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Immunohistochemistry — A Quick ABC Technique Using a Tissue Flotation Bath

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Abstract

A quick method for the avidin-biotin complex (ABC) technique has been developed in which the same results can be obtained in about 1 hour instead of the 2 hours or overnight incubation that is needed according to the protocol provided by the manufacturer. The main processes of this method include:

1. Deparaffinization at 55°C.
2. 3% H_2O_2 for 5 minutes to inhibit nonspecific peroxidase
3. Incubation of primary and secondary antibodies and the chromogen in a flotation bath at 42°C.
4. Slides are mounted in a new mounting medium developed by Nauss and Man.

Introduction

Since the introduction of the avidin-biotin complex (ABC) technique, immunohistochemical preparations have been widely used for many different purposes. According to the protocol provided by the manufacturer, the recommended procedure for the ABC technique requires 2 hours or overnight incubation to complete the whole process. In attempting to get faster results to assist with clinical diagnosis, we have developed a new method that reduces the total procedure time to 1 hour.

Materials and Methods

1. Tissue flotation bath; the water bath that is used for floating paraffin sections with a metal rack placed on top of the glass dish.
2. Primary antibodies:
   - S-100, L-26, Lambda, Desmin, Insulin, Epithelial membrane antigen, human growth hormone, prostate specific antigen, prostatic acid phosphatase, human chorionic gonadotropin, and carcino embryonic antigen were all purchased from Dako Corp., Carpenteria, Calif.

IN THIS ISSUE

Immunohistochemistry — A Quick ABC Technique Using a Tissue Flotation Bath 321
First Aid for Eosin "Bleeding" Problem 324
A Modified Alcian Blue-H&E Technique for Conjunctival Tissue Samples Collected On Cellulose Acetate Filter Paper 326
Cryotomy Tips 327
Noble Named Scientific Editor 328
Set Sail in Monterey NSH Symposium/Convention 329
OSHA's Bloodborne Pathogen Standard Now In Effect 332
Monterey Reminder: September in the Sun 336

No reader should utilize or undertake procedures in *Histo-Logic* articles unless the reader, by reason of education, training, and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished. The procedures discussed in these articles represent the opinions and experiences of the individual authors. Miles Inc. assumes no responsibility or liability in connection with the use of any procedure discussed herein.

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- AE1/AE3 and chromogranin were purchased from Boehringer Mannheim, Indianapolis, Ind.
- Smooth muscle actin was purchased from Sigma Chemical Company, St. Louis, Mo.

3. ABC kits, secondary antibodies, and chromogen were purchased from Biomeda Corp., Foster City, Calif.
4. Sections of formalin-fixed, paraffin-embedded tissues were cut at 4 micrometers. The tissues used were prostate, pituitary, tonsil, heart, liver, lymph node, uterus, skin tumor, pancreas, colon, small bowel, and cardinoid tumor of bowel.
5. Gelatin mounting medium developed by Nauss and Man.

The detailed preparations of the procedure are:

1. Xylene, 55°C, 10 min.
2. Absolute alcohol.
3. Hydrate to water.
4. Hydrogen peroxide/water 3%, 5 min.
5. Wash in running tap water, 1 min.
6. Rinse in PBS (phosphate buffered saline), 3 changes.
7. Primary antibody, 42°C in water bath, 15 min.
8. Wash in distilled water, 10 sec.
9. Places slides in PBS.
10. Biotinylated secondary antibody, 42°C, 10 min.
11. Wash in distilled water, 10 sec.
12. Rinse in PBS.
13. Step-avidin-biotin complex, 42°C, 10 min.
14. Wash in running tap water, 10 sec.
15. Rinse in distilled water, 8 sec.
16. Chromogen (AEC) – Aminoethylcarbozole, 5 to 8 min.
17. Wash in running tap water, 1 min.
18. Counterstain in hematoxylin.
19. Wash in running tap water.
20. Transfer to distilled water.
21. Leave slides in distilled water and mount in gelatin.

Results

This quick method shows results that are comparable to methods that require longer staining times, does not introduce nonspecific staining, and has produced excellent results with all of the antibodies tested.

Figs 1 through 6 show that with our modified method the positive reaction is clearly identified. The tissue shows no evidence of changes from the use of heat for deparaffinization or nonspecific background staining. We compared the results of our method and the method provided by the manufacturer and no noticeable differences were found.

