HISTOLOGIC®
Technical Bulletin for Histotechnology

SAKURA

Vol. XLIII, No. 2 December 2010

Managing Editor, Nancy Klemme
Scientific Editor, Vinnie Della Speranza, MS, HTL(ASCP)HT, MT

Antibody Optimization and Validation

Jim Burchette, HT(ASCP)
Duke University Health System
Durham, NC

Billie Zimmerman, MT(ASCP)QIHC
MCG Health, Inc.
Augusta, GA

burch007@mc.duke.edu

Abstract

Many histotechnologists are perplexed by the myriad of regulatory requirements and the perceived obstacles to achieving an effective antibody optimization program. As immunohistochemistry (IHC) receives greater scrutiny by the College of American Pathologists (CAP), the need for laboratories to develop their own compliant IHC strategies and protocols becomes even more important. There is frequent discussion in the Histonet listserv community as technologists struggle to understand which optimization approaches are compliant with the regulations. In this article we will discuss in some detail the components of an effective optimization program.

Introduction

Antibody optimization is the process where dilution, pretreatment techniques, incubation times, and detection systems are developed for antibody testing in the laboratory. This can be performed in both clinical and research environments. Antibody optimization and validation can range from a simplistic approach to a very complex program depending on the needs of the individual laboratory. A laboratory's staffing, budget, and workload levels may play into which approach is adopted. Some laboratories rely on antibody companies to optimize their protocols and utilize ready-to-use (RTU) antibodies while other

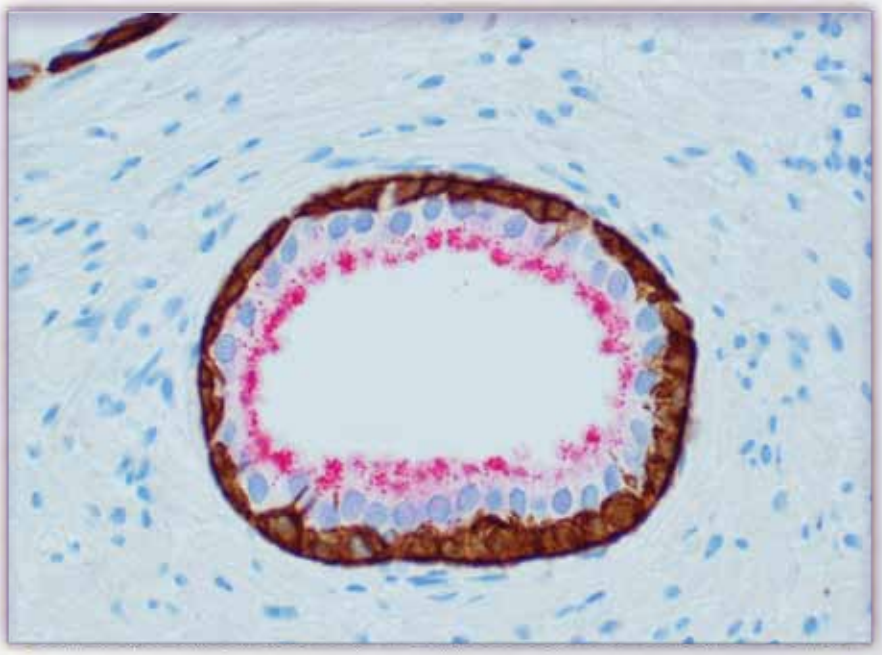

Fig. 1. Prostatic intraepithelial neoplasia (PIN) demonstrated with the PIN-4™ Cocktail (Biocare Medical, Concord, CA) dual-color stain antibody. Prostate basal cells react with a cocktail of CK5, CK14 (brown cytoplasmic staining), and P63 (brown nuclear staining). Mitochondria, predominantly apically located, stain red from reaction with P504S. 400X

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laboratories develop protocols and optimize concentrated antibodies in-house. Regardless of the approach used in your laboratory, documentation of the optimization process and the results of that process are key components, especially when it is time for inspections by regulatory agencies such as CAP, Clinical Laboratory Improvement Amendments (CLIA), state agencies, and internal reviews.

The images that accompany this article are offered to illustrate the optimal staining that can be achieved with a comprehensive antibody optimization and validation program.

Optimization

A major consideration with antibody optimization is determining the proper tissue pretreatment required for exposing a particular epitope. Does the antigen of interest require heat-induced epitope retrieval (HIER), enzymatic digestion, or no pretreatment at all? Attention must also be focused on the type of detection system needed for certain tissue types, such as tissues rich in melanin pigment. An alkaline phosphatase-labeled detection system with fast red chromogen and/or a modified Giemsa counterstain is preferred by some while others choose a 3,3' diaminobenzidine (DAB) chromogen. Sometimes the detection system available is what the supplier offers on its automated platform, but there are always options to take into consideration, such as protocol modifications (horseradish peroxidase versus alkaline phosphatase detection systems), alternate use of chromogens, or chromogen enhancement for specialized applications. A non-biotin-labeled micropolymer detection system is the best option if HIER is required in order to avoid complications that can be caused by tissues rich in endogenous biotin. The use of labeled streptavidin-biotin (LSAB) detection systems should be considered for tissues not requiring HIER pretreatment. Rarely does this type of detection system require an avidin/biotin blocking step when no pretreatment is required; it is required only occasionally following enzymatic pretreatment of formalin-fixed, paraffin-embedded tissue. While not as sensitive as labeled polymer detection reagents, LSAB detection systems are more economical and perfectly acceptable in certain applications. Individual components are available for purchase and are easily prepared in-house for a very economical detection system. All of these variables, if incorporated into your IHC program, should be included in your antibody optimization and validation.

While there are no set guidelines for antibody optimization and validation, there are recommendations set forth by CAP. The CAP question ANP.22750 reads, "Has the laboratory documented evaluation of new antibody lots and new antibodies, prior to use in patient diagnosis?" Question ANP.22760 states, "Are new lots of antibody and detection system reagents tested in parallel with old lots?" (NOTE: New lots of primary antibody and detection system reagents must be compared to the previous lot using an appropriate panel of control tissues.) Interpretation of guidelines among pathologists, technologists, and CAP is often cumbersome and confusing. Yes, antibodies and detection reagents should be tested in parallel with old lots, but think of the ramifications of CAP question ANP.22760. Some automated systems will not allow for programming of this parallel evaluation within a single run. The parallel evaluation might have to be performed in separate staining

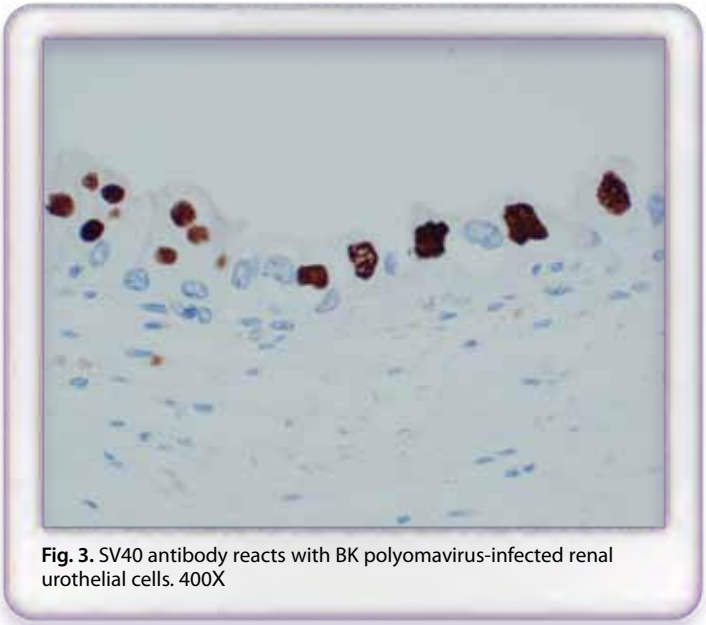


Fig. 3. SV40 antibody reacts with BK polyomavirus-infected renal urothelial cells. 400X

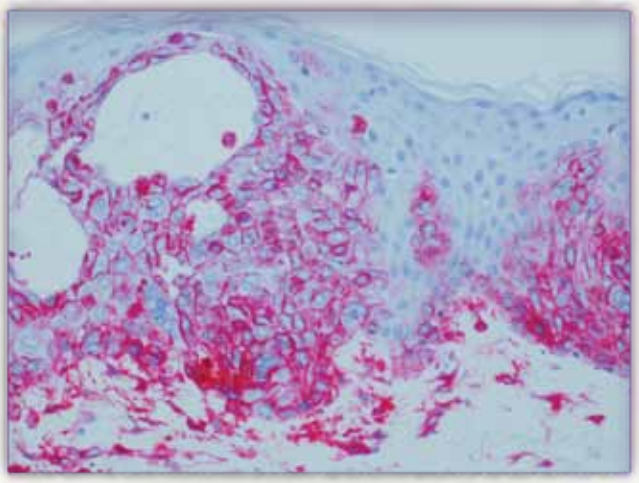


Fig. 2. Varicella zoster virus demonstrated in skin biopsy specimen. 400X

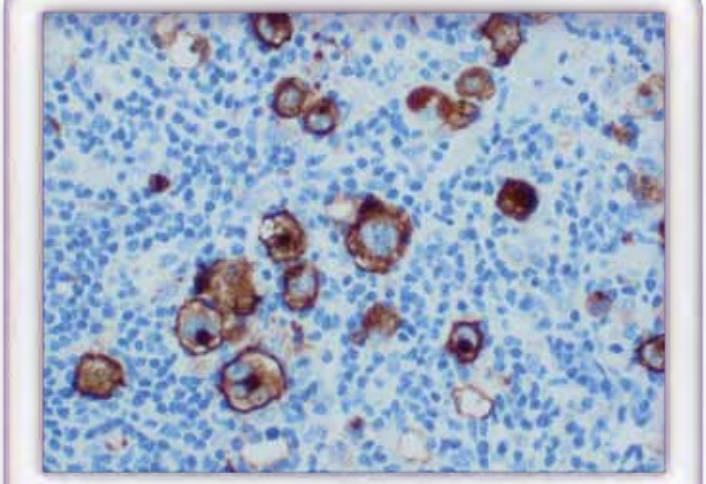


Fig. 4. Reaction of Hodgkin's lymphoma Reed-Sternberg cells with CD15 antibody. 400X

batches. Does that void the evaluation? This certainly presents a problem with interpretation of recommended guidelines and is just one example of the many variables we are faced with in clinical IHC. Showing intent to comply and providing documentation of results are key to achieving compliance.

There is no universal standardization for IHC optimization and validation. Some laboratories prefer to do the optimization themselves while other laboratories depend on services offered by many companies to either optimize or assist in the optimization of IHC tests. It is possible that the latter approach adds to standardization in its own way, with many sites using automated platforms, protocols, and antibody detection systems from a single vendor source. Regardless of the approach taken, proper documentation of the process is required. The companies you purchase from generally perform optimization on preparations of antigens, development and screening of hybridoma cell lines, growth and harvest of polyclonal and monoclonal antibodies, purification, gel and ELISA assays, as well as determining specificity and affinity and all of the legal issues associated with manufacturing and selling products worldwide.

Optimization and Validation Procedure

The following information describes our optimization and validation process. Having been through many CAP inspections without deficiencies, the best course of action is clear documentation of the process and the results obtained at each step. Communication between technologists and pathologists is a vital part of the optimization and validation process and should be included in your documentation. In the end, it is the quality of the IHC on the microscope stage coupled with our contribution to patient care that is the ultimate goal.

Upon receiving an antibody, the product specification sheet (which is usually available on the company website and can be viewed before purchase) should be thoroughly reviewed for the antibody species, immunoglobulin isotype, clone, immunoglobulin and protein concentration, recommended dilution range, and recommended tissue pretreatment. The expected staining pattern should be noted for reaction in neoplastic and non-neoplastic tissues. References are collected, printed, and placed in the paper file created for each antibody. An electronic Word document file should be created for documentation of antibody information, technical notes, communication between staff, and a record of tissues and cases used in the antibody workup. A hard-copy paper file is also maintained for quick reference purposes.

Pathologist involvement is critical in the optimization and validation process. Dr. Neal Goldstein, MD, Director of Surgical Pathology at Clarient, is quoted as saying, "Based on FDA's ruling on class reagents in IHC, the legal responsibility for validation and knowing the relevant parameters is put squarely on the shoulders of the lab director." True, the legal responsibility rests with the director, but technologists have a responsibility to their patients, the pathologists they work with, and their profession to produce IHC results of the highest quality.

