attempts at authorship published here. Believe it or not, it is you, our readers, who have contributed to much of our success because it is not uncommon for individuals to pass along their copies to friends or colleagues. Our mix of technical, news, and feature articles provide the variety that keeps this publication fresh and interesting.

Managing Editor, Gilles Lefebvre
Scientific Editor, Vinnie Della Speranza, MS, HTL (ASCP) HT, MT

I am particularly excited about the changes that we have in store for ***Histo-Logic,*** for as the discipline and its practitioners change in the years ahead, we will continue to work behind the scenes to provide you with a resource that you will want to retain for frequent reference. I invite you to consider participating in the preparation of this publication by contributing your ideas and knowledge in order that they may be

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shared with others in the discipline. Perhaps you have a tip, or use a method in your lab that others could benefit from. I would be happy to offer you assistance in preparing an article to get your ideas into print.

Rest assured that no matter what the outcome on January 1, 2000, we at ***Histo-Logic*** are poised to continue to serve you. While others are stockpiling foods and other goods, I have assembled a large collection of pads and pencils (remember those neat inventions?) so that nothing will prevent our future issues from getting to you. Oh, and yes, I no longer wonder where I will be on New Year's Eve. I'm required to be at work with many of my coworkers, prepared to intervene should anything go awry at 12:01 AM. Many thanks to whoever is responsible for this little blunder. Where will you be when the lights go out?

Atypical Cytokeratin Staining of Sentinel Node Biopsies for Breast Cancer Due to Microwave Pretreatment

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The standard procedure for the assessment of nodal staging in breast cancer has been the axillary lymph node dissection. This is a necessary procedure for patients with early breast cancer in order to predict recurrence and survival.[1,2] The status of the lymph nodes also determines the mode of treatment the patient will receive. Unfortunately, there are complications from this procedure, which include lymphedema, pain, infection and a limitation of shoulder motion.[1] Consequently, a less invasive procedure such as the sentinel lymph node biopsy is becoming the preferred procedure.

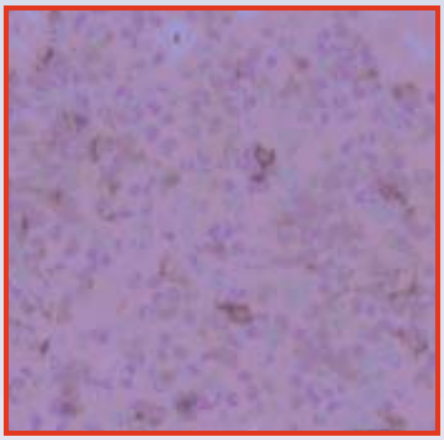

Fig.1. CK22 staining of lymph node dendritic cells using citrate buffer/microwave antigen retrieval method

The sentinel node is the first lymph node that receives lymphatic drainage from the tumor. This is usually determined by the injection of a blue dye or radioisotope around the primary tumor, which then travels to that first draining or sentinel lymph node.[3,4] The node is then excised and sent to the hospital's surgical pathology laboratory where the pathologist examines the specimen, and the tissue is then paraffin processed for microtome cutting. The pathologist will then assess the cut sections to determine if micrometastases are present.[3]

At this hospital, there is a special cutting procedure for sentinel lymph node biopsies. First, level one is cut and a stained H&E is made on one slide, and unstained slides are cut for immunohistochemical testing, such as the cytokeratin antibody stain. Next, the paraffin block is trimmed 40 microns and level two is cut. Again, an H&E is stained and unstained slides are cut for cytokeratin staining. In our lab, we use the pan-cytokeratin antibody called CK22 (Biomeda, Foster City, CA). A pretreatment step is necessary in order to expose epitopes on the cells, which are masked by formalin fixation. For the CK22 antibody, we have always performed a microwave citrate buffer treatment.

When we first started testing the sentinel node biopsies, we noticed that other cells besides the tumor cells were staining, such as dendritic cells. This atypical staining was very disturbing to the pathologists. The initial thought was that something went wrong in the staining process, so we repeated the CK22 test and again the dendritic cells stained. We then contacted another lab for comparison and found that the slides they stained for CK22 had no atypical staining. After discussion with the other lab, it was determined that the only difference in our staining methods was the pretreatment step. They were using an enzyme pretreatment instead of the microwave citrate buffer method. We then tried a trypsin enzyme pretreatment and the atypical staining had disappeared. We theorized that the microwave pretreatment exposed epitopes on these other cells that somehow reacted with the CK22 antibody. This phenomenon did not occur with enzyme pretreatment.

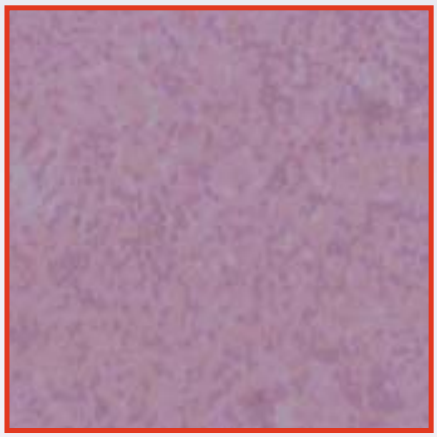

Fig. 2. Proteolytic enzyme antigen retrieval method eliminates dendritic cell staining with CK22

The pathologists were satisfied that they no longer had to disregard the atypical staining while examining their slides. It is important to note that the pathologist is looking for one or two positively stained tumor cells on the CK22-stained sentinel lymph node. So, if they have to weed through atypical-staining cells, it makes their job much harder. As technologists, we must ensure that the final product is the best it can be for the pathologist. Sometimes it is beneficial to compare notes with other labs doing similar testing. Ultimately, the patient's treatment is dependent upon our analysis.

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Adapting a Modified Gram Stain for Use in Resin Embedded Tissues

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Abstract
Our laboratory has been engaged in ongoing studies to evaluate the presence or absence of known Gram-positive bacteria in decalcified paraffin sections of bone. Interest in assessing the impact of infection on bone mineralization or implant materials required us to focus on developing a method to demonstrate bacteria in undecalcified sections. This is a report on our experience with efforts to adapt our routine Gram-positive stain for use in the study of undecalcified bone.

Introduction
Our laboratory has several ongoing studies of osteomyelitis (bone infection) in various models. The primary focus of these studies is the presence or absence of bacteria after treatment. Over the past decade, an increasing number of cure modalities have included growth enhancement constituents, bioerodeable delivery systems, or in some cases, both. Studies of the effect of these cure regimens generally include not only presence/absence of bacteria but also the effect of the materials on the structure and rate of formation of the resulting new or remodeled bone. Additional questions within the orthopedic community center around the influence of biomaterials (most notably metal and polyethylene —those typically associated with joint replacements) on bone formation and infection dynamics. Our lab set out to investigate the feasibility of demonstrating the presence/absence of bacteria after antibiotic treatment in non-decalcified samples of bone. These studies would require processing the specimens into some polymer embedding system. A literature search revealed no significant staining technique being performed in the types of media available in our facility, to demonstrate Gram-positive bacteria in undecalcified bone specimens.

Our earlier investigations of osteomyelitis involved studying paraffin-embedded decalcified samples as per established protocol.[1] A 5% formic decalcification regimen allows us to perform procedures from routine H&E to immunohistochemistry. We use an established modified Gram stain for the particular strains of Gram-positive *Staphylococcus aureus* that are used to induce the disease process.[2] We have found that Gill's II hematoxylin in combination with eosin Y/phloxine counterstain gives the nuclear detail and osteoid seam definition necessary to evaluate bone growth and overall architectural formation. Prior to dissection, the infected rabbit limb is radiographed and the resulting X-ray is evaluated as part of the overall score to categorize the extent of the disease process. The performance of the modified Gram stain across a variety of embedding media became a primary concern. Our intent was to compare the performance of this stain in a decalcified paraffin preparation with a preparation in the resin we use for the study of metallic implants in undecalcified samples.

Materials & Methods
In a standard decalcified paraffin study, osteomyelitic rabbit forelimbs, which were infected with a known strain and quantity of *S aureus,* were harvested, cleaned of nonessential soft tissue, fixed in 10% neutral buffered formalin, and decalcified in 5% formic acid until negative end point determination by the ammonium oxalate test.[1] Decalcified limbs were then split using a disposable microtome blade and processed through graded ethanols, cleared in methyl salicylate, and infiltrated into paraffin as per established protocol.

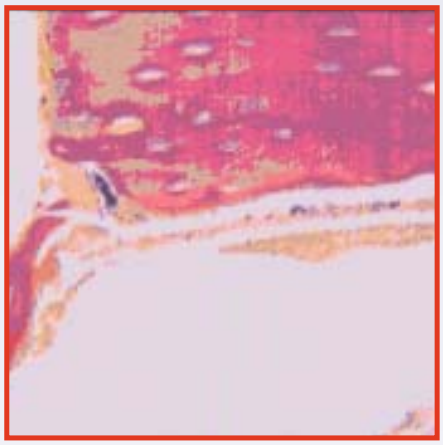

Fig. 1 – Five-micron paraffin section of decalcified rabbit radius stained with routine modified Gram stain including Van Gieson counterstain.[2] Gram-positive staph aureus cocci appear dark blue. Bone is red. Connective tissue and purulence (pus) is yellow. 100×

Five-micron paraffin sections of decalcified bone were hydrated and flooded with 2% crystal violet for 2 minutes followed by decanting the excess crystal violet and flooding with Lugol's iodine for 2 minutes. The slide was then washed in a 50:50 acetone:ethanol mixture until the section was clear. After a five-minute water rinse, the slide was immersed in Van Gieson solution for 3 minutes followed by a water rinse, a dip in saturated picric acid, ethanol dehydration, clearing and mounting.[2] Routine results are depicted in Figure 1.