Discussion

In the process of decreasing the time it takes to produce our immunohistochemistry results, we were able to modify a few of the standard procedures recommended in the protocols provided by the manufacturers that produce antibodies or detection products. Our major modification involves using a flotation water bath as a heat chamber to increase the speed of the reactions. By using this bath we were able to heat the slides with the steam of the water below them without causing the slides to dry, a common problem encountered when using a conventional dry heat oven (Figs 7, 8, and 9). We have compared twelve different tissues with fourteen different antibodies using our method and that recommended by the manufacturer without finding any noticeable differences. The use of heated xylene did not adversely affect the tissue or the quality of the stain. Although several modifications have been introduced by many authors to decrease the time involved to perform the immunohistochemical technique, we felt that this method was not only useful because of the time saved but also because the technique does not involve the purchase of new or expensive equipment, and it produces excellent results.

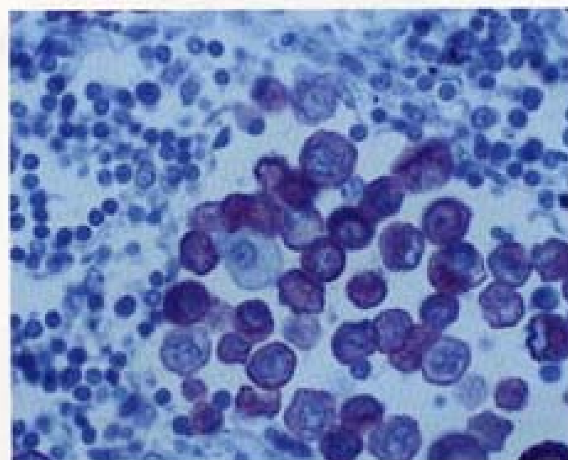

Figure 1: AE1-AE3 positive cells in a section of lymph node. ×400.

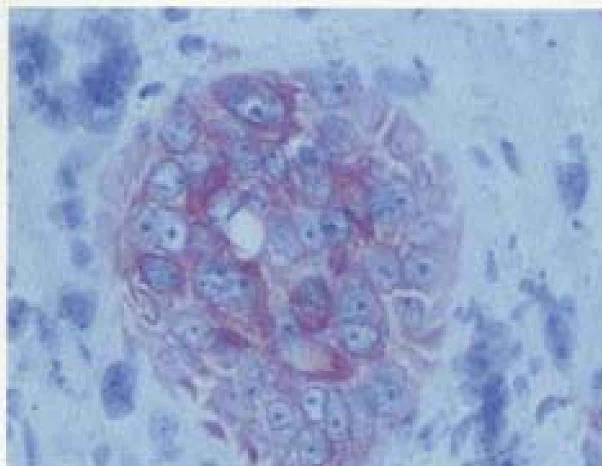

Figure 2: AE1-AE3 positive cells in a section of skin tumor. × 400.

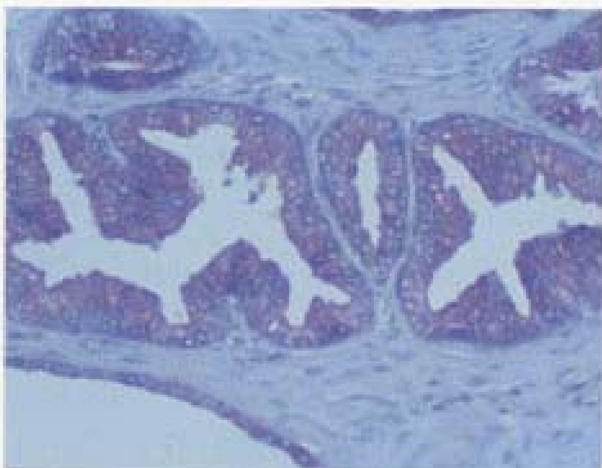

Figure 3: Prostatic acid phosphatase in a section of prostate. × 200.