Reading an antibody specification sheet gives valuable information and a starting point for selecting which tissues to use in the optimization process; however, many technicians and technologists do not understand the overall intended use and application of a particular antibody nor do they have the knowledge of what cases to use for this process. If possible, both strongly and weakly expressing tissues should be used. Availability and quantity of certain control tissues can be problematic. Once the tissue and case selection have been made and obtained, fresh-cut sections are prepared and air dried. A note or profile slide should be labeled with the tissue, case/code identification, and date the sections were prepared. Place this in the slide storage box for reference. A felt-tip marker can be used to mark the slide edge to identify the slide group. When using concentrated antibodies, the manufacturer's recommended antibody dilution is used as a starting point and then other concentrations above and below this dilution are used. As an example, if the recommended dilution is 1:100, prepare 1:50, 1:100, 1:200, and 1:400 dilutions for the initial testing. The recommended antibody working dilution

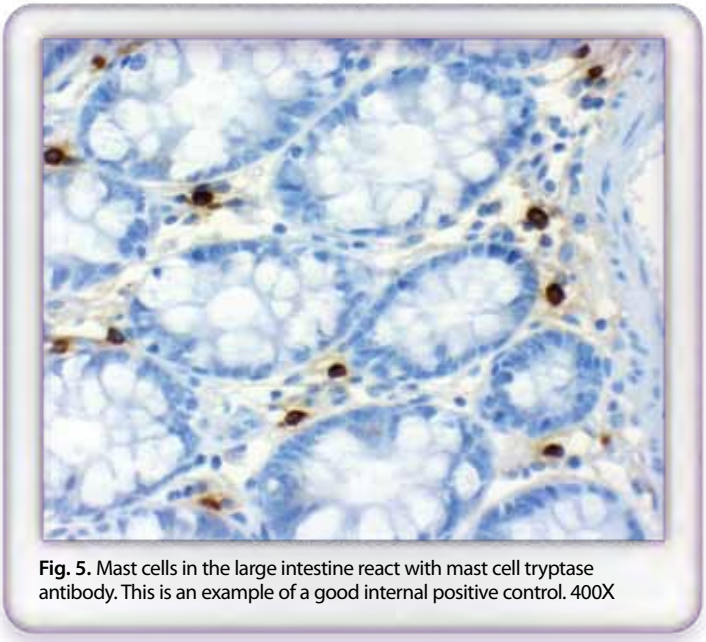


Fig. 5. Mast cells in the large intestine react with mast cell tryptase antibody. This is an example of a good internal positive control. 400X

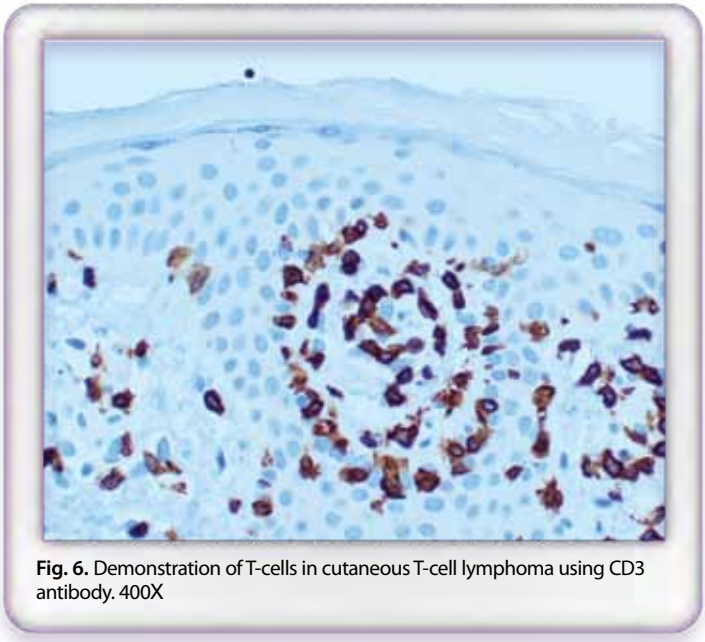


Fig. 6. Demonstration of T-cells in cutaneous T-cell lymphoma using CD3 antibody. 400X

varies depending on the detection system used. If needed, prepare stronger or weaker dilutions after the results are obtained from the initial titration. RTU antibodies are becoming increasingly popular for several reasons. Oftentimes, RTU antibodies are optimized for a particular supplier's detection and staining system, including pretreatment suggestions. RTU antibodies are convenient to use, they save time, and are an alternative to less frequently performed tests, but they will cost more. The user must decide which option—RTU antibody or concentrate—is best for the particular circumstance.

A comparison of different retrieval solutions and heat sources during the initial antibody workup will confirm which methodology works best with your laboratory staining process. If an antibody source recommends "boiling the tissue section for 20 minutes in citrate buffer," you should still take the time to prove to yourself that this is the optimal pretreatment solution. In comparing different HIER pretreatment solutions, use several pH options and vary the time tissues are exposed to the heated solutions. One should not overlook the use of proteolytic enzymes for retrieval purposes. In general, some proteolytic enzymes are less destructive to fixed tissue sections; they provide a faster retrieval process and often the results are comparable to heat retrieval pretreatments. Following the completion of the IHC stain, always review the slides for proper reactivity by viewing internal immunoreactive target structures. Sit down with the pathologist or senior team member and review the slides together. We are a professional team and this is a vital part of communication and learning. Repeat the best result obtained to demonstrate consistency with this new test. Then document the results by the run date to show a chronological timeline. Next, date the slides for filing and retrieval if the slide labeling system does not include the run date.

Documentation forms can be prepared and placed with the folder when the slides are presented to the pathologist, or the forms can be filled out when the slides are reviewed by the technologist and pathologist together. Too often these forms seem to get lost in the shuffle and are not returned to the laboratory. However, many pathologists will respond to an email. This type of communication can be printed, placed in the paper file, and/or copied into an electronic file. Much of the optimization process depends on pathologists' involvement as well as their interaction with the staff. Every institution is different and validation depends on what works in your particular laboratory.

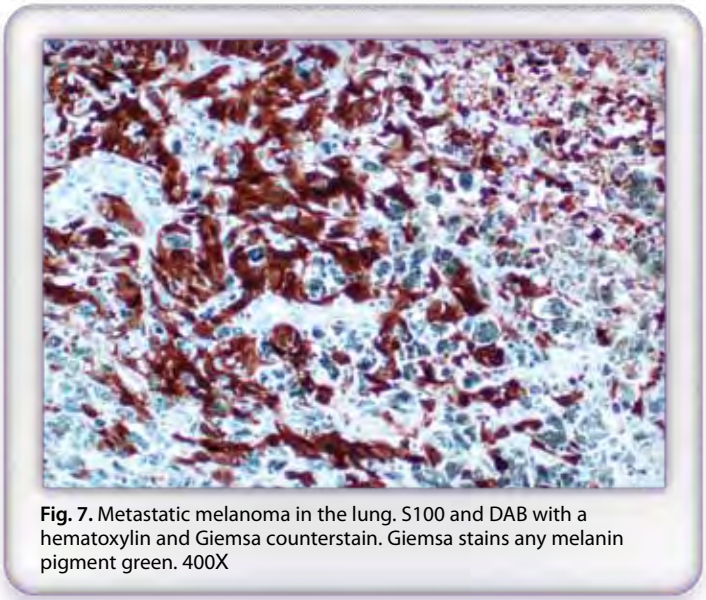


Fig. 7. Metastatic melanoma in the lung. S100 and DAB with a hematoxylin and Giemsa counterstain. Giemsa stains any melanin pigment green. 400X

Once you and the pathologist feel confident with the results, move the test to the routine bench for verification if the optimization process has been performed in a test development area. After the optimal working dilution has been determined with the concentrated antibody, a prediluted antibody supply should be prepared for stability testing. Determine a testing timeline that works for your lab. Is it an antibody that will be in high demand or an antibody that will be used once every 2 weeks or more? The in-house prepared prediluted antibody should be tested once a week with precut control sections taken during the initial workup. In addition, fresh-cut sections can be run for signal comparison. Information such as diluted antibody performance and epitope stability in the stored control section can be obtained with this simple study. Again, document the results.

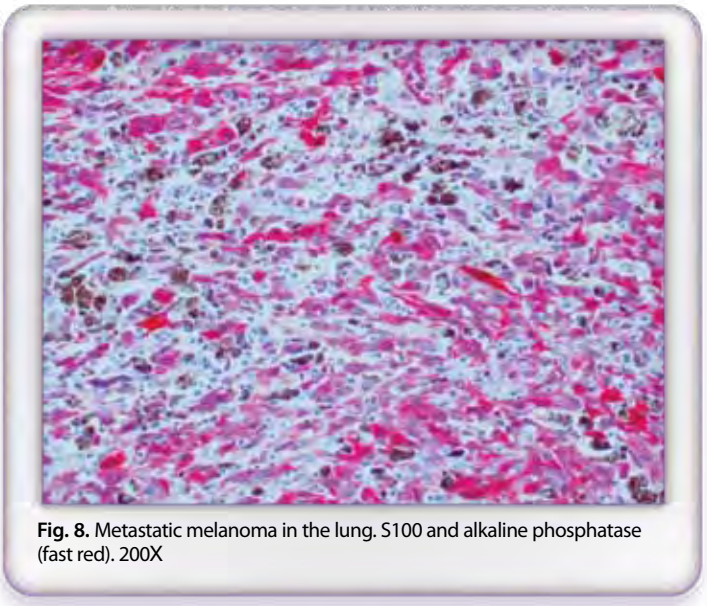


Fig. 8. Metastatic melanoma in the lung. S100 and alkaline phosphatase (fast red). 200X

Validation

Validation is the process of testing a number of cases to ensure the antibody reaction is proper and the results are correct. Validation also ensures correlation of results with other methodologies. Many times an institution doesn't have the resources to test a large number of cases. This could be a result of the type of laboratory service, such as a reference lab versus an academic clinical laboratory. How many cases should be run during the validation process? How many cases are available from the archives that are positive for BK polyomavirus, ALK-1, or cyclin D-1? Not that many, we suspect. Ideally, you should use tissues fixed and processed within your institution when establishing optimization and validation. In reality, many of

us have to purchase or rely on networking with colleagues to obtain tissue blocks or unstained slides for antibody workup. Many workplaces receive outside cases for consultation. Pathologists rely on the dependability and accuracy of their IHC laboratory to properly prepare this material for diagnosis. Outside cases that are properly diagnosed and have correlative support with a panel of antibodies can be used in your validation process.

IHC tests that produce quantitative results, such as the breast tumor markers HER2/neu, ER, and PR, require validation and confirmatory testing by fluorescent or chromogenic in situ hybridization. CAP guidelines should be referenced for the exact number and types of cases required for these breast tumor markers. As you use new antibodies and gain confidence in their performance, add new cases, whether positive or negative, to your lab notes for additional documentation. It may be necessary for a laboratory to send out the requested esoteric test rather than prepare it in-house. Proper validation will give you and your pathologist confidence in the testing and results. Dr. Richard Cartun, PhD, Director of Immunopathology and Histology at Hartford Hospital, is quoted as saying, "Much of antibody validation is dependent on the confidence of the pathologist and quality of the IHC lab." In addition, collaboration between the pathologist and technologist creates a sense of trust and is vital to proper validation and subsequent troubleshooting.