In order to make it possible for us to demonstrate the presence of bacteria in undecalcified bone containing metal implants, our first approach was to investigate if the stain would be successful on cut and ground sections of bone embedded in Kulzer/Exakt 7200 embedding medium. This medium was chosen because of our prior success with histologic studies of undecalcified bone containing titanium and stainless steel implants using the Exakt Macro Cut & Grind System. The lack of staining in earlier specimens with metallic implants that were culture positive for Gram-positive bacteria and embedded in the 7200 medium suggested that the crystal violet stain was not getting into the tissue. We designed a parallel pilot study to examine the utility of staining the infected sample by both a conventional slide staining method as well as a block impregnation method in a manner similar to Bielschowsky's work in 1904.[3]

A 3.5-cm segment of radius was harvested from two operated forelimbs of rabbits infected with *S aureus* (ATCC 49230). The forelimbs were split longitudinally with the Exakt Macro Band System and fixed in 10% buffered formalin. One-half of each specimen was submitted for decalcification, as a control. The remaining halves of these specimens were briefly washed in water and moved to 70% EtOH for storage until processing into the Kulzer/Exakt resin. The two decalcified control specimens were processed into paraffin and stained, as per above, and were found to be positive for bacteria. These tissue blocks were then deparaffinized in xylene and rehydrated through graded ethanols to 70% for storage.

One each of the decalcified and undecalcified specimens were washed in running tap water for 2 hours followed by distilled water. These specimens were then prestained by immersion into filtered crystal violet for 30 minutes followed by a thirty-minute immersion in Lugol's iodine. They were decolorized through five changes of 50:50 acetone:alcohol with a final immersion in 100% EtOH over a period of 30 hours, then processed into Kulzer/Exakt 7200. The two remaining decalcified and undecalcified specimens were not prestained but processed through into the 7200 resin.

For Kulzer/Exakt 7200 processing, the specimens were dehydrated through a series of graded acetone/ethanol mixtures starting at 50:50, and infiltrated through graded ethanol/Kulzer 7200 starting at 50:50, to pure Kulzer 7200. After infiltration under 15 psi vacuum at 4°C, all samples were polymerized overnight by the Exakt Histolux System. Polymerized specimen blocks were then affixed to plastic slides, sawed to a nominal thickness of 100 microns, and then ground and polished on the Exakt Macro Grinder as per manufacturers protocol to a thickness of approximately 45 microns.[4] The slides from previously unstained specimens were then stained with crystal violet for 30 minutes (to compensate for section thickness), Lugol's iodine for 30 minutes, and decolorized with acetone:alcohol.

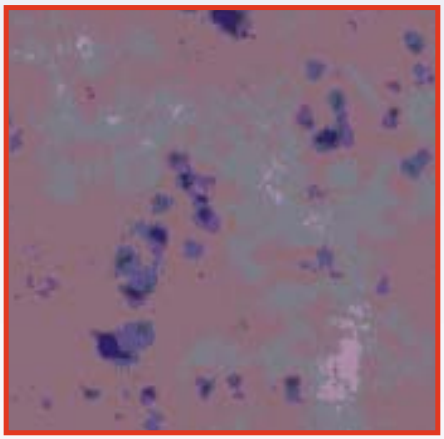

Fig. 2 – Forty-five micron ground section of Kulzer/Exakt 7200 embedded undecalcified rabbit radius Gram stained and decolorized prior to processing. Gram positive staph aureus cocci appear dark blue. Multiple layers of bacteria can be seen. 100×

Microscopic examination revealed Gram-positive cocci in both purulence and bone in the tissues prestained prior to processing into plastic resin. In the sections that were stained after polymerization, the Gram stain did not penetrate the Exakt/Kulzer-infiltrated tissue and no bacteria were seen. Furthermore, decolorization with acetone:alcohol caused visual deformation of the polymerized resin.[5] The results are depicted in Figure 2.

A co-investigator on the same project later inquired about doing undecalcified processing of bone without metallic implants on a smaller and more cost-effective scale. The primary focus of this study was to assess the effect of infection on mineralization and bone remodeling. The earlier Kulzer 7200-metal pilot investigation was to involve large, if not whole, rabbit radii. This new project would study small, specific segments of the radius at the infection site. Because our lab was not equipped at that time with the instrumentation necessary to process and sledge section methylmethacrylate (MMA) samples, we decided to investigate the use of glycol methacrylate (GMA) for this application.

An extensive literature search on Gram stains in GMA sections was conducted. A keyword search of Medline from 1966 through the present was performed. The keyword search pattern looks for the specific word (as it is spelled in the query) in the Title, Abstract, Registry number, word, and MeSH subject headings. Keyword searches were combined using Boolean logic operators "and" and "or." Using the keywords "gram stain," "glycol methacrylate," "plastic," and "histology" in all combinations of Boolean logic, there were no citations (0/52683) that described Gram stain in GMA for the period of 1966 to the present.

Glycol methacrylate was at one time considered a potential water-soluble embedding medium for electron microscopy. The work of Ruddell in 1967 suggested the use of GMA for routine light microscopy.[6] GMA gained favor in the late 1970s as a more rapid

methodology for the clinical study of osteoporosis and osteomalacia in iliac crest biopsies. It became the embedding medium of choice for clinical bone marrow core biopsies at the pathology lab at SUNY Stony Brook during the early 1980s because of the superior cellular detail afforded by the standard 2-micron section. Our prior experience with converting paraffin efficient stains to glycol methacrylate-embedded sections convinced us that such a conversion for this pilot study was very possible.[7,8] Since bacteria colonize surrounding soft tissue as well as bone, we were able to evaluate staining performance in soft tissue embedded in GMA.

In order to answer the basic question of whether the stain would work in GMA, we collected elliptical skin samples containing a central outwardly draining sinus tract located immediately above the infected bone. This tract should contain the Gram-positive bacteria from the infected site within the bone. The sample could then be bisected through the tract to be parallel processed through paraffin as well as GMA. In this way, we could experiment with the alternative processing and staining procedure without compromising any N value for the infected bone generated for the actual project, and not add significantly to the cost.

The paraffin skin sample was processed, as per routine, with the exception that no decalcification was performed. The GMA sample was fixed in formalin, dehydrated through graded ethanols to absolute, and infiltrated in three changes of catalyzed GMA (JB4 kit), before being polymerized via an 8:1 ratio of catalyzed GMA: accelerator, as per kit recommendations.[9] The polymerized samples were sectioned at 2 microns on a JB4 microtome using glass knives.

An initial staining run included both the 5-micron deparaffinized slide and a 2-micron GMA slide from the same skin sample, in parallel, using the times established for paraffin section staining. The results are depicted in Figure 3.

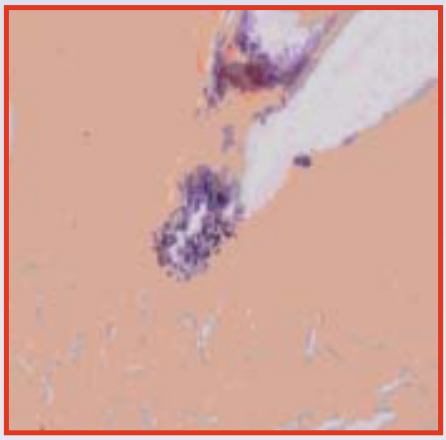

Fig. 3a – 5-micron paraffin section of rabbit skin stained as per Fig. 1. 250×

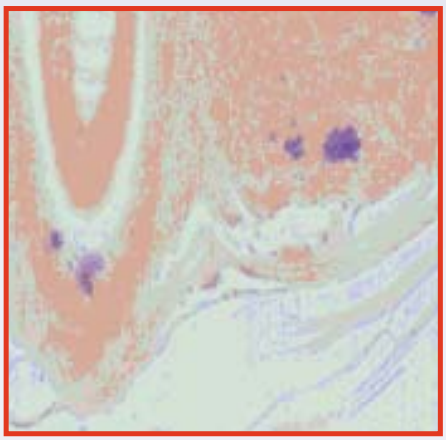

Fig. 3b – 2-micron GMA section of rabbit skin stained as per Fig. 1. Note the similarity of the color quality of the bacteria, the clarity of the 2-micron section, and the faintness of the counterstain compared to 3a. 250×

Results

Figure 2 shows that staining and decolorizing of the fixed tissue prior to processing and embedding successfully demonstrates the presence of Gram-positive bacteria. Note that the section in Fig. 2 is photographed with no counterstain. This was done to afford the investigator maximum visualization of the bacteria with no other distraction. While the staining outcome with this technique was successful, the results were less optimal than the staining of slide-mounted sections.

A comparison between Fig. 3a and Fig. 3b illustrates that the stain successfully demonstrates the presence of bacteria in each section without the need for modifying the paraffin staining procedure. The bacteria are clearly visualized in paraffin decalcified bone (Fig. 1), paraffin skin (Fig. 3a), and GMA skin sections (Fig. 3b). There is a significant difference in the staining intensity of the red component of the Van Gieson counterstain between the paraffin skin and the GMA skin sections. This can be overcome by staining longer in the Van Gieson stain and shortening the dehydration time in order to achieve paraffin-like staining results.