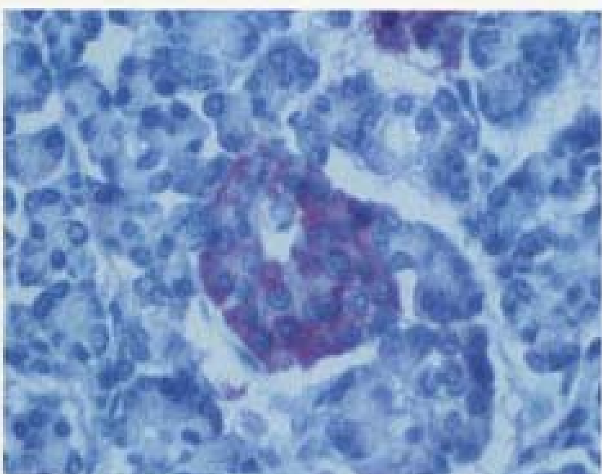

Figure 4: Insulin in islets of pancreas. ×400.

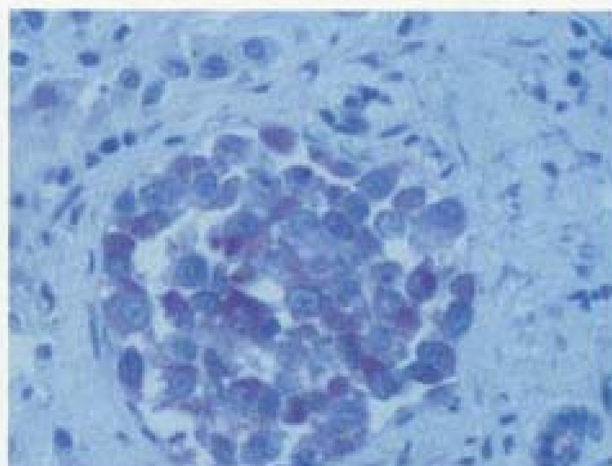

Figure 5: Cells in tumor of liver positive for epithelial membrane antigen. ×400.

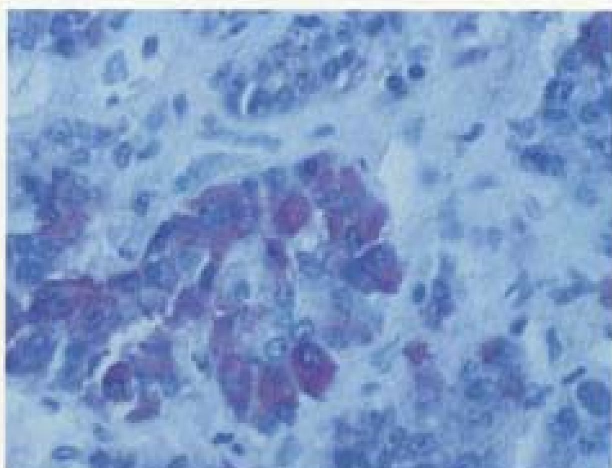

Figure 6: Pituitary tissue showing positive staining for human growth hormone. ×400.

Figure 7: Xylene in water bath.

(continued on page 324)

Figure 8: Water bath with metal rack.

Figure 9: Water bath with plastic cover.

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Acknowledgments

The authors would like to thank Dr Vivian W. Pinn, Chairperson, Department of Pathology, Howard University, for her constant support and encouragement.

First Aid for Eosin "Bleeding" Problem

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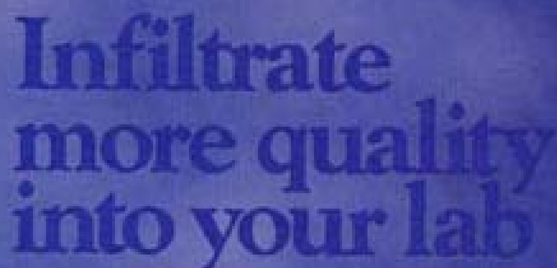
Several years ago, we substituted Propar*(an alkane blend) for xylol and FLEX 100* (a commercial blend of alcohols) for 100% ethanol. The H&E staining quality was acceptable on our automatic stainer, but there was frequent "bleeding" of the eosin into the mounting medium. Several experiments were conducted with various solvents and alcohol blends to alleviate the bleeding problem.

The problem was solved when we added one 100% ethanol station after the two FLEX stations. The stained slides were immersed in the two changes of FLEX for 1 minute each and in the 100% ethanol for 2 minutes. The slides were then immersed in three changes of Propar before coverslipping.

The bleeding stopped and all eosinophilic tissue components retained the eosin.

***Editor's Note:** All potential safety hazards and related possible technical problems should be checked before attempting to use these products.