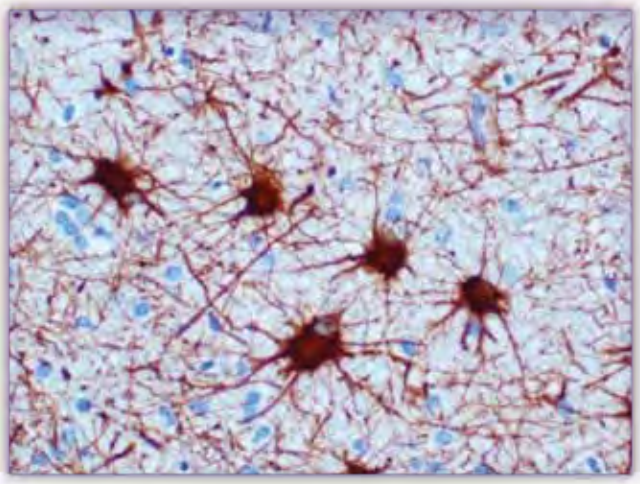


Fig. 9. Reactive astrocytes in brain tissue demonstrated with glial fibrillary acidic protein (GFAP). 400X

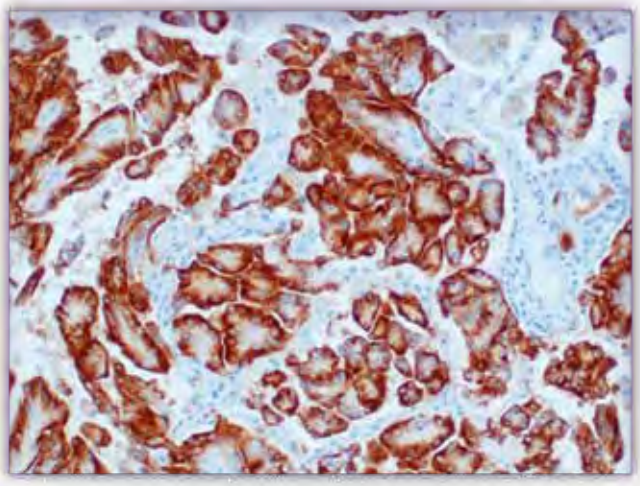


Fig. 10. Renal cell carcinoma metastasized to the lung demonstrated with RCC antibody. 400X

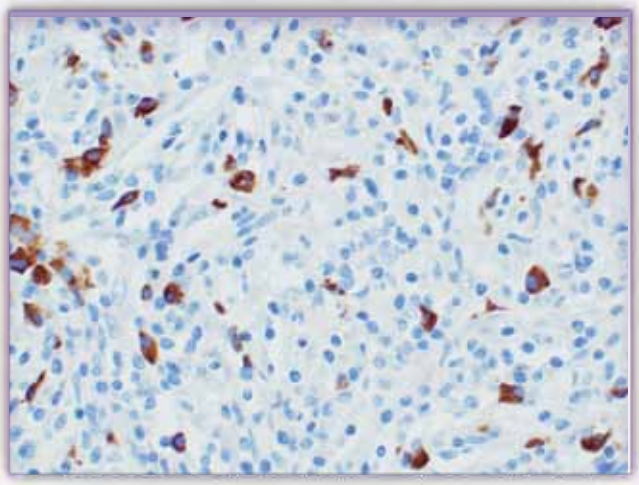


Fig. 11. Plasma cells react with IgG4 antibody in autoimmune pancreatitis. 200X

Demonstration of IHC proficiency can be carried out by subscribing to the CAP proficiency testing programs, or a program can be set up between cooperative hospitals. Results should be compared to the national average. Proficiency programs are also a good source of information about which antibodies are most commonly used.

Establishing an optimization and validation program is a demanding process that presents many variables. The information presented here will not only get you started but will demonstrate that your process is being performed properly, which can provide you with thought-provoking information to improve or implement your own optimization and validation program.

Dermatopathology: All Skin Specimens Are Not the Same

Clifford M. Chapman, MS, HTL(ASCP)QIHC
Technical Director
Strata Pathology Services Inc.
Lexington, MA

clif.chapman@pathsrv.com

Introduction

Whether you work in a histology laboratory located in a hospital, private facility, or research site, chances are that you receive specimens of varying types, including skin specimens. Specimens for dermatopathology require special handling throughout the preparation process in the histology laboratory. In addition, there exists a subset of these and related specimens requiring even more specialized attention. This article will discuss the unique qualities of dermatopathology specimens, along with procedures and techniques used to successfully produce microscope slides of optimum quality. Using these methods will ensure that pathologists receive the best slides possible, resulting in the highest quality of patient care.

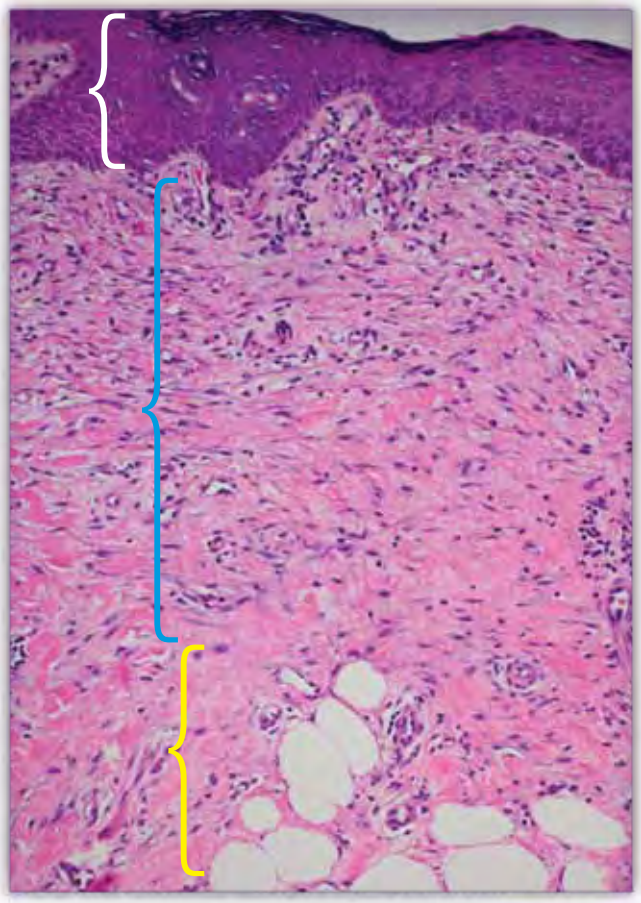


Fig. 1. Section of skin showing epidermis (white bracket), dermis (blue bracket) and adipose layer (yellow bracket). H&E stain. 200X

Why Dermatopathology Specimens Are Different

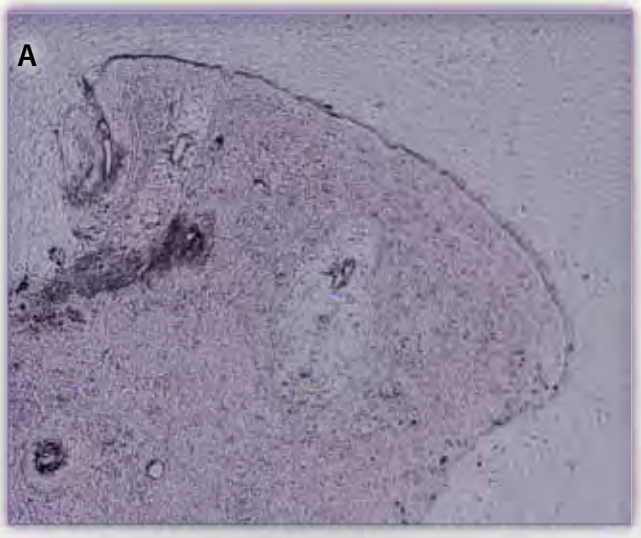
The skin is the largest organ of the body, able to perform multiple functions, including general protection from environmental elements and infection, and body temperature control, as well as being an important component in the immune response. Diseases of the skin can result from a myriad of possible causes because of its varied functions. Skin disorders may result directly from the skin itself, as in skin cancer, or be a symptom of a larger, underlying cause originating elsewhere in the body, such as in an immune reaction. Almost all of these pathologies are manifested in the dermal-epidermal (DE) junction. The DE junction is composed of the epidermis, which rests on a basement membrane, and the underlying dermis. If the specimen is thick enough, an adipose layer may also be present (Fig. 1). The pathologist must be able to view the DE junction in its entirety in order to make an accurate diagnosis. This is the challenge for histologists: to ensure proper orientation of the DE junction in the final microscope slide.

Part 1: Identification of Extraordinary Specimens

Begin at the beginning

Proper handling of dermatopathology specimens begins upon receipt of the specimen in the laboratory. Whoever is responsible for accessioning and grossing must be able to recognize dermatopathology specimens that are out of the ordinary and will require special handling. For example, a skin specimen submitted in Michel's transport medium for immunofluorescence must *never* be sent to the grossing bench, where it might incorrectly be put into formaldehyde. If a skin specimen submitted for direct immunofluorescence is mistakenly put into formaldehyde, the test cannot be performed. Therefore, it is crucial that these specimens be recognized upon receipt so they can be routed to the immunohistochemistry laboratory for proper handling.

Similarly, specimens submitted as a 2 mm punch require special handling. These specimens must be recognized at the grossing bench, where they are submitted in one piece. It is important that when this type of specimen is first placed in a cassette, it must be clearly labeled (eg, "2 mm" written on the side; use of a different color cassette, etc.) so that embedding personnel can easily recognize the presence of a 2 mm specimen inside the cassette after processing. After embedding the specimen on its side to ensure that the DE junction is in the proper orientation, the block is labeled with a colored sticky dot, which is used to alert the cutting staff. Histologists who are cutting a 2 mm specimen are instructed to surface cut the block and pick up the first 3 to 4 sections on a microscope slide. These sections can be viewed unstained under a microscope to ensure that the specimen is correctly embedded, resulting in a correctly oriented DE junction (Fig. 2A). If the specimen has been improperly



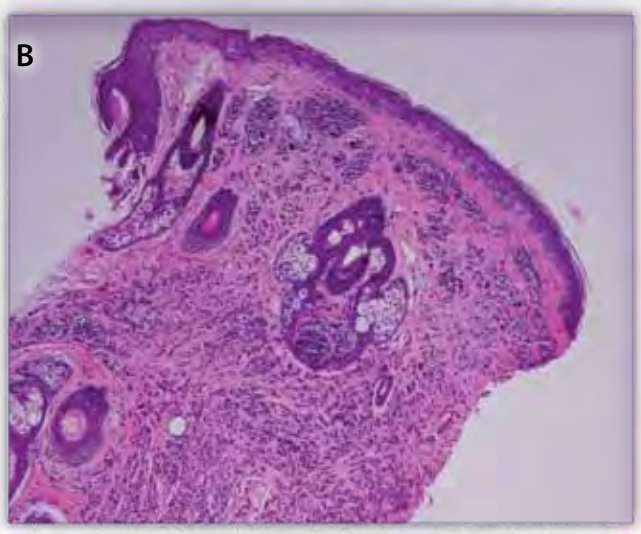


Fig. 2A. Cylindrical punch specimen of skin, unstained. 100X

Fig. 2B. Same cylindrical punch specimen of skin as in 2A, stained with H&E. 100X

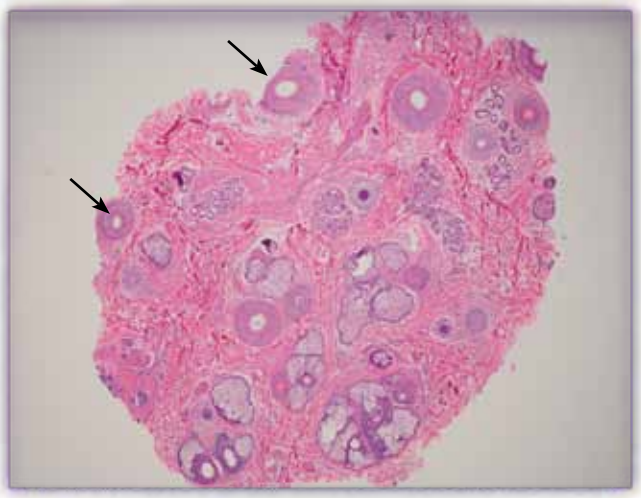


Fig. 3. Cross-section of skin processed using the Headington procedure. Hair follicles are denoted by arrows. H&E stain. 40X

embedded, it can be melted down and re-embedded, as there will still be ample tissue remaining in the block. Only after ensuring correct embedding will the histologist proceed to cut additional ribbons to complete the case (Fig. 2B).

Checking the requisition at accessioning and surgical grossing will also catch specimens that should receive the Headington procedure (named after Dr. J.T. Headington). In these cases, punch specimens from the scalp are submitted for evaluation of alopecia, hair loss, traction, etc. They must be prepared by surgically grossing the tissue into 2 pieces, cutting parallel to the epidermis (which is the opposite of the vertical sectioning required in all other specimens) for viewing the intact DE junction. Figure 3 shows a cross-section of a punch specimen and hair follicles sent for histological and numerical evaluation of the number of hair follicles per square millimeter.

Tiny specimens

When the dermatologist uses the curetting method of sampling a lesion, this can result in one or more tiny fragments of skin present in the specimen bottle, defined as pieces 0.1 mm or smaller. There are several methods that can be used to process these tiny fragments, all with their own advantages and disadvantages.