Discussion

Our experience illustrates that histotechs have opportunities to contribute their expertise in the planning and implementation of projects such as the study described here. Principal investigators have an idea of what they would like to achieve but oftentimes have only vague ideas of how to reach their goal. When we proposed performing the Gram stain in the Kulzer/Exakt resin, the reaction was "who's doing that?" A similar reaction was expressed when the GMA approach was suggested. The answer in both cases was "nobody, that we can determine, so why not be the first?" The original expectation of our investigator to see crisp, clear staining in a cut and ground section (45 microns) while achieving the quality one expects from 5-micron paraffin sections was unrealistic even though the bacteria were clearly demonstrated in both. Whether the total expectations are realistic or not, in circumstances such as these it is generally in the histotech's best interest to work more closely with the PI to establish new methodologies through adaptation of those from other applications.

Acknowledgments:
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Retiring at the Top of His Game

Vinnie Della Speranza, Scientific Editor

Dr. Jules Elias

Earlier this year, Dr. Jules Elias informed the NSH Board of Directors of his plan to retire as editor of the *Journal of Histotechnology* by year's end. This would end a fifteen year stewardship of what has become recognized worldwide as the premier journal for Histotechnology, the culmination of a dream that Jules set for himself when he accepted the role of editor in 1985. It is my privilege to share with our readers some insights into this remarkable man who has been one of the thought leaders of our discipline, and who has made immeasurable contributions to the science of histotechnology.

One of my goals in writing this piece is to say "thank you" to Jules, my former teacher, mentor, counselor, boss and friend, not only for myself but for the many who may not even be aware of his impact on the profession. I recall all too well how, as a neophyte histotech many years ago, I had occasion to hear the names of some of the pioneers of the NSH spoken with reverence, including Lee Luna, Dominic Europa, and Dezna Sheehan, and I regret to admit that I didn't have an appreciation back then for who they were and the significance of their contributions until they were gone.

I had occasion to meet Jules Elias in 1974 as an undergraduate medical technology student at the State University of New York at Stony Brook, when I took the histopathology course he taught in the curriculum. I remember being struck by his energy and passion for sharing his knowledge, which stimulated my interest in histology despite my having devoted two years to train to become a med tech. I casually inquired of him one day after class where I could take additional histology courses after my graduation that summer. His enthusiasm and encouragement set me on a course that would allow me to enjoy a rewarding career of my own.

Like most of us, Dr. Elias began his career at the bench in both research and clinical environments. Armed with a bachelor's degree in Biology, he began he first job at the Jewish Center for Chronic Disease in Brooklyn, N.Y. just a few hours after his first child was born in 1959, where he was involved in diabetes research, performing blood sugars on dogs and rabbits. A maternity leave in the histology lab at the same facility prompted him to learn to cut sections. As he aptly points out, there were no disposable microtome blades back then so one's success was directly linked to one's ability to skillfully sharpen a knife, an art that has all but become lost today. He worked as a histotech for four years before taking a position in electron microscopy. During this time, he began his master's study in hematopathology at Long Island University, and upon completion of his degree in the early 1960s, he set up a histology lab at the federal government's Brookhaven National Laboratory where he prepared animal tissues previously injected with tritiated thymidine to study cell turnover. He worked with an English researcher who brought some of his processed blocks back with her to England. The techs there were startled to discover how easily Jules's blocks cut, and they were eager to learn how he had prepared his tissues. He later went on to study leukemia using both animal and human bone marrow and spleen. His first published paper appeared in *Stain Technology* in 1969.

In 1970, Jules joined the State University of New York at Stony Brook where he established laboratories to work up renal and skeletal muscle biopsies. By the mid-1970s he had established the Thomas A. Edison program for the NSH to permit histotechs in the field to earn credits toward an associate degree by studying at home and taking exams at the NSH symposium, believing with conviction that developing technologies would require at least entry level college study for histotechs. I had the privilege of rejoining him and his talented staff at the university in 1979 to prepare the pathology laboratories for the soon-to-open University Hospital & Medical Center at Stony Brook. He earned his doctorate degree in 1982, the same year that he wrote his first textbook. In the years that followed, he has written over 90 publications, including several textbooks and workbooks.

In 1985, Dr. Elias accepted the editorship of the JOH and set himself on a course to elevate the credibility and esteem of this, "our" journal, working hard to attract the interest and participation of noted

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physicians, researchers, and scholars in medicine around the world. Today, the journal is recognized as a major vehicle for the reporting of significant technological advances in histopathology, and has achieved international repute as evidenced by the number of agencies indexing its articles. Most would agree that Dr. Elias's vision, energy and hard work have put the discipline and the National Society for Histotechnology on the map. Some have expressed that the journal under his tutelage lost sight of its readership, and that its contents were too sophisticated for the needs of the tech at the bench. One has only to examine the journal's editorial board to appreciate that it was never his intention to abandon the tech at the bench, but rather to bring them to the level of knowledge they would need in the decades to come.

Those who have frequented the NSH Symposium/Convention each year can attest that Dr. Elias often presents numerous workshops, and is always eager to share his expertise, despite a rather grueling schedule. By his own admission, one reason that he has been such a valuable resource to the profession is that he has never stopped learning. For all of the years that I have known him, he has always encouraged those around him to read and absorb everything that they could get their hands on and to never stop imagining how methods might be improved.

Since retiring from Stony Brook and moving to the west coast, Jules has been making time to pursue his loves; his music and dancing, teaching and, of course, his family. His life at the moment can hardly be called "retirement." He presently consults once a week at Oregon Health Sciences University, Dept. of Surgery, instructing residents and others about research projects. He also consults for manufacturers of histology instrumentation and supplies, and he is busy writing a wellness book. In his free time, he is principal clarinetist for his community orchestra. He also plays with the Gresham Senior Band, and his own wind quintet, which meets every Saturday morning at his home for rehearsal. It seems clear that he doesn't plan to slow down anytime soon. He tells me that his daughter complains that it is a struggle to fit into his and Renee's busy schedule.

In a year that has left sports fans stunned and reeling from the voids left by the unwelcomed retirements of greats like Michael Jordan and John Elway, it isn't a reach to say that like them, Dr. Jules Elias is retiring at the top of his game. On behalf of all of us who have reaped the benefits of your labors and your contagious love of the profession, Jules, thank you and Godspeed.

Description and Preliminary Results of a Novel Cassette System (Tissue-Tek® Paraform® Cassette System)

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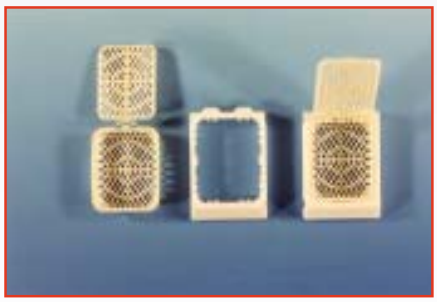
Abstract
A new and unique sectionable cassette system (Tissue-Tek® Paraform® Cassette) and preliminary results of its use are described. The Paraform® system includes an outer frame similar to a traditional cassette with an inner soft material insert with lid. The insert is composed of a material possessing two key characteristics: It is essentially unaltered by fixation and processing, and it can be sectioned in a manner similar to a traditional paraffin block. The soft material is compatible with all commonly used fixatives and solvents.

The tissue is placed in the cassette, oriented, and the lid is closed. The lid firmly holds the tissue against the bottom of the cassette by a series of ratchets. By this mechanism, proper tissue placement and orientation determined at the time of grossing-in is preserved through processing and embedding. At the time of embedding, the inner material insert with lid is displaced downward into an embedding mold. Embedding is achieved without opening the lid or directly manipulating the tissue.

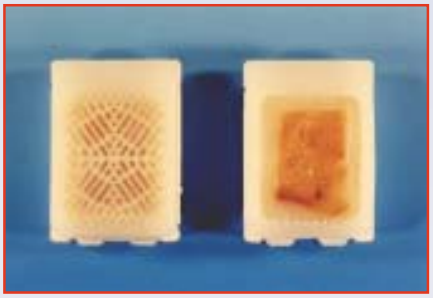
A large tissue cassette has been prototyped and tested. The paraffin blocks produced using the Paraform® system look similar to traditional blocks except for the presence of the soft portion of the cassette within the block. The paraffin block is faced, removing the bottom portion of the cassette. The block is then microtomed in the usual manner. The resulting histologic sections are indistinguishable from those produced from a traditional block. Special stains and immunohistochemical stains are not affected by the Paraform® system.

The Paraform® system offers several advantages compared to the traditional cassette/embedding system. Proper tissue placement and orientation, as determined at the time of grossing-in, are maintained through processing and embedding. Manipulation of cassette lids and the tissue itself is no longer performed, saving time and wear and tear on technologists' hands. Mechanical manipulation of the hard outer frame is possible offering the potential for automation. Designs for biopsy and orientation cassettes (on edge samples such as gall bladder, cyst wall) are in progress. Clinical studies to quantify the efficiencies achieved by the Paraform® system are planned.



Insert and outer frame, separate and assembled

Insert and outer frame, side view. Right image displays downward displacement for embedding step



Paraform blocks, before and after facing

Introduction

The need for cost containment continues unabated throughout the clinical laboratory, including histology. In response to these pressures, some steps in the histology process have been successfully automated in recent years. Automated tissue processing, staining, and coverslipping are the prime examples. Conversely, embedding is a time-consuming step that has resisted innovation. Redundancy of manual effort occurs at the time of grossing when tissue is placed in the cassette, and when the processed tissue is manually removed from the cassette and placed in an embedding mold. Streamlining this process would be highly desirable.

Recently developed synthetic materials resist the chemical environment of fixation and processing, yet possess a soft consistency similar to paraffin, allowing them to be successfully microtomed. The Paraform® cassette system uses one of these new materials to successfully address this challenge.