That's not what I meant by FORMAL Alcohol!



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A Modified Alcian Blue-H&E Technique for Conjunctival Tissue Samples Collected On Cellulose Acetate Filter Paper

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Tissue samples were collected by applying strips (5.0 mm × 10.0 mm) of cellulose acetate filter paper (Millipore HAW P 304) to the conjunctiva of patients' eyes for 2 to 3 seconds and then slowly peeling the filter paper away from the ocular surfaces. These strips were then placed in a teflon sample holder for fixation (10 min) and staining.

A modified alcian blue-hematoxylin and eosin technique was used to evaluate the morphology of the epithelial cells and for quantitating the mucinous material present in the conjunctival sac in normal and abnormal conditions of the eye.

Fixation Acetic-alcoholic formalin (5 mL formaldehyde, 5 mL glacial acetic acid, 90 mL 70% ethanol) for 10 min.

Solutions and Reagents

3.0% Acetic Acid (Stock)

Glacial acetic acid ....9.0 mL
Distilled water ....291.0 mL

0.1% Alcian Blue (Stock)

Alcian blue, 8GX ....0.1 gm
3.0% acetic acid (stock) ....100.0 mL

Alcian Blue (Working)

0.1% alcian blue (stock) ....20.0 mL
3.0% acetic acid (stock) ....40.0 mL

Harris' Hematoxylin (Stock)

Hematoxylin crystals ....5.0 gm
100% alcohol ....50.0 mL
Ammonium or potassium alum ....100.0 gm
Distilled water ....1000.0 mL
Mercuric oxide (red) ....2.5 gm
Glacial acetic acid ....20.0 mL

Dissolve hematoxylin crystals in the 100% alcohol. Using a 2L Erlenmeyer flask, dissolve the alum in the distilled water with the aid of heat and stirring. Add the dissolved hematoxylin to the flask and bring to a boil. Remove the flask from the heat and slowly add the mercuric oxide. Return the flask to the heat and simmer until the solution becomes dark purple. Remove the flask from the heat and immerse in cold water. Allow hematoxylin solution to cool to room temperature and add 20 mL of glacial acetic acid. Filter into storage container.

Harris' Hematoxylin (Working)

An equal portion of the stock hematoxylin is mixed with 3.0% acetic acid (stock) prior to staining and discarded when staining is completed.

Eosin-Phloxine Solution

Eosin (Stock)

Eosin Y, water soluble ....1.0 gm
Distilled water ....100.0 mL

Phloxine (Stock)

Phloxine B ....1.0 gm
Distilled water ....100.0 mL

Eosin-Phloxine (Working)

Eosin (stock) ....50.0 mL
Phloxine (stock) ....5.0 mL
95% alcohol ....390.0 mL
Glacial acetic acid ....2.0 mL

Staining Procedure

1. Two changes of distilled water.
2. 3.0% acetic acid for 3 min.
3. Working alcian blue solution for 10 min.
4. Rinse with tap water for 5 min.
5. Two changes of distilled water.
6. Working hematoxylin solution for 1 min.
7. Rinse with tap water for 10 min.
8. Two changes of distilled water.
9. Eosin-phloxine solution for 1 min.
10. Two changes of 95% alcohol for 1 min each.
11. Two changes of absolute alcohol for 1 min each.
12. Clear filters in xylene and mount on slide with a xylene soluble resin.

Results

Nuclei	deep blue
Cytoplasm	various shades of pink
Goblet cells	light greenish blue
Mucous strands	greenish blue
Mucous granules	greenish blue

Reference

Luna LG, ed. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.* 3rd ed. New York: McGraw-Hill Book Co; 1968:34,36, 163.

Cryotomy Tips

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An area of histotechnology that particularly interests me is cryotomy. Having spent a great deal of time producing frozen sections, I have been able to incorporate several helpful tips that not only shorten our turn-around times but also produce better sections.

Tip # 1 — Preparation
When a specimen needing a frozen section is anticipated, preliminary preparations are made. I try to have the following supplies ready: 1) a number of slides lined up and ready to go, 2) a box of Kleenex, 3) a paintbrush, and 4) the water-soluble embedding media.

Tip # 2 — Pre-cooled Cryostat Chucks
One of the most time-consuming aspects of cryotomy is waiting for the embedding medium and embedded tissue specimen to freeze. I have found that if the object holders (cryostat chucks) are stored in the cryostat, freezing takes less time.