One method uses processing sponges, which are easy to use. The fragment is simply placed on a sponge and sandwiched between another sponge before placing it into a tissue processing cassette. The disadvantages of this method include possible loss of the specimen into the sponge itself, fragmentation of the specimen during the embedding process, and solution carryover in the tissue processors due to retention of processing fluids by the sponges themselves.

Alternatively, the specimen can be wrapped in lens paper or placed into a nylon bag prior to placing it into a tissue processing cassette. While the specimen may not get lost in the lens paper as it might in a sponge, it may still be damaged through fragmentation during the embedding process, as mentioned above.

A third method involves the use of HistoGel™ (Richard-Allan Scientific, Kalamazoo, MI), a gelatin-like substance that is liquid when heated and solid at room temperature. The tiny fragment is embedded into the HistoGel during surgical grossing; as a result, the fragment becomes trapped inside the hardened protective HistoGel. The disadvantages are that the HistoGel must be heated prior to use, and it may not be left heated for prolonged periods (longer than 48 hours). The advantages are that the fragment cannot be lost during processing, and it is not handled directly during the embedding process. This method works extremely well for cell blocks and other small, fragile specimens.

Part 2: Specimens Requiring Special Handling

Suspected fungal infection

A large percentage of specimens submitted for dermatopathology are suspected of having fungal infection. Usually, the periodic acid-Schiff stain with diastase (PAS+D)

is used to stain these organisms. Likewise, the Gomori methenamine silver (GMS) stain may be used.

Skin specimens are usually submitted as a cylindrical punch biopsy of the suspected area. Routine formalin fixation and paraffin embedding are used to produce a paraffin block. At the time of cutting the slide for hematoxylin and eosin (H&E) staining, additional unstained slides can be cut for PAS+D and/or GMS staining. Positive staining of fungal hyphae can be observed on the surface of the epidermis and sometimes within hair follicles. Skin has a built-in PAS positive control in that the basement membrane will stain positive as well (Fig. 4).

Nail specimens from the fingers and toes may also be submitted for suspected fungal infection. Since these tissues contain very hard keratin proteins, a softening step must be used prior to processing. Upon receipt, the formalin-fixed or unfixed nail specimens should be grossed into a tissue processing cassette and then immersed in the following nail softening solution:

Nail Softening Solution—5% TWEEN 85
Tween 85 (Sigma catalog # P4634).....5.0 mL
10% formaldehyde.....95.0 mL

It is best to hold the specimen overnight while the keratin softens and the tissue continues to fix. The next day, the specimen can be loaded into the tissue processor for routine processing. During cutting, the sections should be picked up on gelatin-coated slides (slides coated with a 0.5% aqueous gelatin solution) and then dried, for both H&E and special stains (eg, PAS+D, GMS, etc). This procedure ensures both optimal cutting of the nail specimen and section adherence during staining. The method also ensures adherence of sections during immunohistochemical staining for melanoma markers in nail-bed specimens.

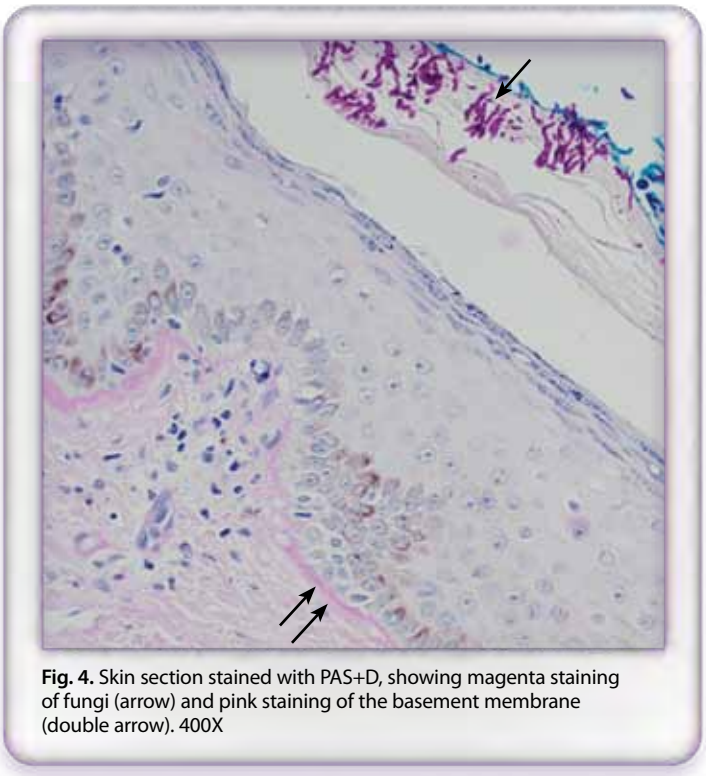


Fig. 4. Skin section stained with PAS+D, showing magenta staining of fungi (arrow) and pink staining of the basement membrane (double arrow). 400X

Melanin bleaching

Many specimens submitted for dermatopathology are suspected skin cancers, such as malignant melanoma. Immunohistochemical stains such as S100 and MelA may be performed to detect melanoma markers. Sometimes the H&E stain can be obscured by the presence of a high concentration of melanin. When this happens, the histologist can remove the melanin using a melanin bleaching technique. In this method, an unstained slide can be hydrated and treated with 0.25% potassium permanganate for 1 hour, followed by a rinse in 4% oxalic acid for 5 minutes. Then the slide can be stained with H&E and viewed without the presence of melanin.

When silver stains go wrong

Some dermatopathology cases require silver staining to confirm the diagnosis. Some examples of these silver stains include the Fontana-Masson, GMS, Warthin-Starry, and modified Steiner stains. Given the fact that many skin specimens are tiny and may be the only sample from the lesion, it is imperative that the stains work consistently. Infrequently, a silver stain may go awry, resulting in silver precipitate coating the microscope slide. If this happens, the precipitate can be removed by using the following solution:

Silver Removal Working Solution
1% potassium ferricyanide.....10 mL
(1 g potassium ferricyanide in 100 mL deionized water)

5% sodium thiosulfate.....40 mL
(5 g sodium thiosulfate in 100 mL deionized water)

Immerse the slide in this solution for 1 hour, during which time the silver precipitate will be removed. Then rinse the slide in distilled water and return it to the working solution for the specific silver stain method.

Summary

Skin specimens received for dermatopathology are composed of epidermis, dermis, and adipose tissue, which can cause many issues for histologists to consider during processing of the tissue into microscope slides. Additionally, there are several different types of skin specimens that require special handling in order to make an accurate diagnosis. The techniques outlined in this article will help to produce slides of optimum quality.

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Acknowledgments

The author would like to acknowledge the technical expertise of Patricia Almon, Nancy Collins, and Ann Costa in the preparation of microscope slides used for the photomicrographs in this article.

Lessons Learned From Exposure Incidents: Your Work Can Make You Ill!

Maureen Doran, MS, HTL(ASCP)
Southern Illinois University School of Medicine
Histology Center
Carbondale, IL

mdoran@siumed.edu

It is estimated that 50,0000 laboratory workers in the United States are at risk of exposure to microbiological agents that cause disease.[1] News of occupational exposure incidents to biological agents rarely makes headlines. Most often, these reports go unnoticed by the general public but also by those who work in the clinical and research setting. Case histories can play an important role in hazard assessment and safety awareness. The realization that "this could happen to me" is a powerful motivator for safety compliance. Our willingness to follow safety guidelines is influenced by our perceived risk of exposure. In 1991, OSHA published the bloodborne pathogen standard because it recognized that a significant percentage of healthcare professionals developed occupational-acquired diseases from contact with blood and/or body fluids.[2] These guidelines did heighten awareness to the risk of exposures and significantly reduced occupational infections. However, through such things as the globalization of our planet, the illegal animal trade, and research with "select agents," we are increasing our potential exposure to pathogens that can, and will, challenge our safety protocols and threaten the safety of our work environment.

Zoonoses are infections that are transmissible from animals to humans. Many diseases have been identified as being shared between man and animals. Approximately 75% of emerging infectious diseases are zoonotic so it is no wonder that exposure risks are increasing.[3] Select agents are bacteria, viruses, and toxins determined to be used as weapons in biological warfare. Several of these agents are zoonotic. Although the cause of most laboratory-associated infections is unknown, data indicate that animals or animal tissues are involved in 30% to 40% of the infections acquired in the laboratory.[4] An early study of occupational exposures discovered that, of the 1303 bacterial infections reported, 126 resulted from contact with animals, 28 from an animal bite, and 95 while performing a necropsy.[4] The risks are probably greater than we think if we take into account that there are more than 400 labs registered to handle and use select agents for research. The risks are not only to those involved in research—any human exposure from a research setting soon becomes a clinical exposure. These data are especially noteworthy because many histotechnologists work in settings where animal tissues are studied.

There are some concerns regarding employee safety and accident reporting in research laboratories handling select agents. Officials at the University of Texas (UT) at Austin have acknowledged that they failed to report 10 out of 13 laboratory accidents to federal authorities in the last 7 years.[5] In September 2007, UT Austin revealed a series of accidents that occurred in 2002, 2003, 2004, and 2005 that resulted in at least 4 lab-acquired infections that were not properly documented, investigated, or reported.[5] The infections involved *Shigella*, an infectious bacterium, which may have been genetically engineered. In one incident, a vial containing human embryonic kidney cells exploded and sent pieces of plastic into a worker's eye. In another, a lab worker taking a blood sample from an animal infected with *Shigella* was squirted in the eye with blood. Of the 5 exposures involving *Shigella*, 4 resulted in worker illnesses but all the workers have recovered. The *Shigella* infections follow a serious 2006 accident at UT Austin involving a genetically engineered influenza that crossed bird flu (H5N1) with a common flu type (H3N2).[6] The lack of accident reports misrepresents the safety of these lab environments and downplays the potential risk to employees and the community.

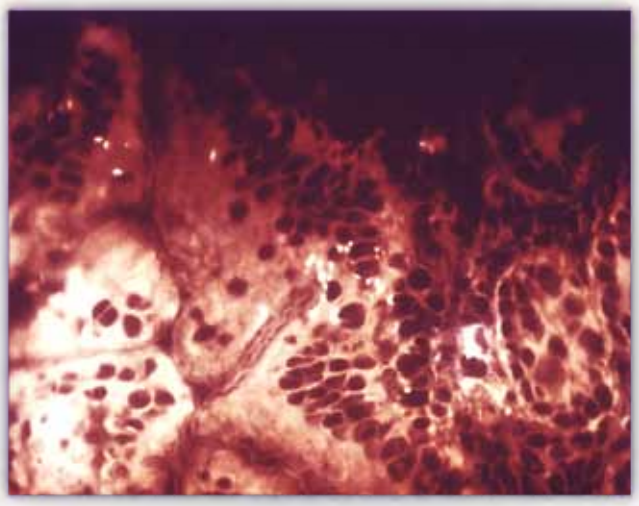

Fig. 1. This micrograph demonstrates 2nd stage shigellosis progression, where the *Shigella* bacteria have penetrated the intestinal mucosa.

Photo credit: CDC Public Health Image Library (image #5159); Dr. Eugene Gangarosa and Dr. Sam Formal, Walter Reed Army Institution of Research.

Anthrax (Fig. 2) has been recognized as an important disease of livestock for many years. Recently, more severe anthrax outbreaks have occurred in the US at game ranches with nontraditional species such as white-tailed deer.[7] Anthrax can be transmitted by ingestion or inhalation, or through cutaneous exposure. Anthrax became headline news in the popular media in the aftermath of the 9/11 attacks. In 2001, weaponized anthrax was delivered in letters sent in the US mail, resulting in 11 cases of inhalational anthrax and 11 cases of cutaneous anthrax.[8] Five people with inhalational anthrax died. During this time, a Texas lab worker did contract the cutaneous form of anthrax from testing an anthrax specimen.[9] In 2004, live anthrax was mistakenly shipped to Children's Hospital Oakland Research Institute.[10] The researchers actually handled the live anthrax bacterium thinking the sample contained dead anthrax. Those exposed included 1 of the primary researchers, 2 laboratory technicians, and an animal handler. Fortunately, no one developed any signs of illness. On April 13, 2006, workers at the UT Health Science Center in Houston were potentially exposed to aerosolized *Bacillus anthracis* when liquid from vials leaked inside an unshielded tabletop centrifuge.[11]

Officials notified the appropriate authorities; the Centers for Disease Control and Prevention (CDC) told the safety team that the institutional response was appropriate. A total of 7 workers were treated with antibiotics as a precautionary measure.