Description of System

The Paraform® cassette designed for a single large piece of tissue or several large pieces of tissue is described. The Paraform® cassette system has a rigid outer frame similar to that of a traditional cassette. The outer frame is easily manipulated by hand and has a slanted surface for handwriting or machine printing of an accession number.

An inner insert composed of a soft flexible material forms the bottom and inner sides of the cassette. The same material forms the lid. The insert bottom, sides, and lid are fenestrated similar to a traditional cassette to allow passage of solutions.

Ratchets along the inner side of the insert permit each corner of the lid to be lowered to the appropriate height to securely hold the tissue in place between the lid and the bottom of the insert. By this means, the proper placement and orientation of the tissue, determined at the time of grossing, are maintained throughout the remainder of the process.

The inner insert is held in place by small tabs on the outer frame. To manually embed, the cassette is placed over an embedding mold. The insert with lid is pushed as a unit downward which releases the insert from the outer frame. The freed insert is pushed downward until contact is made with the bottom of the well. The well is filled with paraffin, and the block is released as usual after cooling.

Preliminary Results

The material forming the inner insert and lid resists the harsh chemical environment of fixation and processing. No significant swelling or change in consistency has been observed during fixation with formalin or other commonly used fixatives. The material is also resistant to various decalcification solutions. The material has been tested with various processing cycles using xylene, xylene substitutes, and acetone, without significant swelling or change in consistency.

The paraffin blocks produced using the Paraform® cassettes are similar in appearance to traditional blocks, except that the insert is visible in the block. The material has a soft consistency similar to paraffin, which allows it to be microtomed without dulling of blades more quickly than would be expected by cutting traditional blocks. Microtomy of blocks made using the Paraform® system requires minimal change from the usual procedure. The block is faced which removes the bottom of the insert and exposes the tissue. After this is accomplished, ribbons are made in the usual manner, mounted, and prepared for staining.

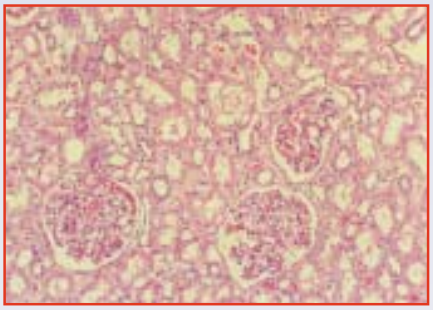
The material has little affinity for stains. With routine H&E stain, the material may be faintly visible on the periphery of the slide, but would likely remain unnoticed if one were not specifically looking for it. No interference with routine histochemical or immunohistochemical stains has been observed.

The Paraform® system has the potential to positively impact work flow. Time is saved during the embedding step because histotechnologists do not have to reopen cassettes or directly manipulate tissue. The amount of time saved may be substantial with cassettes containing multiple pieces of tissue, i.e., prostatic chips, disc fragments, etc.

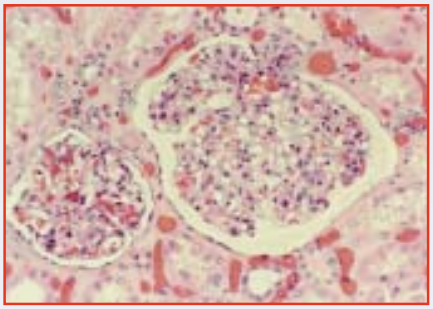
Future Directions

The large tissue cassette is designed for large flat or irregular pieces of tissue (uterus, breast, prostatic chips, autopsy sections, etc.) which generally require only proper tissue placement in the cassette. Additional cassette designs are in progress appropriate for small biopsy specimens and larger pieces of tissue requiring orientation on edge such as gall bladder wall or cyst wall. The majority of tissue routinely encountered can likely be appropriately handled by only a few cassette designs.

Since the Paraform® cassette system maintains tissue placement/orientation once the lid is closed, automation of the embedding step becomes possible. Machine operations required include manipulation of cassettes by means of the rigid outer frame, downward displacement of the inner insert into a mold, dispensing of paraffin, and ejection of the block. A prototype system is in development.

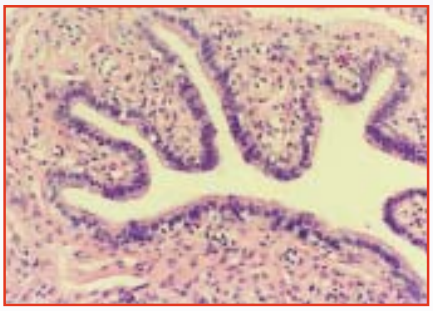


Kidney, H&E, 10×

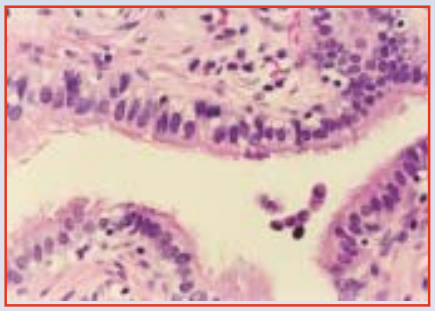


Kidney, H&E, 20×

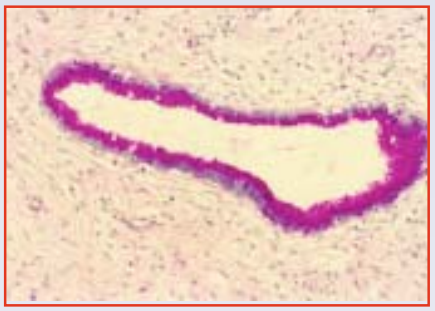
Fallopian tube, Keratin, 10×



Fallopian tube, H&E, 20×



Fallopian tube, H&E, 50×



Endocervix, PAS, 20×

Conclusion

The Paraform® cassette system holds promise to improve work flow in the histology laboratory by reducing redundancy in the grossing and embedding steps. Time is saved in the manual mode, but greater efficiency is anticipated by automating the process. Time savings can address such issues as total direct costs and the shortage of trained, experienced histotechnologists for both routine work and special techniques.

All in a Day's Work !

Vinnie Della Speranza, Scientific Editor

All in a Day's Work! *is a salute to the efforts of histotechs everywhere, those unsung heroes who often go unrecognized for their contributions to the advancement of health and science. Please contact the editor if you would like to see your work featured in this column.*

Nowhere is the "art" in histotechnology more apparent than at **Ward's Natural Science Establishment, Inc.** of Rochester, New York. While most of us may not be familiar with Ward's, few of us have gone untouched by this remarkable facility. Remember those frogs you might have dissected in junior high school, or those microscopic preparations of parasite ovum you had to memorize for the parasit final in college? Well, there is a very good chance that your school obtained those materials and countless others at Ward's.

Sounds simple enough until you stop and think about the huge volume of biologic (and other) specimens and materials required to satisfy the needs of educational facilities around the country, and in fact, the globe! Ward's is one of only two suppliers for such material in the country but is unique in that, unlike it's competitor, Ward's will custom prepare teaching materials to your specifications. Another attribute that is somewhat unique in this modern electronic age, is that you will always speak directly to a customer service agent at Ward's when you call during regular business hours. So your needs receive their immediate attention.

Anna Woolston and Kathy Stone checking the final product before shipping.

This past Spring, **Mary Georger**, Manager for the Microscope Slide Department at Ward's, invited me to visit her workplace. There they produce over 600,000 slides each year, representing over 1800 different catalog items ranging from Amoebae to Zygnema. Prior to my arrival there, I wondered what could be so special about this place, especially as I drove up to this non-descript building set back

well off the road. I thought that I'd pretty much seen all that there is to see as far as histology labs go, but I would venture to say that Ward's is like no environment you can possibly imagine. My tour there rivals any museum I've visited, only better because here I had the chance to go behind the scenes to witness this amazing operation.

Bob Conlon mounting whole mounts.

Speaking of those frogs I mentioned earlier, during my tour through the Preserved Materials section, we came to a large room with stainless steel troughs about 30 feet long by 6 feet wide and 4 feet deep, one which seemed to contain a gazillion bull frogs, and other small toads, bringing images of Budweiser commercials immediately to mind. They will be sacrificed and injected with a pigmented latex material into the veins and arteries to permit easy demonstration of the circulatory system to students. The facility also provides mammals and fish, in addition to amphibians, for student comparative anatomy labs.

In Osteology, bones, including human ones are bleached and reassembled into skeletons. In Geology there literally were rocks and minerals everywhere, waiting to be catalogued and packaged for shipping. In another area, entymology specimens (insects) are organized into teaching collections. If you would like a treat, call and ask for their catalogue on CD-ROM.

Ward's gets its name from its founder Henry Ward, a real life "Indiana Jones," who traveled around the world seven times, sat atop Mount Sinai, survived smallpox and an Arab desert battle, all before the turn of the century. As a young boy, Henry developed a thirst for travel and adventure that never ceased. At the age of twenty, Henry went to Europe to tutor a boyhood friend, Charles Wadsworth. Together, they traveled throughout Europe, then crossed the Mediterranean to Egypt and descended the Nile. The collection of fossils and minerals Ward gathered during this eight-month excursion can be viewed today at Buffalo's Natural History Museum. Henry financed his studies in geological studies at the Paris School of Mines by selling fossils.