Tip # 3 — Application of Water Soluble Embedding Medium
The embedding medium should be applied sparingly, using only enough to cover the tissue on the chuck. This technique will not only shorten the freezing time but also shorten the time spent rough cutting.

Tip # 4 — Heat Extractor
When a heat extractor (quick freezing apparatus) is used, it is a good idea to keep the contact area free of frost. After removing frost and wiping with a dry towel, I lightly cover the contact area with cryostat lubricating oil. When the heat extractor is placed on the embedded tissue block and freezing is accomplished, the frozen block can be easily removed from the heat extractor.

Tip # 5 — Reattaching Frozen Tissue Block
If the frozen tissue block becomes loose or is dislodged from the chuck, there is no need to thaw the tissue, re-embed, and then freeze again. Simply place a drop of water on the backside of the frozen block and press it firmly on the chuck. Adherence of the frozen tissue block to the chuck is almost immediate and cryotomy may resume.

Tip # 6 — Attaching the Section to the Slide
After picking up the frozen section on a clean glass slide, I place the backside of the slide against my glove-covered wrist for a couple of seconds. This technique seems to help provide better adherence of the section. Using this simple technique lessens the chances of losing the tissue during staining. The slides are then stained with a Hematoxylin and Eosin procedure.*

***Hematoxylin and Eosin Procedure for Frozen Sections**

1.	100% ethanol	10 dips
2.	95% ethanol	10 dips
3.	Tap water	10 dips
4.	Hematoxylin	30 to 60 dips
5.	Tap water	10 dips
6.	Ammonia water	10 dips
7.	Tap water	10 dips
8.	Eosin	10 dips
9.	95% ethanol	10 dips
10.	100% ethanol	20 dips
11.	Xylene	20 dips
12.	Coverslip	

Cryotomy is a challenge because there are always new ways to expedite the process and increase the quality of slides for diagnosis.

Noble Named Scientific Editor

Brent Riley
Managing Editor

Leonard Noble, HT (ASCP), HTL, was recently named scientific editor of *Histo-Logic*. He follows in the footsteps of Lee Luna, who founded *Histo-Logic* 21 years ago and remained scientific editor until his death earlier this year. Noble will be responsible for the scientific content of the newsletter, including soliciting and editing articles from the field.

Leonard Noble thrives on challenge. Of course, for him, challenge is synonymous with opportunity. And when Noble is presented with a new opportunity, he seldom turns it down. So, naturally, when he was approached to become the new scientific editor of *Histo-Logic*, he saw a huge opportunity.

"I plan on maintaining the level at which Lee Luna has built *Histo-Logic*," Noble announced. "I'm going to set many goals and work toward them just as Lee did. I'll be looking at the needs of the reader and the profession and establishing goals based on those needs. To do that, I will have to get input and feedback from the profession and from my peers.

"In the tradition established by Mr Luna, we will look at ways of soliciting those types of articles that are important in today's laboratory," he said. "As things change and new techniques and procedures are implemented, we will keep up. But we also want to include articles about other aspects of histotechnology, things like government regulations, safety, and management."

Noble values continuity. He believes that once people get to know and like a publication, they don't want to see drastic changes.

From the time he entered the histotechnology profession, Noble's career has been nonstop. His first day on the job in the histology lab at Duke University Medical Center was his first exposure to histotechnology. "I was thrown into it right away," he said, "in an environment that was not only a service laboratory but also an academic laboratory." But Noble saw it as a challenge and an opportunity. Within a short period of time, Noble was teaching histological techniques to medical technology and pathology assistant students. Later, he was in charge of the histochemistry section and was a technical adviser for graduate histochemistry classes at the medical center.

Noble has always been interested in writing. He has published multiple articles, including five in *Histo-Logic*, and assisted with many others.

Noble's first published article appeared in *Laboratory Management* in 1977. It was titled "Histochemical Staining Gives Muscle Biopsies New Utility." He had previously provided technical assistance on a number of published articles authored by others.

"My interest in scientific writing came from my work experience at Duke University. Phillip Pickett was my supervisor and mentor in histology. He has authored numerous publications about innovations in histotechnology. My work experience with Pickett, combined with the atmosphere at Duke, gave me an excellent education, not only in histotechnology and related laboratory sciences but also in developing and writing scientific articles."