In June 2000, a comatose man was admitted to the hospital with renal failure and fever. He was treated with doxycycline for a possible tickborne illness but his condition worsened and he died.[12] His autopsy specimens were sent to the histology lab and blood was sent to be cultured. Two weeks later a microbiologist became ill and was diagnosed with possible food poisoning. At the same time, blood cultures from the comatose patient were reported as positive for tularemia, an illness caused by the bacterium *Francisella tularensis* (Fig. 3), which is found in animals and is typically transmitted to humans through tick bites. The ill microbiologist had handled the tularemia-positive cultures.[12] As a result, the microbiologist's blood cultures also were positive for tularemia. Viable tularemia was also found on the outside of specimen jars stored in a pathology cooler. Autopsy and histology technicians were later screened for infection. Tularemia can survive for some time in a cool, moist environment. In 2006 at UT San Antonio, workers entered a tularemia lab to inspect malfunctioning air filters without wearing gloves or any respiratory protection.[11] No infections resulted, although several workers received antibiotic treatment as a precautionary measure.

Three Texas A&M University researchers were infected with the biological weapons agent known as Q fever *(Coxiella burnetii)* (Fig. 4) in 2006.[13] The infections were confirmed in April of that year, but Texas A&M officials did not report them to the CDC, as required by law. Three individuals from a lab that conducts aerosol challenges of pigs and other studies with the Q fever bacterium visited the hospital at the same time the lab workers tested positive for Q fever.[13] According to federal law, Texas A&M was required to report the infections immediately upon their discovery. By not reporting the infections to the government, Texas A&M thus violated (again) the Select Agent Rule.

In November 2006, there were 3 cases of brucellosis (Fig. 5) in microbiologists at 2 clinical labs.[14] As a result, 146 workers were exposed. These cases emphasize the importance of following

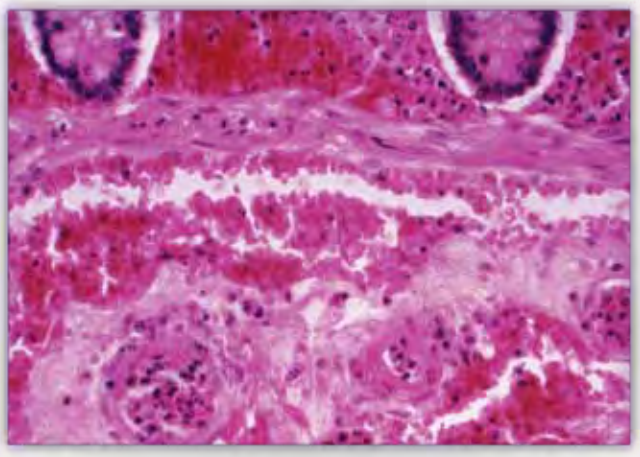

Fig. 2. This micrograph demonstrates submucosal hemorrhage in the small intestine due to fatal human anthrax infection. H&E stain, 240X.

Photo credit: CDC Public Health Image Library (image #4629); Dr. Marshal Fox.

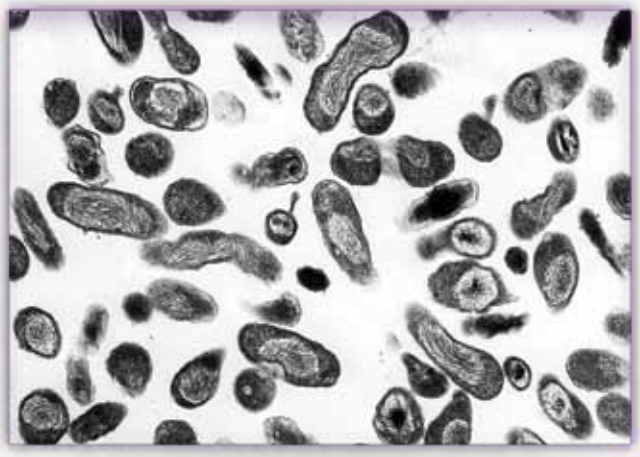

Fig. 4. This photomicrograph demonstrates *Coxiella burnetii*, the bacteria responsible for Q fever.

Photo credit: Rocky Mountain Laboratories, NIAID Biodefense Image Library, and NIH, made available through Wikimedia Commons.

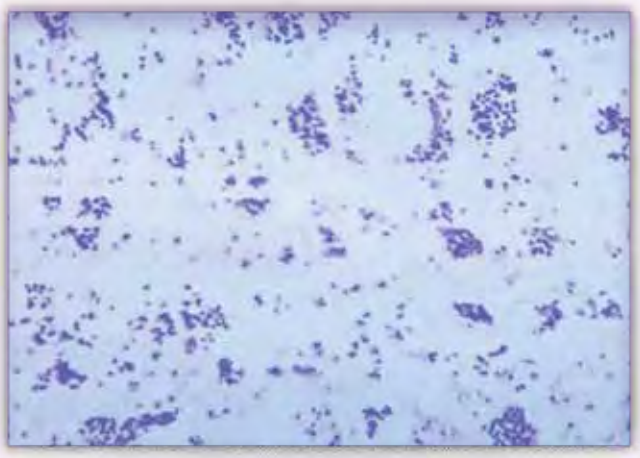

Fig. 3. This photomicrograph demonstrates *Francisella tularensis*, the bacteria responsible for tularemia. Cells were stained with methylene blue.

Photo credit: CDC Public Health Image Library (image #2985); Dr. PB Smith.

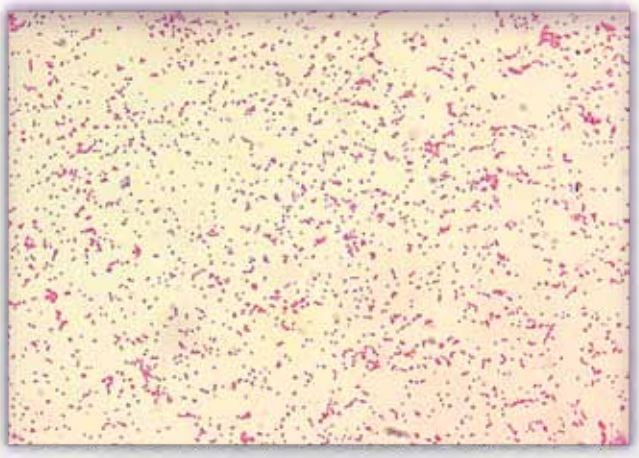

Fig. 5. This micrograph demonstrates *Brucella spp*, which are poorly staining, small gram-negative coccobacilli responsible for brucellosis infections.

Photo credit: CDC Public Health Image Library (image #1901); Larry Stauffer, Oregon State Public Health Laboratory.

recommended biosafety practices, including documenting exposure incidents and rapid implementation of response protocols.

In May 2004, a Russian scientist at the former Soviet biological weapons laboratory, Vector, in Siberia, died after accidentally sticking herself with a needle infected with Ebola virus[15] (Fig. 6). The accident was not reported for 2 weeks. An American scientist was involved in a similar accident with Ebola at the US Army's leading biodefense lab at Fort Detrick, Frederick, MD, but she did not contract the disease.[16] The lab disclosed the accident within 48 hours, officials said.

Vector has been a leading recipient of aid in an American program to help former Soviet scientists and labs convert to peaceful research. Vector is also 1 of 2 repositories in the world of the deadly smallpox virus (Fig. 7); the other is in the US at the CDC in Atlanta, GA. Janet Parker, a photographer in England, was the last documented death attributed to smallpox in 1978.[17] She was exposed to the virus when it escaped from a lab located below her darkroom. Faulty ventilation was determined to be the cause of her exposure. Smallpox appeared once again when a surprising discovery was made in 2004: an envelope containing scabs from 19th century smallpox vaccinations was found in a university library in a book on Civil War medicine.[18]

West Nile virus (WNV) (Fig. 8) is a mosquito-borne arbovirus from the *Flaviviridae* family, which first appeared in North America around the summer of 1999. It is a human, equine, and avian neuropathogen. Since the 2002 WNV epidemic in the US, the number of laboratories and laboratory workers involved in arboviral diagnostic and reference activities has increased substantially.[19] As a result, the potential for laboratory-acquired WNV infections has also increased. Laboratory-acquired arboviral infections are most likely underreported. In August 2002, a microbiologist working in a US laboratory was performing a necropsy on a blue jay submitted as part of a state's WNV surveillance program.[20] The microbiologist worked in a Class II laminar flow biosafety cabinet (Fig. 9) under biosafety level 2 (BSL-2) conditions; he lacerated a thumb while using a scalpel

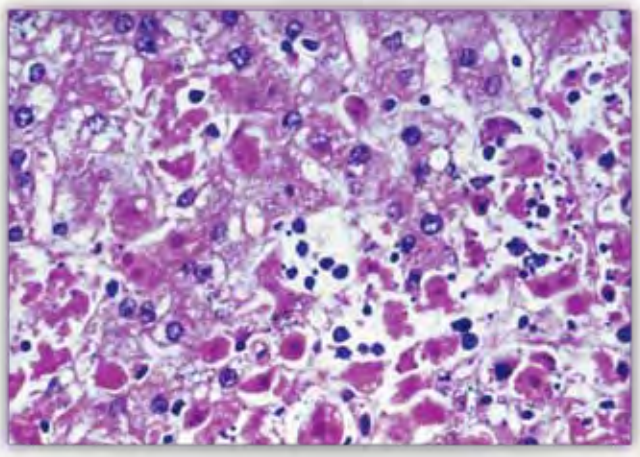

Fig. 6. This micrograph is of human liver infected with Ebola virus, the cause of Ebola hemorrhagic fever.

Photo credit: CDC Public Health Image Library (image #7058); Dr. Lyle Conrad.

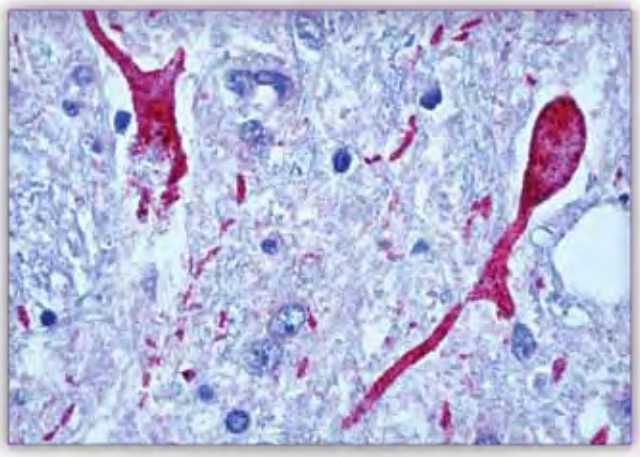

Fig. 8. This photomicrograph of human brain tissue shows antigen-positive neurons and neuronal processes (red) indicative of West Nile virus from a patient with West Nile encephalitis.

Photo credit: CDC Public Health Image Library (image #1845); W-J Shieh and S Zaki.

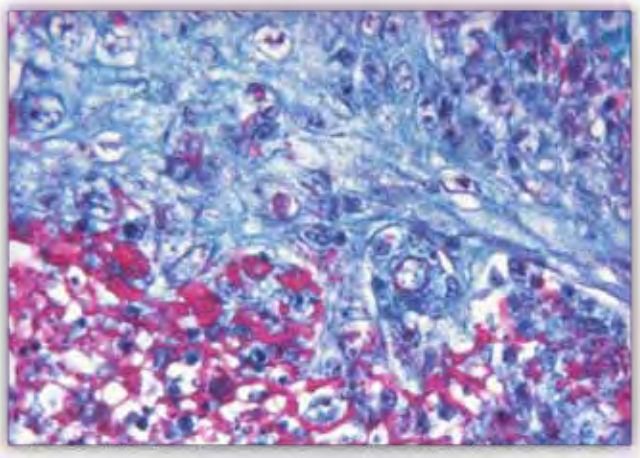

Fig. 7. This micrograph shows the histologic changes in human skin infected with the smallpox variola virus.

Photo credit: CDC Public Health Image Library (image #3226).