During his travels as a young man, Ward learned that natural science research was enthusiastically supported in Europe. He was so impressed with the work he saw there that he brought talented osteologists and taxidermists from France and Germany to work for him in the U.S. When American colleges and universities finally caught "collection fever," Ward's Natural Science Establishment had its formal beginnings and over time, attracted many scientists who went on to become world famous. In 1862, Vassar Women's College commissioned Ward to prepare a collection, the first one that he would offer for sale, which was assembled on the campus of the University of Rochester. Among the famous characters that Ward came in contact with was William F. Cody, better known as Buffalo Bill, who came to Rochester to engage Ward in the preservation of buffalo heads. Ward also did business with P.T. Barnum of circus fame. Barnum's elephant, Jumbo, was the largest in captivity. After Jumbo's death, Ward was commissioned to mount Jumbo's skin and skeleton, a project that took almost two years, to preserve the 12-foot high, 14-foot long, 6-ton corpse.

Dubbed the "Great Museum Builder," Henry Ward's last collecting passion was meteorites. His passion took him to Europe, Persia, Mexico, and Columbia at the age of 71. In the early days of supplying museums, taxidermy and paleontology were the largest departments. By the turn of the century, many museums had preparatory staffs of their own. Then schools began to teach science, creating the need for all kinds of specimens and teaching aids. Today, the Biology department is composed of Preserved Materials, Osteology, Live Materials, Biotechnology, and the Microscope Slide Department.

Carla Thompson cutting serial sections in the Embryology Section.

If you think that your lab produces high quality slides, you must see the perfection prepared at Ward's Natural Science Establishment. I had an opportunity to view a number of microscopic preparations, and the talent in the laboratories at Ward's was immediately apparent. The Microscope Slide Department is composed of Whole Mounts, Botany, Embryology, Zoology, and Histology. **Carla** and **Lori** in the embryology section often prepare whole mounts of entire embryos. Many items are serially sectioned, with absolutely no room for error. One lost section is doom for the entire block. One popular item, whitefish eggs used to demonstrate the various stages of mitosis, is prepared here. Three sections of each mitotic stage are mounted on a slide, for a total of

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9 sections per slide. They sell more than 2,000 of these slides each year, requiring the use of motorized microtomes to get the job done. The problem is that none of the commercially available motorized microtomes are fast enough, so they have adapted sewing machine motors for their use.

All those who make it happen in the Microscope Slide Department at Wards.

If you are looking for new challenges, come work in histology, where they may be silver staining 400 slides at a time, preparing 12,000 blood smears, or sectioning celloidin blocks, a technique many of us have only read about, on any given day. Or try the whole mount section where **Kathy, Anna, Phyllis,** or **Bob** may ask you to dissect out the mouth parts of grasshoppers. Healthcare advancements in industrialized nations make it necessary for Ward's to get many of its specimens of infectious agents, including parasites, from the far reaches of the globe. In some cases, as with the frog embryology line in the Zoology section, **Ina** may be growing her own aerated fertilized frog's eggs in the lab for her work at Ward's. Of course, you won't find local sources when you need camel fleas so…well as a matter of fact, where does one get camel fleas from Mary?

Words really cannot do justice to my experience at Ward's. I wish that I could bring all of you back with me for a visit. I do wish however to congratulate all of the techs working with Mary who produce the finest work imaginable. Their talent and pride shine through in every specimen they prepare.

Today, Ward's Natural Science Establishment strives to continue in the spirit of its founder, with his infinite sense of wonder, innovation, and dedication to enlightening students of the natural sciences. All of Ward's products come with a lifetime guarantee. Mary recently received a frantic call from a client who had attempted to clean with xylene over 200 slides of celloidin-embedded material that were about 40 years old, but upon receiving them, she determined that they couldn't be salvaged so they will be replaced with new stock at no cost to the client—unbelievable customer support in this day and age. I have concluded from my visit that if you need something Ward's doesn't have, it probably doesn't exist.

If you'd like to learn more about Ward's Natural Science Establishment or its product line, you can visit their web site at www.wardsci.com or call 1-800-962-2660.

Special thanks to Mary Georger for providing the background information used in this article.

Using Frozen Sections for High Throughput Tissue Analysis in the Age of Molecular Pathology

Deborah Lawson
Millennium Pharmaceuticals
Cambridge, Massachusetts

Why Use Frozens?
Preservation of RNA for molecular pathological analysis of disease is becoming more and more important as a tool for prediction, diagnosis, and prognosis of human disease. Progress in all areas of medical research and new drug discoveries are mainly due to the increased capabilities of scientists to analyze cells on a molecular basis; they are looking at why mutations occur in DNA or RNA to make cells normal or pathological. Frozen sections preserve these structures best, sacrificing some morphology to look at genetic and protein regulation. Utilization of frozen sections allows us to better understand genetic regulation in tissues by better preserving these regulating proteins, enzymes, and cell signaling pieces of RNA, which give us clues into how disease processes work.

Many believe that frozen tissue always looks like Swiss cheese and is loaded with artifact. Not true if done properly. Frozen tissue is used routinely in industry as a way of getting data quickly and efficiently. If frozen properly, tissue can look almost as good as paraffin sections. Frozen tissue is useful for doing in situ hybridization, as well as immunohistochemistry, quickly and efficiently. Freezing has the advantage of minimizing the loss of RNA in situ, and it also minimizes the masking of antigen binding sites in immunohistochemistry when looking for low signal probes and hard-to-stain antibodies.

Tissue can be frozen either unfixed or fixed. An advantage to using unfixed frozen sections is that tissue does not need to go through any time-consuming processing. If freezing is done quickly and efficiently at the correct temperatures, it has excellent morphological preservation and little artifact. Shrinkage found in formalin-fixed, paraffin-embedded tissue is avoided in frozen tissue so analysis of structure is more easily accomplished, especially when measuring structures such as the size of infarct, as in the case of ischemic brain. Fixed frozen tissue also has the advantage of a controlled, minimally fixed cross link in paraformaldehyde so one does not have to use antigen retrieval as in formalin-fixed paraffin sections.

Want to shorten your turnaround time? Freeze everything, then process in paraffin as a backup.

Freezing Unfixed Tissue
Freezing tissues properly can be done using a variety of methods. These include the use of dry ice, isopentane over liquid nitrogen, or ethanol on dry ice. There are advantages and disadvantages to each of these. The method of freezing should be carefully considered, tailoring it to the structures one wants to examine.

The simplest of the above methods is to take fresh tissue immediately after excision and freeze it in powdered dry ice for about 5-10 minutes, depending on the size of the tissue. Then tissue may be transferred to the cryostat and sectioned unembedded. This technique is very good for isotopic in situ hybridization using low signal probes, and works well for rodent tissues, especially brain. Sections are cut and placed onto superfrost plus or gold plus slides (Erie Scientific), with no subbing needed. Slides are then laid flat at room temperature until the sections are just dried onto the slides, collected at –20°C then transferred to –80°C at the end of the cutting session for storage. Sections can be stored in tightly closed boxes for up to eight months in some cases. Probes and antibodies must always be run with proper controls to make sure no loss of signal has occurred with long term storage.

After cutting, tissue can be stored in small labeled plastic whirl-pak bags at –80°C for up to 1 year. Slides can then be removed from the –80°C when needed, brought to room temperature, and fixed in your fixative of choice, such as cold acetone, ethanol, or paraformaldehyde, depending on the protocol to be used. The dry ice method of freezing may produce some artifact, but for screening tissues quickly, both in a clinical and research setting, it is far superior than simply freezing in the cryostat. Storage of tissue and slides has the one drawback of having to use up extensive space in a –80°C freezer.

Unfixed tissue can also be embedded in OCT™ if it is small enough and does not have moisture on the surface. Moisture on the surface tends to make tissue pull away from the OCT,™ especially when dealing with brain including the attached dura. Never try to embed unfixed brain and then cut it. One is much better off with no embedding at all in the case of brain. If using unfixed tissue, the internal temperature of the cryostat is also an important consideration, taking into account tissue type, species, and age of tissue (adult, postnatal, or embryonic). If sectioning adult brain, for example, the optimum cutting temperature is –16° to –18°C, but embryonic brain may be better at –20°C due to the higher water content. It is best to freeze tissue embedded in OCT™ rapidly over an ethanol/dry ice slurry, floating cryomolds (Tissue Tek), or peel away molds in the slurry. Freezing will take minutes. The only drawback to this method is that writing on the molds tends to come right off.

Liquid nitrogen-cooled isopentane produces the least amount of artifact but is cumbersome to use. One must use a plastic cup or other vessel that will not crack at these temperatures. The isopentane is placed in the cup and the cup is immersed in liquid nitrogen that is placed in a dewar. Muscle biopsies are routinely frozen in this way with beautiful results. The –160°C temperature snap freezes and preserves enzymes in the tissue. Brain can also be done this way, but chill the isopentane only to –120°C using a low-temperature thermometer to monitor the temperature. Lower temperatures tend to crack sensitive nervous tissue.

Immersion of unembedded tissue in liquid nitrogen is not a desirable way to freeze tissue. The tissue will crack, and bubbles from the nitrogen will produce artifacts. However, if tissue is first embedded in OCT™ and dropped into liquid nitrogen, this will work fine. Problems with this method include difficulty in fishing the specimen out of the liquid nitrogen quickly, before it over-freezes, and orientation of the specimen can be difficult because the sample tends to sink to the bottom of the nitrogen while freezing.

Freezing Fixed Tissue
Processing fixed tissue for frozen sections can be a long process, but for certain situations where excellent results are needed with a hard-to-stain antibody, the results are worth it. The fixative of choice is usually 4.0% paraformaldehyde that is freshly prepared. In our lab we always make all of our processing solutions with diethylpyrocarbonate (depc)-treated water to better preserve RNA, since we do in situ processing of all of our specimens.