Today, Noble is the manager of anatomic pathology at North Carolina Baptist Hospital/Bowman Gray School of Medicine of Wake Forest University Medical Center in Winston-Salem, North Carolina. He is responsible for the technical operations of the histology, cytology, and molecular diagnostics laboratories, the autopsy services, and the medical transcriptionist staff.

Noble has been in his position at the medical center for 15 years and the laboratories are well organized and operating very efficiently.

The medical center's anatomic pathology section is 43 strong. The center itself has 8500 employees. The anatomic pathology labs perform the full range of cytological, histological, histochemical, and immuno procedures," Noble remarked.

When he was approached by Brent Riley of Miles Inc. and asked to be scientific editor of *Histo-Logic*, Noble did have to think about it, but not for long. "I feel that this is the right time in my career to take on this responsibility," he said.

"I didn't have to think about it long because it was quite the honor," Noble said. "This, to me, is just as big an honor as anything I've ever done or any award I've ever received." And he has received some prestigious awards during his career, including the Golden Forceps Award in 1990 and the J.B. McCormick Award in 1989. He was also chosen as the NSH exchange speaker for the Swiss Society of Histotechnology in 1991.

Noble says one main goal is to continue to produce a high-caliber publication. "I deeply respected Lee, what he stood for, and everything he accomplished in his career," Noble said. "I will still rely on Lee's family for their expertise and help," he continued. "They are still a very important part of *Histo-Logic*."

One of Luna's goals was to make *Histo-Logic* more of an international publication. Noble plans to continue those efforts by soliciting more articles from histotechnologists in other countries. Through his trip to Switzerland, he was able to establish many contacts who may help him accomplish this.

Noble also maintains a busy lifestyle outside of histotechnology. His 16-year-old son and 9-year-old daughter keep him and his wife "on the go" constantly. "My son plays football and my daughter is into dancing and gymnastics," he said. Noble also has a dog and a cat and is actively involved in church and community work. He enjoys gardening and has collected Indian artifacts for more than 20 years.

Noble has always been very active and visible as a histotechnologist. He travels throughout the country to give lectures and conduct workshops. He is a charter member of the National Society for Histotechnology and is still very involved with the society. He has been the NSH vice president for 4 years and has also served on a number of NSH committees, including 6 years as chairman of the Awards Committee.

Noble encourages histotechnologists to write. He is currently developing three articles of his own for publication. "I like the current format of *Histo-Logic*," Noble said. "I want it always to be a very readable publication. I plan to work behind the scenes and make *Histo-Logic* and every article published in *Histo-Logic* the best it can possibly be." Above all, Noble wants to carry on the tradition that Lee Luna established.

With that in mind, Leonard Noble gladly accepts the challenge and opportunity that *Histo-Logic* presents. In fact, he's thrilled.

Set Sail in Monterey at the NSH Symposium/Convention

Brent Riley
Managing Editor

If you plan to attend the 1992 NSH Symposium/Convention in Monterey, California, you have a lot to look forward to. "It will be the perfect time of the year to be in Monterey," proclaimed Kerry Crabb, convention vice chairman. "They say that if you're going to Monterey, the weather in September is ideal."

Both indoors and out, conditions should be perfect in Monterey on September 12-18 — perfect for learning, sharing information, making friends, and having a great time.

More than 1000 registrants are expected at the event — one of the largest ever. "This will be the first time we have used a conference center for the meeting," explained Crabb. Events will be held at the Doubletree and Marriott hotels as well as the Monterey Conference Center.

Among the registrants, about 300 first-timers are expected to attend. A welcoming reception will be held for newcomers on Saturday night.

(continued on page 330)

The theme of the symposium/convention is "Set Sail Into the Future With Histotechnology." This theme ties in with the flavor of Monterey yet is representative of a concept established for histotechnology 3 years ago when the 90s were deemed the "Decade of Progress."

The exhibit hall is fully booked. One hundred twelve booths will be managed by about 300 representatives from 63 companies, 17 of which will be attending for the first time. Here, you can see and touch the latest in histopathology equipment and supplies. Among the exhibits, two booths will be set up specifically for recruitment of histology professionals. The exhibit hall will be open Tuesday at 4 pm and will close Thursday at 1 pm.