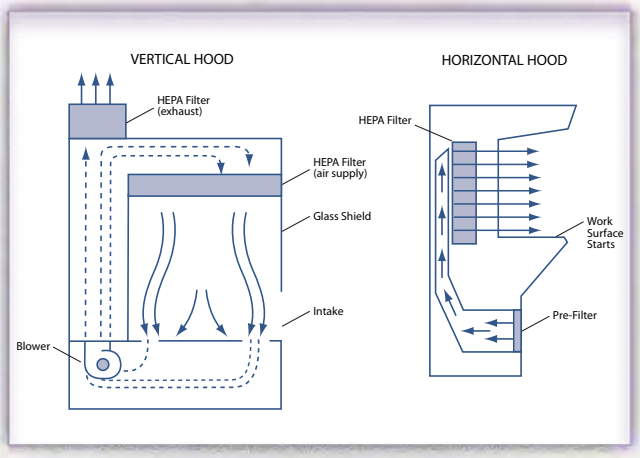

Fig. 9. Schematic drawing of HEPA-filtered air flow in vertical and horizontal laminar flow hoods.

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to remove the bird's brain. Four days after the injury, the microbiologist had acute symptoms of headache and malaise followed by chills and sweats. Two days later, the microbiologist noted a maculopapular rash and sought medical care from a physician but reported no history of recent mosquito bites, prolonged outdoor activities, or recent blood transfusion.[20] These cases confirm that laboratory workers are at risk for occupationally acquired WNV infection. Participating laboratory employees should receive training that reinforces awareness of potential occupational hazards and risks, and that stresses the importance of timely reporting of all injuries and illnesses of suspected occupational origin.

An estimated 10 to 20 Americans contract plague each year, with about 1 in 7 cases being fatal.[21] Bubonic plague (Fig. 10), also known as the Black Death, killed an estimated 25 million people in Europe between 1346 and 1351. The last major urban outbreak in the US occurred in Los Angeles in 1924-1925, when at least 30 people died.[22] Bubonic plague is not contagious, but if left untreated the bacteria can invade the bloodstream where they then get carried throughout the body. Plague bacteria that settle in the lungs cause the pneumonic form of plague (Fig. 11), which can be spread from person to person through an infected person's cough. Bubonic plague is usually transmitted to humans from the bites of fleas infected by diseased rodents. In 1984, a veterinarian in Claremont, CA developed plague pneumonia from treating an infected cat.[23] In April 2006, there were 2 plague deaths in New Mexico and a laboratory worker also contracted plague.[24] On October 27, 2007, Erik York, a 37-year-old wildlife biologist for the National Park Service, performed a necropsy on a mountain lion. Three days later he began experiencing flu-like symptoms. He was treated at a local clinic but was not diagnosed with any serious ailment. On November 2, 2007, York was found dead in his home at Grand Canyon National Park. Forty-nine people who had contact with York were given aggressive antibiotic treatment. No one became ill. Necropsy results of the mountain lion indicated it was infected with *Yersinia pestis*, the causative agent of plague. Autopsy results for York confirmed that he was infected with the same plague bacteria.[25] In September 2009, a researcher from the University of Chicago, Malcolm Casadaban, died after exposure to "a weakened and ordinarily harmless strain of the bacteria that causes plague."[26] Healthcare personnel should use appropriate isolation precautions when caring for patients with evidence of respiratory involvement compatible with plague. The plague is considered a bioterrorism agent and state law requires that doctors report suspected cases to local health departments.

Preventative measures are required to decrease the incidence and severity of laboratory-acquired infections. Safe handling of potentially infectious materials requires knowledge of signs and symptoms of exposure and routes of transmission.

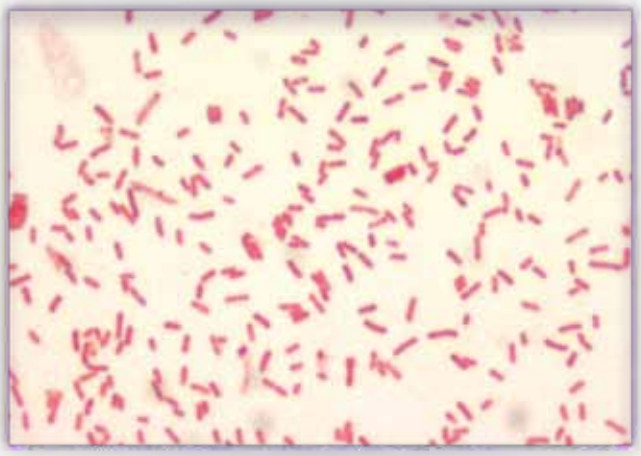

Fig. 10. This micrograph demonstrates *Yersinia pestis*, the gram-negative bacillus responsible for infection with bubonic plague. Bipolar staining occurs when using Wayson, Wright, Giemsa, or methylene blue stain. 1000X.

Photo credit: CDC Public Health Image Library (image #1914); Larry Stauffer, Oregon State Public Health Laboratory.

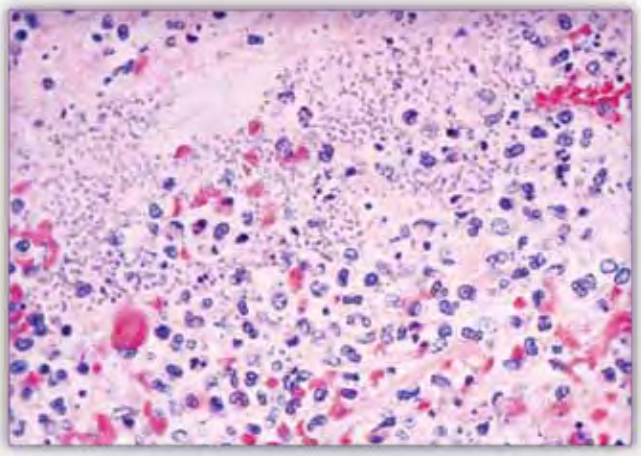

Fig. 11. This photomicrograph demonstrates *Yersinia pestis* in human lung from a patient infected with a fatal case of the pneumonic form of plague bacteria that settled in the lung.

Photo credit: CDC Public Health Image Library (image #1956).

Appropriate risk assessment and reporting are key elements of biosafety programs along with taking safety seriously. Diligence is necessary to maintain safe standard operating procedures. There is no universal requirement for reporting laboratory-acquired infections. "Those who do not remember the past are condemned to repeat it."[27]

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Optimization of the Automated Periodic Acid-Schiff Hematoxylin (PASH) Stain for Spermatozoal Acrosomes Using the Sakura Tissue-Tek® Prisma® Stainer and the Sakura Tissue-Tek® Glas™ *g2* Coverslipper

Pamela Mercer, HT (ASCP); Leslie Duncan, HT (ASCP)

Bristol-Myers Squibb Co.
Research and Development
Department of Pathology
Mount Vernon, IN

pamela.k.mercer@bms.com
leslie.duncan@bms.com

Abstract

Histopathological evaluation of rodent testes for preclinical toxicology studies is routinely performed using hematoxylin and eosin (H&E) staining; however, the H&E stain does not provide ideal contrast for optimal visualization of the acrosome of the spermatid, which is essential in distinguishing specific stages of spermatogenesis. Recognition of the morphologic alterations in the structure of the acrosome as the spermatid matures is essential in evaluating the various developmental maturations that occur during spermatogenesis. Therefore, many pharmaceutical companies commonly utilize a periodic acid-Schiff hematoxylin (PASH) stain for short-term (≤3 months) rodent toxicology studies to more easily visualize the acrosome. PASH stains are commonly performed manually, ie, without the use of automated tissue stainers. We have developed an optimized method to perform this staining procedure using automated equipment without sacrificing staining quality while decreasing both the time required to do this task and the costs of laboratory staining reagents.[1-4]

Introduction

Our histopathology laboratory performs routine H&E staining using a Sakura Tissue-Tek® Prisma® automated slide stainer and the Tissue-Tek® Glas™ *g2* coverslipper (Sakura Finetek USA, Inc., Torrance, CA). Since the PASH is routinely performed on rodent testes for investigational new drug (IND) and reproductive toxicology studies, we developed a method for an automated PASH staining procedure that would: (1) produce staining quality comparable to our current manual method, (2) run simultaneously with our routine H&E staining protocol to ensure no loss in production time of study slides, and (3) be economically feasible. In order to accomplish all of these objectives, we developed a staining protocol (configuration) for the automated stainer, subjectively evaluated the staining quality of slides produced using the manual and automated methods, and evaluated the cost and time savings for performing the manual versus automated PASH staining procedure. Additionally, we determined the reagent endpoint (specifically for Schiff reagent and Mayer's hematoxylin) when performing the automated stain.

Materials and Methods

Tissue fixation is a key factor in the evaluation of the testes. Our laboratory uses modified Davidson's fixative (mDF) as the primary fixative for rodent testes instead of 10% neutral buffered formalin (NBF) or Bouin's fluid. Fixation time is generally 48 to 96 hours in mDF, and then the testes are subsequently transferred to 10% NBF for further fixation or storage prior to processing. It is our experience that this fixation time in mDF is a critical component of optimal fixation for cell morphology.[1,2]

For this study, rat testes were fixed in mDF for approximately 48 hours, then rinsed and transferred to 10% NBF on the processor for a minimum of 1 hour. The testes were processed overnight using a Sakura Tissue-Tek® VIP E300™ processor, embedded in paraffin blocks, and sectioned at 4 microns on an automated rotary microtome. The slides were dried in a slide dryer at approximately 60°C for 1 hour.

Our PASH staining method combines periodic acid and Schiff reagent, and is counterstained with Mayer's hematoxylin. Mayer's hematoxylin achieves nuclear staining without obscuring the PAS-positive stained tissue. For this project, the following reagents were purchased commercially for staining consistency: Schiff reagent (Mallinckrodt Baker, Inc., Phillipsburg, PA, or Electron Microscopy Sciences, Hatfield, PA), Mayer's hematoxylin (Electron Microscopy Sciences), 0.5% periodic acid (Poly-Scientific R&D Corp, Bayshore, NY), 95% and 100% ethyl alcohol, and xylene.

Manual Method

Reagent containers (300 mL volume) were filled with approximately 250 mL of the appropriate reagent and placed under a ventilated hood. Slide racks containing 20 slides each were moved from one staining container to the next, with each slide remaining in the container for the appropriate time as determined by the manual staining procedure. The technician manually moved the slide racks to each container or washing station, and provided agitation (if desired) by moving the slide rack up and down in the staining container.

Automated Method

For the automated method, the Sakura Tissue-Tek® Prisma® stainer with the automatic transfer to the Sakura Tissue-Tek® Glas™ *g2* coverslipper was used to stain and coverslip the slides. The staining protocol for the PASH stain was programmed using the standard configuration on the Prisma® stainer. Standard reservoirs were filled with approximately 700 mL of each reagent. Up to 120 slides per staining run were loaded into the start stations on the stainer, and the PASH program was selected (Table 1).[5] Additional slides were loaded into the start stations and the routine H&E program was selected to run concurrently with the PASH stain.

Table 1. Automated PASH Staining Protocol for Acrosomes

Step	Reagent Reservoir	Reagent	Time
1	Start	Program start	—
2	01	Xylene	3 minutes
3	02	Xylene	3 minutes
4	03	Xylene	3 minutes
5	09	100% ethyl alcohol	2 minutes
6	10	100% ethyl alcohol	1 minute
7	11	95% ethyl alcohol	1 minute
8	12	70% ethyl alcohol	1 minute
9	28	Distilled water	1 minute
10	27	0.5% periodic acid	5 minutes
11	Wash	Tap water	1 minute
12	24	Schiff reagent*	30 minutes
13	Wash	Tap water	10 minutes
14	28	Distilled water	1 minute
15	17	Mayer's hematoxylin†	1 minute
16	Wash	Tap water	3 minutes
17	12	70% ethyl alcohol	1 minute
18	11	95% ethyl alcohol	2 minutes
19	19	100% ethyl alcohol	4 minutes
20	18	Xylene	4 minutes
21	End	Xylene	—
22	Automatic transfer to the Sakura Tissue-Tek® Glas™ *g2* coverslipper		

Our laboratory does not use the heating or drying stations.
The "mix" is turned on to agitate the reagents for all stations.

*Store at approximately 4°C; bring to ambient temperature prior to use.

†Filtered before use with 24.0 cm filter paper.

Results

Slide Quality

Slides stained using the automated PASH staining protocol were independently compared microscopically to slides stained using the manual method by two board-certified veterinary pathologists. Slides stained using the automated method were equivalent (if not identical) to slides stained manually (Fig. 1). All structures expected to stain with PAS were positive, the acrosomes were easily visible, and the nuclei stained a light blue. The automated procedure also reduced variability in staining quality that is commonly experienced when manual staining is performed by a variety of technicians.