Equipment
- 4.0% paraformaldehyde in depc/PBS
- PBS/depc
- 10% sucrose, 20% sucrose, 30% sucrose made in depc/PBS
- OCT™ (Sakura Finetek)
- Cassettes for processing, if tracking is necessary
- Pencils for labeling cassettes
- Permanent markers
- Disposable plastic containers
- Dissecting instruments of choice
- Plastic bags for tissue storage, and freezer boxes
- Aluminum foil
- Peel away molds and cryomolds
- Latex or nitrile gloves
- Lab coat
- 95% ethanol, 70% ethanol
- brain matrix
- powdered dry ice
- Safety glasses
- 10% bleach
- –80°C storage boxes

Procedure

1. Dissect out tissue and fix in freshly prepared 4.0% paraformaldehyde for 2-48 hours at 4°C (depending on size of tissue). This can be done with perfused or unperfused tissue. For brain, it is best to slice with a brain matrix before immersion fixation for better infiltration and preservation. Fixation time will depend on size of tissue.
2. Rinse tissue at room temperature in 1X PBS/depc on shaker, with 3 changes, 30 minutes each.
3. Place in 10%, 20% and 30% sucrose, respectively, at 4°C until tissue sinks in each. Can take each step up to overnight.
4. Place in OCT™ for an hour to infiltrate at 4°C.
5. Place in fresh OCT™ and freeze on an ethanol/dry ice slurry.
6. Store well wrapped in foil and plastic bags at –80°C until use.

In Conclusion

In this age of high throughput work, frozen sections are underutilized as a tool to get work out faster. Quality does not have to be compromised in the process, but the technologist needs to pay careful attention to detail in utilizing freezing techniques. Frozen tissue will be used more and more in the future because of the Chip or Array technology that is being developed. Preservation of RNA for Array analysis, as well as the morphological tissue analysis, will become part of the molecular pathology workup for us all in the very near future.

Acknowledgments

I owe the example of freezing in powdered dry ice to my friend and colleague at Millennium, Pei Ge. I am grateful for all of her expert advice in the area of in situ hybridization.

I owe the idea of the importance of the ideas of immunohistochemistry and molecular pathology to Dr. Jules Elias, whose enthusiastic attitude for these subjects has infected me during the past 26 years.

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How to Perform an Electronic Literature Search, or Answer the Question "Has Anyone Ever Done This Stain Before?"

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Introduction

Ever been asked this question? Ever asked it yourself? My boss asked me the other day if anyone had ever published a method for doing Gram's stain in plastic (see associated article). As I started to answer his question, it occurred to me that others might have this problem arise and need to know how to attack it. So, here follows the attack plan I used that day and some associated background comments.

Background

Manual Method vs. Computer Searches. In the early Searchizoic Era, you had to find that elusive Stonehenge-like building somewhere on a university campus called a library. You've been in them before, those stuffy old buildings with thousands of books. In the central prayer place called the Reference Section, these buildings contain row upon row of sacred texts like, *Annals Index, Biological Abstracts, Chemical Abstracts, Science Citation Index, Current Contents* and the Most Holy, *Index Medicus.* In order to find an article on a particular stain, you had to look in each volume of each set of abstracts for the stain, by both subject and author. To be comprehensive, you also should look in the index of several histology texts (see References and Suggested Reading) for the staining procedure and the tissue of interest. These printed procedures should have references that you can track down. This activity assumes you know something about the stain and the person associated with the procedure. You know what happens when you assume. After several days and several less strands of hair, you may finally arrive at the conclusion that either (1) NO ONE has ever done this before or (2) you simply can't find any references about this stain. Now what? Cry? Pray? Find another profession? Jump for joy since you're about to be famous? Write your article, get it published and read in the next issue's Letters to the Editor (Elias, 1990) you aren't the first after all? Bummer.

The advent of personal computers has greatly accelerated the search process and decreased the amount of false negative and false positive searches. The U.S. National Library of Medicine (NLM), on the campus of the National Institutes of Health (NIH) in Bethesda, Maryland, houses over 5 million items related to biomedical sciences. The personnel of the NLM created the MEDLARS system and the MEDLINE® database. MEDLINE is a computer-based search device that lists citations and abstracts from over 4,000 journals (all those listed in *Index Medicus*) published from 1966 to the present. That's over 9 million references! This is a free search service, so if you have access to a computer and access to the worldwide web, you can search the journals in this system at the URL www.nlm.nih.gov at no additional charge. If you want the full-text version of the article you find, sometimes you can get it through the publisher or NLM for a modest fee. Usually, I print a list of the articles the search finds for me and then camp out at my local library until I find all the articles or run out of bread crumbs, whichever comes first. Several other search databases are available through the NLM, including compilations of AIDS clinical trials, chemicals, toxicology studies, and many more. Computer searches are, by far, faster and more thorough.

Specific Example or How To

MEDLINE may be searched by Author, Subject Heading, Journal, Title, Search Field, or Keyword. The keyword search pattern looks for the specific word (as it is spelled in the query, see note[1]) in the Title, Abstract, Registry number word, and MeSH subject headings. This method of searching the database has been, in my experience, the most effective strategy; i.e. returned the largest number of articles (hits). However, you do have to be sure to use a variety of words to cover your topic since authors express their ideas and techniques differently. Also, take advantage of the truncation symbol offered with MEDLINE to capture various suffixes: adjectives as well as nouns, plural as well as singular, etc. For example, "pollut" will pick up pollute, polluting, polluted, pollution, and therefore is more comprehensive than just using the word pollution. Keyword searches can be combined using Boolean logic operators "and" and "or." This helps narrow the query to answer the specific question at hand.

My boss asked, "Has anyone ever done a Gram's stain in plastic?"

I asked, "What kind of plastic, GMA, MMA, or other?"

He said, "Yeah, any of those."

There are several keywords to try in our search: "Gram stain," "glycol methacrylate," "plastic," and "histology." Type these into the computer search page and you'll get the following hits from MEDLINE 1966 to present:

Table 1. Keyword Search

Keyword	Hits
Gram stain	979
glycol methacrylate	362
plastic	24,608
histology	26,894

Great! Now what. Do I have to read all these abstracts? No. Now you narrow your search fields using logic. Do any of the articles list both "Gram stain" and "glycol methacrylate" as keywords in their title or abstract? Repeat this question with all keywords in all combinations. The results are:

Table 2. Narrowing the Search

Combined Search Terms	Hits
Gram stain AND glycol methacrylate	0[2]
Gram stain AND plastic	3
Gram stain AND histology	14
Glycol methacrylate OR plastic	24,970
Plastic AND histology	97
Gram stain AND (glycol methacrylate OR plastic)	3
Gram stain AND (glycol methacrylate OR plastic OR histology)	17

Conclusion

So now all I have to look at and read are 20 article abstracts to see if any of them are related to the question my boss originally asked. What was that again? "Has anyone ever done a Gram's stain on sections from tissues or tissue implant composites embedded in any kind of plastic media?" No. Nobody has ever published anything in the journals covered by MEDLINE on this subject from 1966 to the present.

1 Spelling is important. Ask anyone who has tried to "unsuscribe" from the Histonet. DCM

2 Notice in Table 1 that Gram stain returned 979 hits and that glycol methacrylate returned 362 hits. If you looked at each of these abstracts, all 1341, you'd be blind. Asking the computer to compare all the abstracts and list only those with BOTH Gram stain AND glycol methacrylate in them nets you 0 abstracts. The pattern of combined queries is referred to as Boolean logic.

Acknowledgments

Thanks are in order to Ms. Rena Sheffer of the University of Arkansas Medical Sciences Library for pointing me to the National Library of Medicine's website. We have a direct link to MEDLINE from our desktop computers through the library on campus. I use it daily. According to the NLM, over 350,000 literature searches are done using MEDLINE each day. A special thank you goes out to the lady who taught the best and most helpful course I took in college, Intro. to Library Research and Resources, Mrs. Kathy Sanders.

References and Suggested Reading

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Correction

Histo-Logic wishes to thank Dr. Roy Korson of Burlington, VT for informing us of an error that appeared in the timeline "Countdown to the 21st Century" in our Spring '99 issue. We reported erroneously that William Harvey described the circulation of blood in 1828. Dr. Korson points out that "*Wm. Harvey lived from 1578 – 1657 and that his famous treatise on the motion of the heart and the circulation of the blood first appeared in 1628.*" It seems that Harvey's ideas were slow to catch on, and experimental proof confirming his theories didn't appear until 1828, two hundred years later, according to Dr. Korson. This illustrates the need to verify information obtained from the internet and we thank Dr. Korson for sharing his insights on the matter.

Histonet: The List Server for Histotechnology

Linda R. Margraf, MD
Associate Professor of Pathology and Histonet Administrator
Herbert K. Hagler, PhD
Associate Professor of Pathology and Histonet Administrator
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Dallas, Texas 75235

Introduction

Have you ever had one of these days? Two technicians have called in sick. One of the pathologists, visibly agitated, has just returned a tray of

the diagnostic gastrointestinal biopsy slides to the lab. He grumbles that the slides have a horrible bubble artifact and he can't interpret them. They will need to be repeated as soon as possible. The chief of Pathology was just on the phone wanting to know if you can perform an alcian yellow stain by Wednesday, only problem is you've never heard of an alcian yellow stain and it is not in the books on the shelf. Now, one of the university researchers has come into the lab saying he needs you to find an antibody for interleukin-2 immunohistochemistry that will work in rat tissues. What on earth are you to do? One easy and fast (and free) solution is to turn to Histonet, the electronic mail (email) list server that specifically addresses issues in histotechnology. This article will describe what a list server is and how it can improve the function of your histology or research laboratory. It will describe Histonet, one of the first and still most active lists devoted to the broad range of topics in histotechnology.