Seventy workshops will be conducted, beginning Saturday and continuing through Tuesday. The workshops will cover innovative as well as traditional histological techniques, lab management procedures, and personal performance management.

Clinical and veterinary, industrial, research (V.I.R.) scientific sessions will begin on Wednesday and continue through Friday morning. These sessions will begin with the Professor C.F.A. Culling Memorial Lecture. This year the speaker will be Clive R. Taylor, MD, PhD, from the University of Southern California School of Medicine. His topic will be "Diagnostic Immunohistochemistry – Standards of Practice." This will be the tenth anniversary of the memorial lecture.

Official NSH committee meetings, region meetings, and board of directors meetings will be held throughout the week. Most of these meetings are open to all attendees.

As always, social and hospitality events will be plentiful at the convention. The Awards Banquet will be held on Thursday night, preceded by a cocktail party sponsored by Miles Inc. The theme for the awards banquet is "Monarch Magic" in recognition of the millions of monarch butterflies that come to Monterey each winter. According to Vivian McClure and Coleen Roush, banquet chairpersons, the purpose of the banquet is to honor outstanding histotechnologists as well as the many companies that help support the NSH. Six hundred fifty people are expected to attend the semiformal banquet.

Miles Inc. will also sponsor a party on Tuesday night called "MTV Night." Attendees are expected to come dressed as their favorite rock star, singer, or musician. Many are expected to come as rock groups, such as the Beatles, the Rolling Stones, or perhaps even Guns and Roses.

The local chairman of the symposium/convention is Ben Shelkowsky. He has been working with about 10 other area histology professionals to plan and prepare for the event.

If you have an extra day before or after the convention, plan to take in some of the attractions in and around Monterey. The hotels are only about a block from spectacular Monterey Bay and Fisherman's Wharf. You can also walk to Cannery Row, the inspiration for the John Steinbeck novel. The nearby Monterey Aquarium is also worth visiting. And, if you have a car, you can take a short drive to Pebble Beach, Carmel, Point Lobos, or Big Sur.

Crabb had some other excellent advice for all those who plan to attend the symposium/convention. "Talk with your peers," he advised. "Socializing during the breaks and during the workshops and in the evening at the hospitalities is an important part of the experience," he continued. "We are a friendly group that enjoys talking about our work and sharing information with each other. At the convention, you can develop a network of close friends on whom you can call for assistance if you ever have a question or a problem. The information you can get from the interchange with your peers is just as valuable as the information you get from the workshops and lectures."

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OSHA's Bloodborne Pathogen Standard Now In Effect

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Modern-day laboratory personnel have routinely been made aware of the risk of exposure to bloodborne pathogens (BBP), and subsequent safe techniques to minimize exposure to these pathogens have been developed. Blood and blood products are usually thought of as the vehicles for these bloodborne pathogens. However, it must be remembered that exposure to other body fluids visibly contaminated with blood (ie, cerebrospinal fluid, amniotic fluid, urine, etc) and unfixed tissue can also be vehicles for bloodborne pathogens.

Concerns about workplace exposure to two specific bloodborne pathogens, HIV (Human Immunodeficiency Virus) and HBV (Hepatitis B Virus), led the Occupational Safety and Health Administration (OSHA) to revise its exposure control regulations. Although infection by HIV in healthcare workers has not significantly increased in the past 4 years, the reported incidence of infection by HBV rose dramatically by 37% among the general population from 1979 to 1989.

Essentially, the new OSHA Standard (29CFR 1910.1030),* which became effective in March 1992, expresses requirements that are meant to minimize worker exposure to bloodborne pathogens. The Standard mandates that all laboratories establish stringent work practices and procedures that will ensure minimal exposure to these pathogens. Compliance with the Standard was required by July 1992.

Requirements of the new OSHA Standard include the following:

I. AN EFFECTIVE TRAINING AND EDUCATIONAL PROGRAM must be implemented. The program must include:
   - A. The effective dates for compliance of the BBP Standard and an explanation of the contents of the Standard.
   - B. Training that is suitable to the audience.
   - C. An instructor who can answer questions or who can give explanations is required. A simple impersonal video or sign-off document is not acceptable.
   - D. Instruction must be given on the general epidemiology, modes of transmission, and symptoms of BBP diseases with an emphasis on HIV and HBV related diseases.