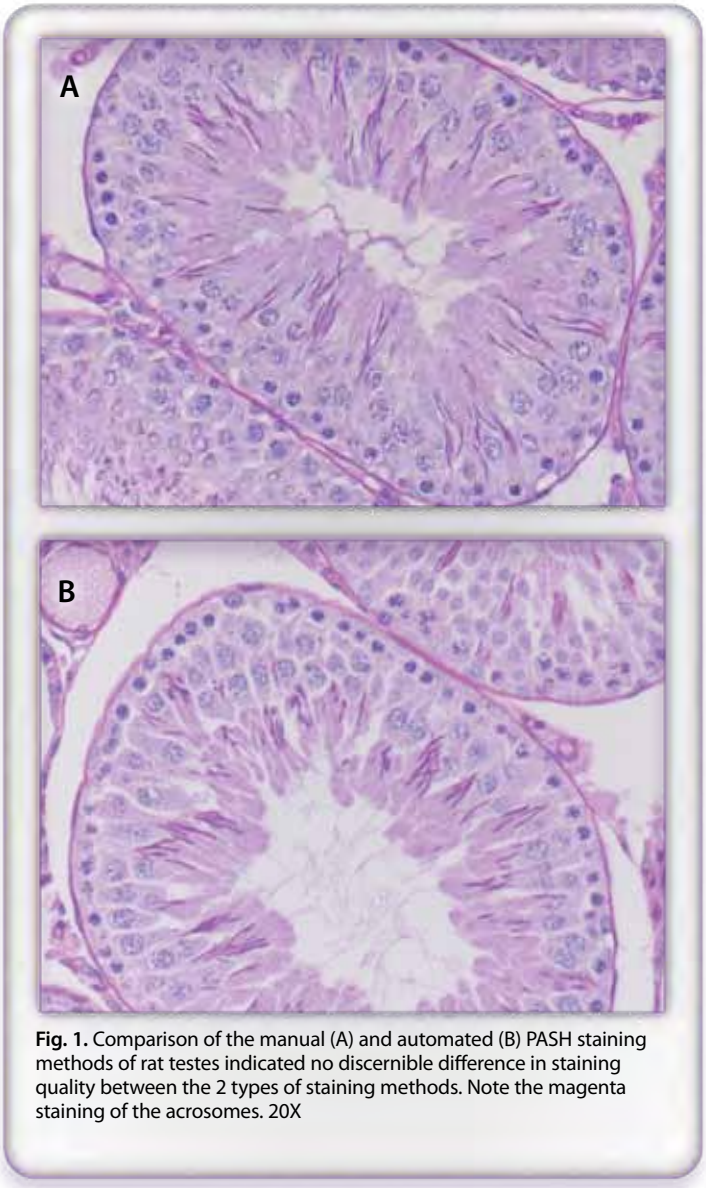

Fig. 1. Comparison of the manual (A) and automated (B) PASH staining methods of rat testes indicated no discernible difference in staining quality between the 2 types of staining methods. Note the magenta staining of the acrosomes. 20X

Time Savings

With the ever-increasing workload in the histology laboratory, it is of paramount importance to find ways to reduce technician time for a given task, which will result in increasing efficiency. With the manual (bench) method of the PASH stain, a technician spends approximately 2 hours performing the stain, and has limited time to perform other tasks during this 2-hour period. The automated procedure utilizes approximately 20 minutes of a technician's time (fill reagent containers, start program, and remove the slides from the coverslipper). Once the PASH program is started on the automated stainer, the technician can walk away from the machine and perform other duties, and return to the machine to retrieve the finished slides when the protocol is completed. This study demonstrated that the automated stainer could accommodate both H&E and PASH stains concurrently, which resulted in increased efficiency of the equipment.

Cost Savings

In the manual method, the costs incurred for a single use of some reagents (Schiff reagent, Mayer's hematoxylin, and periodic acid) were greater (per 200 slides) compared to the costs for the automated method, which allowed for the greatest cost savings (Table 2). Based on previous experiences of performing this stain manually, our laboratory routinely discarded all staining reagents after each manual run, except for the Schiff reagent, which was used one additional time and then discarded. This methods development project demonstrated that the Schiff reagent could be used for at least 200 slides in the automated method, with the stain quality of the last (200th) slide (Fig. 2B) being comparable to the first slide (Fig. 2A). The 0.5% periodic acid was changed after each staining run using the automated method; however, the Mayer's hematoxylin was used for 200 slides, producing comparable staining results from the first to the last slide. Utilizing the staining reagents for multiple staining runs in the automated stainer resulted in a cost savings of 61% ($446.60 vs $175.60) (Table 2).

Alcohol and xylene costs are minimal since the same reagent reservoirs are used for the deparaffinization and dehydration steps for both the H&E and PASH stains. An even larger cost

Table 2. Cost Comparison: Manual vs Automated Staining

Reagent	Manual (per 200 slides)	Automated (per 200 slides)
Schiff reagent	$90.00	$50.40
Mayer's hematoxylin	$165.00	$46.20
0.5% periodic acid	$97.30	$65.80
100% ethyl alcohol	$25.20	$3.50
95% ethyl alcohol	$24.10	$3.40
Xylene	$45.00	$6.30
Total Costs:	**$446.60**	**$175.60**

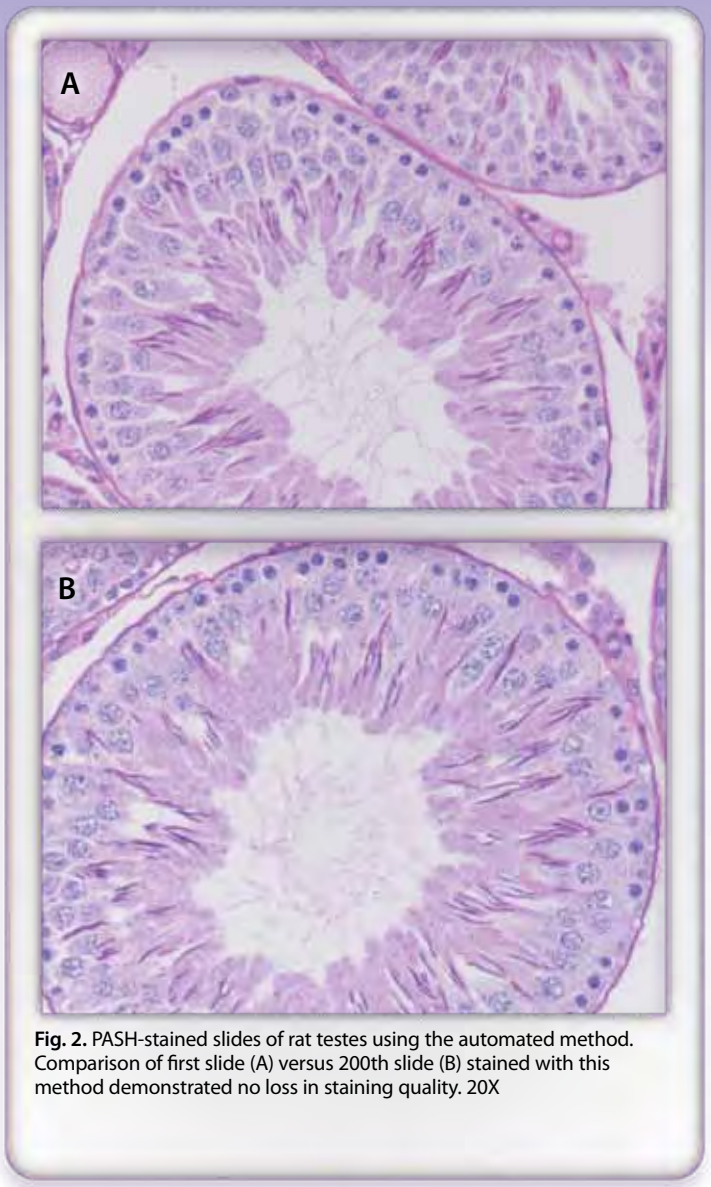

Fig. 2. PASH-stained slides of rat testes using the automated method. Comparison of first slide (A) versus 200th slide (B) stained with this method demonstrated no loss in staining quality. 20X

savings could be incurred if the 0.5% periodic acid was prepared in the laboratory; however, for this study, this staining reagent was purchased commercially.

A total cost savings of $271.00 per 200 slides was realized using the automated staining method compared to the manual method.

Discussion

This study demonstrated that the automated PASH using the Sakura Tissue-Tek® Prisma® stainer produced results comparable to those obtained by the manual method. It also demonstrated that the stainer could stain both H&E and PASH slides at the same time, thereby increasing production/output of study slides. With the reduction of technician time required to perform the PASH stain, technicians are able to perform other laboratory functions, thus increasing overall efficiency in the laboratory. In addition, our laboratory was able to increase productivity as well as reduce laboratory staining reagent costs by considering a novel approach to a conventional procedure.

References

1. Lanning LL, Creasy DM, Chapin RE, et al. Recommended approaches for the evaluation of testicular and epididymal toxicity. *Toxicol Pathol*. 2002;30(4):507-520.
2. Latendresse JR, Warbrittion AR, Jonassen H, Creasy DM. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. *Toxicol Pathol*. 2002;30(4):524-533.
3. Pecht D, Proctor JE, Litz D. *Staining of Acrosomes With Modified Periodic Acid-Schiff (PAS) Reagent*.
4. Latendresse JR. What a testis means to me. Paper presented at: VIR Seminar; October 18, 1999.
5. *Sakura Tissue-Tek® Prisma™ Automated Slide Stainer Operation Manual*. Torrance, CA: Sakura Finetek USA, Inc; 2005.

Acknowledgments

The authors would like to acknowledge Kristen Jones, a senior attending the University of Southern Indiana, for participating in our summer internship at Bristol-Myers Squibb in Mount Vernon, IN. The methods development procedure described in this article fulfilled a portion of her requirements for our internship program.

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Global Celebrations Planned in Honor of Histotechnology Professionals Day

Vinnie Della Speranza, MS, HTL(ASCP)
Immediate Past President
National Society for Histotechnology
Columbia, MD

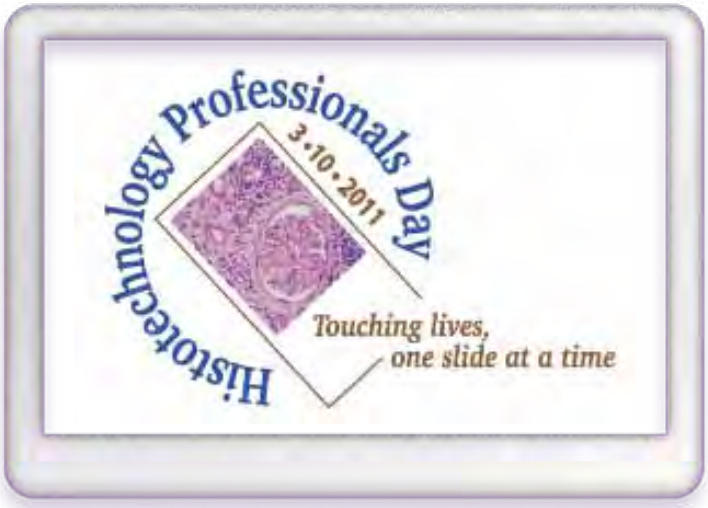
dellav@musc.edu

At its annual Symposium/Convention in Seattle, WA, this past September, the National Society for Histotechnology announced that the second annual worldwide ***Histotechnology Professionals Day*** will be celebrated March 10, 2011. This celebration is intended to bring to light the important contributions that have been made in science and medicine by those who have devoted their professional lives to this largely unknown discipline. By all accounts, the inaugural event held March 10, 2010 was a great success.

While the history of histotechnology is often attributed to the work of individuals such as Robert Hooke and Antonie van Leeuwenhoek, it is clear that the many scientific developments that followed hinged on the further development of the microscope and the histochemical techniques that refined the preservation and staining of biological samples, all necessary to reveal the microscopic structure of normal and diseased tissues. While more sophisticated techniques employing the use of antibodies and nucleic acid probes emerged in recent years, many of the methods developed in the 18th and 19th centuries remain in widespread use today in modern histology laboratories.