Linda Margraf, MD and Herb Hagler, PhD keep Histonet up and running.

List Server Basics

A list server is simply a computer that runs software which will receive incoming email messages and automatically reroute a copy of each message to everyone on the subscriber list. Email uses the vast expanse of the internet to allow almost instantaneous communication between networked computers around the world. Some list servers are designed to disseminate information only to the subscribers on the list. Other lists, however, encourage ongoing dialog between the subscribers so that anyone with comments about a topic can contribute to the discussion. This allows a researcher in New Zealand to get input from labs in Japan, Germany, Great Britain, and California for the best parameters to set up a new immunostaining protocol without ever leaving his desk. Most list servers allow the subscribers to receive the messages immediately as they filter through the server ("real time") or compiled as a batch of messages, known as a digest, usually issued every 24 hours. Many list servers have additional functions, such as archives and FAQs (frequently asked questions) files so previous comments regarding a topic can be retrieved. There are now active lists covering a vast range of topics from archeology to zebra fish. Medical lists have become very popular with physicians and medical organizations. Most medical specialties have at least one email list discussing the optimal diagnoses, treatment modalities, and latest research findings in their area of interest. Several lists are now dedicated to topics pertaining to medical technology and selected areas in histopathology.

There are a few important words of caution to consider before jumping immediately on a list. Lists, on occasion, can be the targets of spam or unsolicited mailings that can become irritating, if not frustrating, to eliminate. Most lists pertaining to medical and professional topics are fairly free of this problem compared to lists covering more general topics. It is important to remember that simply reading an email message cannot cause a virus to infect your system, however, attachments should be carefully screened with an up-to-date virus detection program prior to opening. Some lists, such as Histonet, block attachments from going through the system to minimize the risks to subscribers. And, as is always a concern with information posted on the internet, the validity of the information or methodologies presented on a list should be verified before making any changes in your laboratory.

Each email list has its own set of rules and operating protocols so it is best to remain a silent observer for a while before jumping into the conversations on-line. Some lists will aggressively spurn any obvious neophytes or "newbies." Email lists differ from chat rooms in that the audience remains fairly stable, and most subscribers only comment on topics of shared interests. Histonet has been fortunate enough to attract a number of noted authorities in histology who will take the time to provide detailed answers to questions about their particular areas of interest. This includes people who can readily answer questions about eliminating bubble artifact in biopsies, send protocols for alcian yellow stains, and find antibodies for immunostains on rat tissue.

Histonet

The Histonet list, which has been in operation since January of 1996, now has over 1100 subscribers from all over the world. Subscribers include hospital employees from major urban centers and small, isolated locales, university researchers, botanists, laboratory workers in government agencies, veterinary facilities, and a wide variety of commercial industrial ventures. The questions prompting topics of discussion are as diverse as the participants. On a given day, a discussion of the best procedures for antigen retrieval for immunohistochemistry may be interspersed with comments regarding dinosaur bone sectioning, and butterfly preservation methods. Most topics however, pertain to optimizing routine diagnostic histologic procedures, equipment selection, laboratory management, and histotechnologist employment opportunities.

Histonet is run using hardware and software owned by the University of Texas Southwestern Medical School

Department of Pathology in Dallas, Texas. Currently, Histonet uses the LISTSTAR software from Quarterdeck Corporation (California) and, with the present number of subscribers, it processes more than 50,000 outbound messages a day. A single old Macintosh, a cast-off from a departmental computer upgrade, busily receives and distributes the messages around the clock to the subscribers from nearly fifty countries around the globe. The system is not without perils. Every one of the fairly frequent glitches in the University's own servers or power supplies, affectionately known as "intermittent power interruptions" send a ripple of problems through the system, creating repeating messages or temporary "quiet spells." By frequently monitoring the system, most of the problems are never noticed by the subscribers. Additional challenges to the list administrators include keeping the address lists updated (it is amazing how frequently people change addresses), and assisting in the spelling of the word "SUBSCRIBE." Perhaps the most important challenge in keeping a list such as Histonet running, is determining the appropriate focus and content of the list's messages. Aside from assuring the list remain free of unsolicited advertising, the administrators have allowed the subscribers to determine what they feel is an appropriate use of the forum. Histonet appears to succeed so well as an open, friendly exchange of information because it has been embraced by a congenial and knowledgeable community of histotechnologists. Though controversies regarding the appropriateness of messages occasionally arise, they rarely detract from the usefulness of the service. It is hoped the program (and the poor, tired, old Macintosh) will keep running for years to come.

If you are interested in subscribing to Histonet, send an email to Histonet@pathology.swmed.edu and put "subscribe" (spelled correctly (!) and without quotation marks) in the SUBJECT LINE of the message. If you have any questions about Histonet, please contact Linda Margraf, MD at Lmargraf@childmed.dallas.tx.us.

WHAT IS CYTOLOGY ANYWAY?

Kathleen A. DaSilva, SCT(ASCP)
Cytology Supervisor
University Hospital
Stony Brook, N.Y.

Caller: Cytology Department, please.

Operator: Psychology Department. Let me connect you.

Caller: No, not the Psychology Department, the cytology department, C-Y-T-O-L-O-G-Y.

Operator: Oh, the cytology department. Let me connect you.

I've lost count how many times I have heard that scenario during my career. In fact I am constantly amazed that even some fellow medical professionals are not aware of what a cytologist really does. Hopefully this article will shed some light on the mystery of what cytology is about and how far the field has come over the past 50 years.

Fig 1. Dr. George Papanicolaou

It all began with a doctor by the name of George Papanicolaou. Dr. Papanicolaou was born in Greece in 1883 and immigrated to America in 1913. He obtained employment with the Department of Anatomy at Cornell University Medical College. While studying the menstrual cycle in guinea pigs, Dr. Papanicolaou obtained cell samples from the vagina using a pediatric speculum. In studying the squamous cells, he found changes in their morphology that corresponded to the different phases of the menstrual cycle.

Dr. Papanicolaou began to apply what he had learned about the morphologic cell changes in guinea pigs and use it to study the hormonal status of human patients. He joined forces with a gynecologist named Herbert Traut. Together they began to collect human samples, but Dr. Papanicolaou soon made a startling discovery. He found bizarre cells in the smear from a woman with cervical cancer. He then realized that with this new technique, he was not only able to study the hormonal status of patients but was also able to detect cervical cancer in asymptomatic patients at an early and curable stage! Their findings were published in the American Journal of Obstetrics and Gynecology in 1941.

Since that landmark paper in 1941 there has been a greater than 70% decline in the cervical cancer death rate. The Pap smear has been such a success that, until recently, this screening technique has remained virtually unchanged since 1941.

Specimens are obtained using a speculum that is inserted into the vagina to permit the cervix to be visualized. A sample is taken using any one of a variety of collection devices that have been developed over the years, including the Ayre spatula, which was introduced in 1944, the cytobrush which appeared in the 1980s, or the "broom" or papette which is the most recent device to appear on the scene. The

collection device is inserted into the cervical os, or opening, and rotated to gently collect superficial cells from the cervix and endocervix. The cells are then smeared onto a glass slide and fixed immediately. The fixatives of choice include ethanol (ethyl alcohol), which, although it provides better nuclear detail, is inconvenient to transport, or a spray fixative that is used to coat the cells on the slides which are much easier and less hazardous to transport. The fixed slides are then sent off to the cytology lab for processing.

Fig 2. Pap smear collection devices, courtesy of Medical University of South Carolina, Charleston SC

At the laboratory the slides are stained with the traditional Papanicolaou stain, a staining method devised by Dr. Papanicolaou himself, specifically for Pap smears. The Pap stain is a modification of the conventional hematoxylin-eosin (H&E) stain. Alcohol-based counterstains (OG & EA) are used to highlight variations in cell morphology (ie. cell maturity and metabolic activity). There are several brands of these Pap stains on the market, which are slight variants of Dr. Pap's original formula, depending on how intense a blue stain you prefer. Over the years, there have been a number of refinements in cyto prep technique including the cytospin for hypocellular body fluids, to automatic stainers and coverslippers, which have helped tremendously to reduce turnaround time.

Most recently, we have witnessed the appearance of what is probably the most significant refinement to the Pap smear to occur since its inception, the development of monolayer technology. Instead of a physician smearing the cells onto a glass slide after collection, the sample is placed into a liquid fixative solution. In the laboratory, the sample may be placed onto an instrument to prepare the monolayer preparation, or it may be prepared by hand, depending upon the technology that a laboratory chooses to use. The use of monolayer technology offers wonderful advantages that increase the likelihood that abnormal cells will be discovered by the cytotech during screening. First, by avoiding the use of smears prepared during the patient's pelvic exam, we can eliminate variability in smear thickness and overall quality inherent in a method that has traditionally been performed by many different physicians. With monolayer technology, all patient samples are consistently prepared, regardless of who the patient's physician is. Another important advantage is that monolayer technology eliminates blood, inflammation, and mucus commonly found in smears, which can hinder interpretation. But perhaps most important of all, it has been discovered that with the traditional Pap smear, up to 80% of the collected cells are discarded on the collection device. With monolayer technology, the cells are transferred into the preservative fluid, and the collection device can be sent to the laboratory as well. As a result, the cellular yield and the potential for discovering an abnormality is greatly increased.