II. Laboratories must establish an EXPOSURE CONTROL PLAN. All personnel must have knowledge of this plan and have access to the plan at all times. The Exposure Control Plan is an integral part of this Standard and must be designed and implemented with several important considerations:
   - A. Determination of exposure potential or TASK CATEGORIES must be established, listing all job descriptions and defining their potential for exposure.
   - B. The Exposure Plan requires that HBV vaccinations be made available to all personnel who may be exposed to potentially infectious material.
   - C. Procedures for the evaluation of circumstances surrounding exposure incidents and appropriate plans of action in the event of an exposure must be established.
   - D. The Exposure Plan must be reviewed and updated at least annually.
   - E. METHODS OF COMPLIANCE. A control strategy with appropriate documentation must be established. Initially, a policy of UNIVERSAL PRECAUTIONS must be implemented. Universal Precautions state that ALL human and other potentially infectious materials are treated as if they were infectious. The Control Strategy must include:
     1. Engineering controls, such as biological safety cabinets, autoclaves, puncture proof sharps containers, specially designed ventilation systems, and splash guards.
     2. Personal Protective Equipment (PPE), such as face shields, goggles, gloves, masks, gowns, and aprons.
     3. Establishment of Work Practices to minimize exposures.
        - a. Define procedures to minimize splashing, spraying, and aerosolizing.
        - b. Establish systems of waste disposal in closable, leakproof, labeled containers.
        - c. Develop sharp handling and disposal procedures. Needles must not be sheared, bent, broken, or recapped by hand.

(continued on page 334)

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- Constructed of welded steel for stackability and durability
- Available with either single- or double-slide drawers for flexibility and easy retrieval
- Choose olive or beige

d. Establish glassware handling procedures.

e. Establish personal hygiene policies such as no eating, drinking, smoking, or application of cosmetics in the laboratory. Use of lip balm or handling of contact lenses is also prohibited. Regular handwashing is mandatory after removing gloves.

f. Determine the use and policy for PPE, especially gloves, lab coats, goggles, safety glasses, and face shields. These policies will depend on the nature of the potential exposure, (ie, masks in combination with eye protection devices). Surgical caps or hoods and shoe covers shall be worn in instances when gross contamination can be anticipated (eg, autopsies). All PPE shall be removed prior to leaving the work area.

g. Establish housekeeping procedures with scheduled specific disinfecting practices, including bench top, equipment, and other environmental surfaces. Procedures for safe handling of laundry must be established.

h. Establish and implement procedures for LABELS and SIGNS as appropriate. All containers of blood or potentially infectious materials must be labeled. Appropriate labels should be placed on refrigerators, freezers, and waste containers. Red bags and red containers may be used in lieu of physical labeling. Labels must display the biohazard symbol and the word, "BIOHAZARD." Labels and signs that are fluorescent orange or orange-red with blue, black, or green color symbols or lettering are appropriate.

III. A RECORD OF TRAINING and EXPOSURES must be maintained. Records of training must be maintained for 3 years from the date of training. Records of exposures must be maintained for at least the duration of employment plus 30 years.

OSHA's Bloodborne Pathogen Standard is a detailed and very specific document that will have a significant impact on the overall operation of our laboratories. Establishing procedures and practices for compliance must be carefully determined on an individual laboratory basis. A lengthy and detailed examination of the Standard was not the intention of this article but rather an overview of significant highlights of the Standard. The reader is encouraged to obtain a copy of the Standard† for reference when establishing practices and procedures for their particular laboratories.

It should be emphasized that OSHA's Bloodborne Pathogen Standard, as with their other regulations, is a performance-oriented standard. This means that when your compliance program is formed it may differ from other programs because of individual situations. Like chocolate chip cookies, there are hundreds of recipes, but in the end, they are all good cookies.

In their efforts to comply, many safety managers, supervisors, and laboratory directors frequently bury themselves and their employees in a deluge of minute details because of their lack of understanding of the saying, "The main thing is to keep the main thing the main thing." The most important consideration when formulating your individual program should be the purpose rather than mere compliance with the regulation.

"Employers shall provide a workplace free from recognized hazards" (OSHA ACT Section 5 A1).

* HIV and HBV Research Laboratories and Production Facilities have an entire set of special precautions and requirements under this law. They will not be discussed in this article but the reader should consult the Standard for detailed information.

† Occupational Safety and Health Administration
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200 Constitution Avenue, NW
Washington, DC 20210
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