The largely obscure discipline (histotechnology) that emerged from the collective efforts of biologists, chemists, lens makers, and later, physicians, gained greater awareness as surgical pathology emerged as a distinct medical discipline in the early years of the 20th century. Prior to that time, surgeons served as their own pathologists, making the diagnosis in the operating theatre based solely upon the gross appearance of an excised lesion. By the mid 1920s, the American College of Surgeons endorsed the necessity of microscopic evaluation of excised tissues, which led to the emergence of anatomic pathology as a distinct laboratory discipline. The American Society of Clinical Pathology, first formed in 1922, began certifying histotechnicians in 1948. Over the years that followed, the skills of histotechnicians and histotechnologists were sought after in various laboratory settings outside of the medical arena so that today their work is indispensible in marine biology, pharmacology, veterinary medicine, forensics, biotechnology, and bioengineering research. It is without dispute that the work of histotechnologists has been essential to the advancement of those and other important scientific disciplines.

Why celebrate Histotechnology Professionals Day? The contributions of histotechnologists remain largely unknown to those outside the science community despite the important impact their work has had on society. As the techniques performed on tissue in the histology laboratory have grown more sophisticated, so too have the skills and knowledge required of the histotechnologist. As the demand for trained histology professionals increases, the importance of educating the public about our discipline and its role in society becomes ever more apparent. Each of you has a story to tell. If that story reaches young people who are considering their career options, it is quite possible that your experiences will entice others to investigate histotechnology further. I hope that you will begin planning your Histotechnology Professionals Day celebration to help lay people understand why your work is so important. If we don't get our stories out, where will the next generation of histology professionals come from?



Visit **www.nsh.org** to obtain free tools to help you plan your Histotechnology Professionals Day celebration.

You can learn more about the history of the discipline from the following sources:

Clark C, Kasten G. *History of Staining*. 3rd ed. Baltimore, MD: Williams & Wilkins; 1983.

Rosai J, ed. *Guiding the Surgeon's Hand: The History of American Surgical Pathology*. Washington, DC: US Armed Forces Institute of Pathology; 1997.

Lyle L. Baker

Patriot, Friend, Colleague, Teacher

Mr. Lyle L. Baker was born June 21, 1937, in Mahaska County, Iowa. Lyle served in the US Navy for 20 years as a medical technologist. In addition, he served on the destroyer USS Beale. One of the highlights of his career was assisting astronauts as a Navy medic at NASA in a test program (SWEAT) in 1973. Upon retiring from the Navy, Lyle joined the pathology department at Moses Cone Health System in Greensboro, NC, as Chief Histotechnologist, a position he held until retiring in 2005.

Lyle had a great love for histotechnology. He enjoyed explaining his work and profession to students, residents, pathologists, and colleagues. It was always a pleasure to call him and hear him answer the telephone, "Histology, Mr. Baker." He had a great sense of humor, unusual manner of wit, and a warm, friendly greeting to all those he came into contact with.

Lyle enjoyed educating others about histotechnology. He often presented lectures and workshops at local, state, and national conferences, sharing his expertise and knowledge to advance the field of histotechnology. While he held a number of positions in the North Carolina Society for Histopathology Technologists, he appeared to enjoy serving as Vendor Liaison most. Just this past April at the North Carolina meeting at Research Triangle Park, he shared that "he began histotechnology at 37 years of age and lived long enough to reverse the numbers to 73." He truly loved assisting and being involved with the vendors and sales force. He often helped the new sales people get a firm foundation in the profession of histotechnology by educating them to go out and truly know the market and their products. Lyle often assisted in helping companies bring in educational seminars and opportunities for local histotechs and students.

During his career, Lyle served the National Society for Histotechnology (NSH) in many capacities. He was Region III Director from 1982-1988, participated in numerous teleconferences, and he served as Awards Chairperson from 1988-1998 and again in 2002. Lyle was awarded the J. B. McCormick, MD, Award in 1997, the Histotechnologist of the Year Award in 2002, and the Lee G. Luna Foreign Scholarship Award in 2005. He served as a delegate in the NSH House of Delegates for many years, adding his voice to those who helped to form and shape our national organization. Lyle truly loved histotechnology, the North Carolina Society for Histopathology Technologists, and the NSH, which was so evident from his enthusiastic participation and generous gift of his time, support, and attendance. Seeing his face at each meeting, hearing his voice during the sessions, and his kind words, witty gestures, and unique stories at the NSH awards ceremonies have given us fond and wonderful memories that will last forever. He loved sharing his photography, talking about his family, particularly his grandchildren, passing on many gardening tips and "tricks of the trade," and telling stories of his travels and of his wonderful life with his wife, Linda. We will miss Lyle but we also know that he will greet us again someday with his warm smile, ready humor, and his loving spirit.

—M. Lamar Jones

✔ Mark Your Calendar!
Educational Opportunities in 2011

JANUARY

21 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Catching More Fish from the Stream: Metabolic Biomarkers Using Preservation by Extraction and Fixation (PREF)**
Speaker: Dean Troyer, MD
Adjunct Professor, Department of Pathology
University of Texas Health Sciences Center
San Antonio, TX

26 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Overview of Spirochete Stains**
Speaker: Robert Lott, BS, HTL(ASCP)
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

FEBRUARY

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Personality Types in the Histology Laboratory**
Speaker: Glenda Hood, MEd, HT(ASCP)
Histotechnology Program Director
Tarleton State University
Fort Worth, TX

23 **NSH Teleconference 1:00 pm Eastern Time**
Title: **A Palette of Nuclear Stains**
Speakers: Ada Feldman, MS HTL(ASCP) and
Dee Wolfe, HT(ASCP)
Anatech Ltd
Battlecreek, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

MARCH

4-5 **Indiana Society for Histotechnology**
Site: Hilton North
Indianapolis, IN
www.indianapolisnorth.hilton.com
Contact: Debra Wood
Phone: (317) 491-6311
Email: demwood@iupui.edu

10 **Histotechnology Professionals Day**

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Safety in the Histology Laboratory**
Speaker: Clifford M. Chapman, MS, HTL(ASCP)QIHC
Strata Pathology Services
Lexington, MA

23 **NSH Teleconference 1:00 pm Eastern Time**
Title: **The Evolution of Digital Pathology**
Speaker: Elizabeth Chlipala, BS, HTL(ASCP)QIHC
Premier Laboratory
Boulder, CO
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

25-27 **Georgia Society for Histotechnology**
Site: Callaway Gardens
Pine Mountain, GA
Contact: Mike Ayers
Phone: (770) 304-4065
Email: mayers@newnanhospital.org

26 **Rhode Island Society for Histotechnology**
Contact: Nancy Heath
Phone: (401) 444-3246
Email: nheath@lifespan.org

31-Apr 3 **Texas Society for Histotechnology**
Site: Marriott Plano
Plano, TX
Contact: Kathy Dwyer or Veronica Davis
Phone: (972) 768-6279 or (972) 579-8291
Email: kdwyer@ameripath.com
veronida@baylorhealth.edu

APRIL

8-9 **Region III Meeting Hosted by Tennessee Society**
Site: Hilton Garden Inn
Nashville, TN
Contact: Charlene Henry
Phone: (901) 595-3191
Email: Charlene.henry@stjude.org

15 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Histology Process Improvement: Managing the Change Process**
Speaker: William DeSalvo, BS, HTL(ASCP)
Chair, NSH Quality Control Committee; System Production Manager, Laboratory Sciences of Arizona
Tempe, AZ

26 **Delaware Society for Histotechnology Dinner/Lecture**
Contact: Michelle Hart
Email: MHart@christianacare.org

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **The Effect of Pre-Analytical Factors on IHC Quality and the Optimization of Antibodies for Formalin Fixed Paraffin Embedded Tissue**
Speaker: Mark Rees, PhD
Leica Microsystems
Bannockburn, IL
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

27-29 **Tri-State Meeting (Wisconsin, Minnesota, Iowa)**
Site: Doubletree Hotel (formerly Radisson)
Rochester, MN
Contact: Sheri Blair or Judy Stasko
Email: sherinc2@comcast.net
judith.stasko@ars.usda.gov

28-30 **North Carolina Society of Histopathology Technologists**
Site: Shell Island Resort
Wrightsville Beach, NC
Contact: Lisa Gates
Email: lisa.d.gates@gsk.com

MAY

12-13 **Illinois Society for Histotechnology**
Site: ParADice Hotel and Casino
East Peoria, IL
Contact: Maureen Doran
Phone: (618) 453-1584
Email: mdoran@siumed.edu

13-15 **California Society for Histotechnology**
Site: Hilton Concord
Concord, CA
Contact: Lydia Figueroa or Robin Simpkins
Phone: (800) 725-8723 ext. 7863
Email: lefigueroa90501@yahoo.com
rsimpkins@biocare.net

19-21 **Region I Meeting**
Site: Hilton Garden Inn
Bangor, ME
Contact: Clare Thornton
Phone: (207) 941-8262
Email: cthornton@dahlchase.com

19-22 **Florida Society for Histotechnology**
Site: Grand Hyatt
Tampa Bay, FL
Contact: Susan Clark
Phone: (954) 562-4862
Email: dotmr@bellsouth.net

20 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Mitigating Contamination in the H&E Process**
Speaker: Angie Cahill, HTL(ASCP), Marketing Manager
Ventana Medical Systems
Oro Valley, AZ

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Educational Opportunities in 2011

MAY (cont.)

25 **NSH Teleconference 1:00 pm Eastern Time**
Title: **What's So Big About Macrosections in the Histology Laboratory?**
Speaker: Jennifer Lehmann, BS, HTL(ASCP)
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

28 **NSH Region IX Education Day**
Contact: Ann Lynde or Mark Elliott
Email: ann@nshregionix.org or mark@nshregionix.org

JUNE

2-4 **Missouri Society for Histotechnology**
Site: Resort at Port Arrowhead
Lake of the Ozarks, MO
Contact: Sharon Walsh
Email: userwalsh@sbcglobal.net

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Issues in Processing Breast Specimens**
Speaker: I-Tien Yeh, MD, Professor, Dept of Pathology
University of Texas Health Sciences Center
San Antonio, TX

22 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Clone Wars: Muridae vs Leporidae (Are You Team Mouse or Team Rabbit?)**
Speaker: Jeff Gordon, BS
Cell Marque
Rocklin, CA
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

24-26 **Mississippi Society for Histotechnology**
Site: Lake Terrace Convention Center
Hattiesburg, MS
Contact: Kimberly Wright
Phone: (601) 288-1064
Email: KWright@forrestgeneral.com

JULY

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Understanding the Finances of Laboratory Management**
Speaker: Elizabeth Sheppard, MBA, HT(ASCP)
Ventana Medical Systems Inc.
Tucson, AZ

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **The Cell Cycle and Cancer**
Speaker: Traci DeGeer, BS, HTL(ASCP)QIHC
Ventana Medical Systems Inc.
Tucson, AZ
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

AUGUST

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **A Systems Approach to H&E Staining**
Speaker: H. Skip Brown, MDiv, HT(ASCP)
Leica BioSystems
St. Louis, MO

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Estrogen and Progesterone Testing Standardization for Breast Cancer**
Speaker: Elizabeth Sheppard, MBA, HT(ASCP)
Ventana Medical Systems Inc
Tucson, AZ
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

SEPTEMBER

16 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **IHC Tips and Troubleshooting from a Preclinical Research Perspective**
Speaker: Anne C. Lewin, BS, HT(ASCP)QIHC
Senior Research Scientist, Bristol-Myers Squibb
Princeton, NJ

16-21 National Society for Histotechnology Symposium/Convention
Site: Duke Energy Convention Center
Cincinnati, OH
Contact: Aubrey Wanner
Phone: (443) 535-4060 or register online at www.nsh.org
Fax: (443) 535-4055
Email: aubrey@nsh.org

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Lower G.I. Biopsies: Tissue Identification, Diseases and Stains**
Speaker: Mitual Amin, MD
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

OCTOBER

21 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **The Cryostat and Technique**
Speaker: Mari Ann Mailhiot, BS, HT(ASCP)
Leica Microsystems
Deerfield, IL

26 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Taking It to the Bio "Bank": The Journey of a BioSpecimen to a BioMarker**
Speaker: Barbara Pruetz, BS, HTL(ASCP)
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

NOVEMBER

16 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Violence in the Workplace: What Can We Do to Prepare?**
Speaker: David Tate, MS
Purdue University
West Lafayette, IN
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: ***In-situ* Hybridization for Beginners**
Speaker: Bonnie Whitaker, HT(ASCP)QIHC
Ohio State University Medical Center
Columbus, OH

DECEMBER

21 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Safe Storage of Laboratory Chemicals**
Speaker: Peggy A. Wenk, HTL(ASCP)SLS
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Vinnie Della Speranza, ***HistoLogic***® Scientific Editor, 165 Ashley Avenue, Suite 309, Charleston, SC 29425. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.