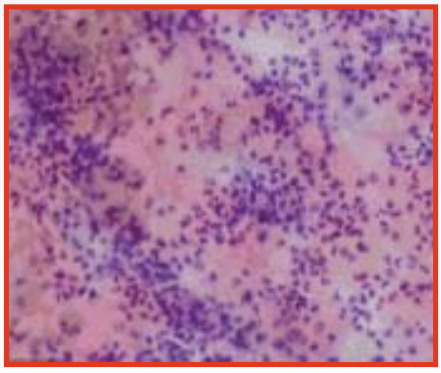

Fig 3. Conventional pap smear. 20×

After the slides are stained and coverslipped they are sent to the cytologist for screening. The cytologist must look for changes in cell morphology, which may indicate the presence of precancerous or cancerous lesions. In essence, the cytologist must look at every single cell on the slide, the equivalent of looking for a needle in a haystack, in order to be certain that any abnormal cells that may be present are found and identified. While the method a cytologist uses to screen a slide hasn't changed much over the years, the microscopes that they use have gone through a number of changes over the last 5 years. Most significantly, modern microscopes are much more ergonomic, which makes it possible to avoid job-related maladies, such as carpal tunnel syndrome, that can come from hours spent at a microscope screening slides. Newest on the scene, cytology has witnessed the appearance of computerized rescreening devices, which are, with the exception of one instrument, FDA approved only for quality control (rescreening a slide previously screened by a cytologist). While there are some who argue that computer rescreening affords greater protection to the patient because humans may fail to find abnormal cells because of fatigue, eyestrain, or other distractions, microscopic screening, regardless of how it is performed, can only be as good as the slide being screened. A computer is no better at finding abnormal cells in a poorly made slide than a human is, which reinforces the importance and value of monolayer technology.

Cytodiagnosis can be conducted on samples from virtually any part of the body, utilizing a variety of techniques to isolate cells from body fluids, mucus linings, and the sampling of even deep-seated lesions with fine needle aspiration biopsy, all of which offer the patient important alternatives to surgery for diagnosis.

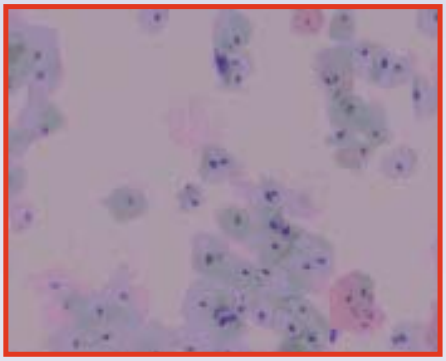

Fig. 4. Monolayer preparation. 20×

Cytology has come a long way in just 50 short years. The value of cytodiagnosis as an inexpensive tool to identify cellular abnormalities has been embraced by the medical community, and in fact is relied upon quite heavily today. With the technological advances that have occurred in recent years and those appearing on the horizon, it would seem that cytology has a very bright future ahead of it.

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NSHers Party Like It's 1999

The National Society for Histotechnology (NSH) chose Providence, Rhode Island for this year's truly historic event — the 26th and last NSH meeting of the century.

Sakura, **Allegiance**, and **Richard Allen** were sponsors of an exciting Millennium Party held on October 16 that attracted more than 900 partygoers. Five different decades were saluted — the 50s through the 90s — with colorful costumes, including varsity sweaters reminiscent of the 50s and Beach styles of the 60s. Life-size cardboard cut-outs of James Dean, Marilyn Monroe, Austin Powers, and other notables represented the decades and added to the fun.

In addition to a delectable buffet and bracing refreshments, popular games and activities kept everyone moving — from Twister to Bungee Run, a Hula Hoop Contest to Dance Lessons (Swing, Electric Slide, Charleston), pool games to hair braiding. Even tattooing (fake ones, of course). What's more, four lucky NSHers were selected to enter a unique Cash Cube filled with tons of swirling money. The participants had seconds to grab as much cash as they could. When the event was over, more than $800 had been given away!

An incredible live performance by Takashi Tsuzuki from Sakura made everyone believe that Elvis was truly in the house. And at midnight, partygoers counted down to the new century — out with the old millennium and in with the new — as balloons cascaded down from the ceiling. It was a great ending to a terrific party.

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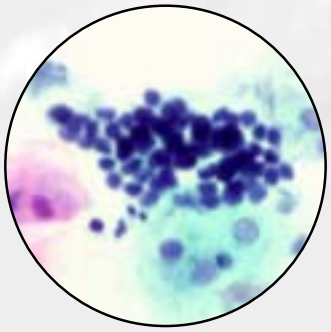

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Feb 18 **University of Texas Health Sciences Center/San Antonio**
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Speaker: Gwendolyn Goss, BA, HT(ASCP)
"Hollande's Solution as a Routine Fixative"
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Mar 4 **Colorado Society for Histotechnology**
Site: University of Colorado Health Science Center
Contact: Sara Williams
303-861-6177
Email: boowilliams@earthlink.net

Mar 10 **American Society of Clinical Pathologists**
Teleconference, 12:00 pm Central Time
Speaker: Glenda Hoye, HT(ASCP)
"Reviewing Connective Tissue Stain Procedures and Results"—for more details, contact ASCP at (312) 738-1336 or Email info@ascp.org

Mar 11 **Arkansas Society of Histotechnology**
Site: Baptist Medical Center, Little Rock, AR
Contact: Louellen McLemore
2394 Grant 73, Sheridan, AR 72150
(W) 870-541-7541 (H) 870-942-4483

Mar 15-18 **Florida Society of Histotechnology and NSH Region III Meeting**
Site: Delta Orlando Resort, Maingate Universal Studios, Orlando, FL
Contact: Jerry Santiago
Veterans Affairs Medical Center
1601 SW Archer Road (113), Gainesville, FL 32608
(W) 352-376-1611 ext. 6432

Mar 17 **University of Texas Health Sciences Center/San Antonio**
Teleconference, 12:00 pm Central Time
Speaker: Clifford M. Chapman, MS,HTL(ASCP)
"Dermatopathology: A Guide for the Histotechnologist"
1-800-982-8868

Mar 17-18 **Kentucky Society of Histotechnology Symposium**
Site: Holiday Inn, Louisville, KY
Contact: Renee Matherly
502-852-5587
Email:rmath0516@aol.com

Mar 17-19 **NSH Region II Meeting**
Site: Holiday Inn & Holidome, Downtown Williamsburg, VA
Contact: Frances Freund
804-288-6172 or 804-267-8134
(weekdays 9:00am-4:00pm)
Fax: 804-267-8831

Mar 31 - Apr 1 **New York State Histotechnological Society**
Site: Holiday Inn, Saratoga Springs, NY
Contact: Sandra Cummings
PO Box 81, Esperance, NY 12066
(W) 518-875-6304
Email: Toosandyd@aol.com

Apr 7 **American Society of Clinical Pathologists**
Teleconference, 12:00 pm Central Time
Speaker: Gwendolyn Goss, HT(ASCP)
"Regulations and Liabilities – Safeguarding the Laboratory Professional and Pathologist"—for more details, contact ASCP at (312) 738-1336 or Email info@ascp.org

Apr 8-11 **American Society of Clinical Pathologists**
Spring 2000 meeting—Boston, MA
for more details, contact ASCP at (312) 738-1336 or Email info@ascp.org

Apr 13-15 **Texas Society for Histotechnology State Meeting**
Site: Camino Real Hotel, El Paso, TX
Contact: Kathy Dwyer
(W) 214-947-3538 (H) 972-223-5105

May 11-13 **Histology Society of Ohio State Meeting**
Site: Clarion Westgate, Toledo, OH
Contact: Susan Black
5526 Whiteford Road, Sylvania, OH 43560
(H) 419-885-8266 (W) 419-471-3702
Fax: 419-479-6916
Email: SBHT31@aol.com

May 17-21 **California Society for Histotechnology Symposium/Convention**
Site: Doubletree Hotel, San Jose, CA
Contact: Linda McGlothlen
2325 Brindlewood Dr., Rancho Cordova, CA 95670
(H) 916-635-3240 (W) 916-734-2534
Email: lindamcgloth@gateway.com

May 18-20 **Georgia Society for Histotechnology**
Site: The Georgia Center, University of Georgia, Athens, GA
Contact: Connie Wavrin
(H) 770-452-8310 (W) 770-686-2385
Email: connie_wavrin@emory.org

May 18-20 **Illinois Society for Histotechnologists State Meeting**
Site: Radisson Hotel, Schaumburg, IL
Contact: Cathy Locallo
Anatomic Pathology, University of Chicago Hospital
5841 S Maryland Ave MC6101, Chicago, IL 60637
(W) 773-702-8492
Email: clocallo@mcis.bsd.uchicago.edu

May 19-20 **Missouri Society for Histotechnology**
Site: Elms Resort and SPA
Excelsior Springs, MO
Contact: Janet Kliethelmes
913-339-0484

Jun 2 **American Society of Clinical Pathologists**
Teleconference: 12:00 pm Central Time
Speaker: Richard W. Brown, MD
"Special Stains in the Histology Laboratory – an Update,"—for more details, contact ASCP at (312) 738-1336 or Email info@ascp.org

Jun 8-10 **Arizona Society for Histotechnology**
Site: Doubletree Guest Suites, Phoenix, AZ
Contact: Ethel Macrea
Ventana Medical Systems, Inc.
3865 N. Business Center Drive, Tucson, AZ 85705
(W) 520-690-2739
Email: dawnbrok@flash.net

Jun 16 **University of Texas Health Sciences Center/San Antonio**
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Speaker: Fiona E. Craig, MD
"Bone Marrow Specimen"
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Contact: NSH Office
4201 Northview Drive, Suite 502
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