HISTOLOGIC®
Technical Bulletin for Histotechnology

SAKURA

Vol. XLII, No. 2 December 2009

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

Precipitating Chromogen Optimization for the Localization of CD68 in Melanin-Impregnated Metastatic Murine Lung Tumors

John Lydon, BS; Jennifer Johnson, BS; Denise Griffiths, BS, HT(ASCP); Kenneth Munroe, BS; Jay Harper, PhD; Beth L. Thurberg, MD, PhD
Genzyme Corporation
Framingham, MA

John.Lydon@genzyme.com

Abstract

3,3´-Diaminobenzidine, or DAB, is the most commonly used precipitating chromogen for immunohistochemical staining in our lab today. However, there are times when DAB is not appropriate to use. In samples such as skin or tumor that contain high levels of melanin, DAB/antibody staining makes it nearly impossible to distinguish CD68 from the endogenous pigment present within the tissues. In those cases, a different chromogen is not only beneficial, but also required to make the proper analysis possible.

This study explored some of the different chromogens available that can be used instead of DAB in order to distinguish between real staining of CD68 in this experimental murine model of a highly metastatic lung melanoma, heavily infiltrated with melanin, and staining due strictly to endogenous pigment (Figs. 1 and 2).

Introduction

Along with increases in pigmentation and vascularization, CD68 positive macrophages have been used as diagnostic markers for determining the stage and progression of different melanomas, including malignant human melanomas and uveal melanomas.[1-3] Our group was interested in making similar correlations

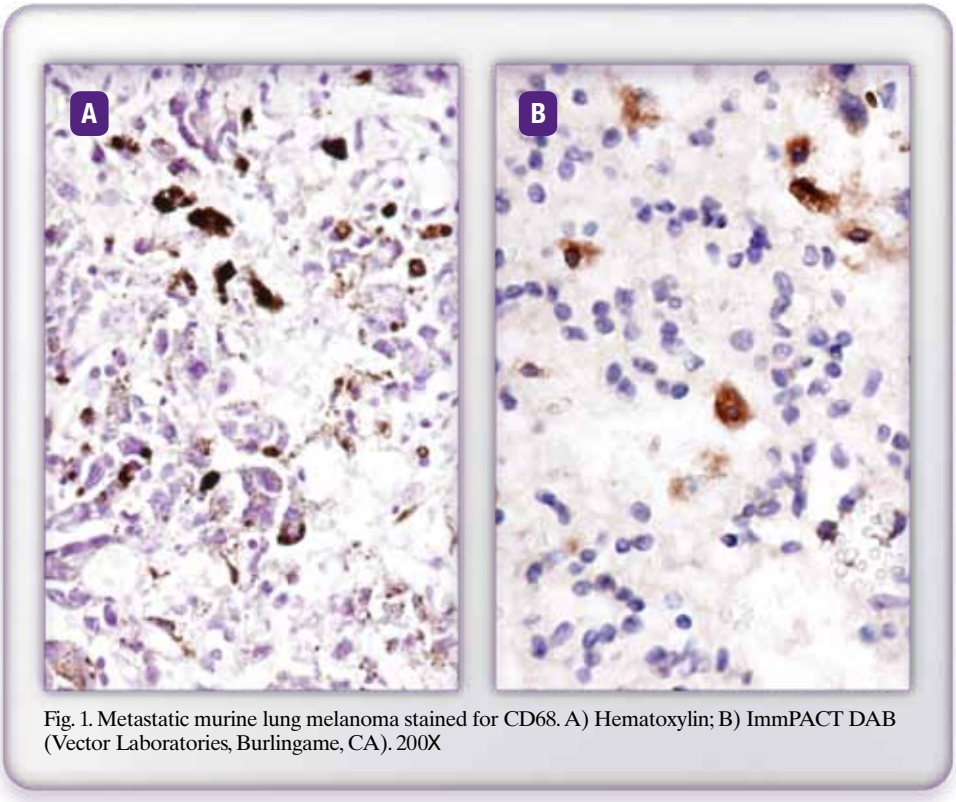


Fig. 1. Metastatic murine lung melanoma stained for CD68. A) Hematoxylin; B) ImmPACT DAB (Vector Laboratories, Burlingame, CA). 200X

IN THIS ISSUE

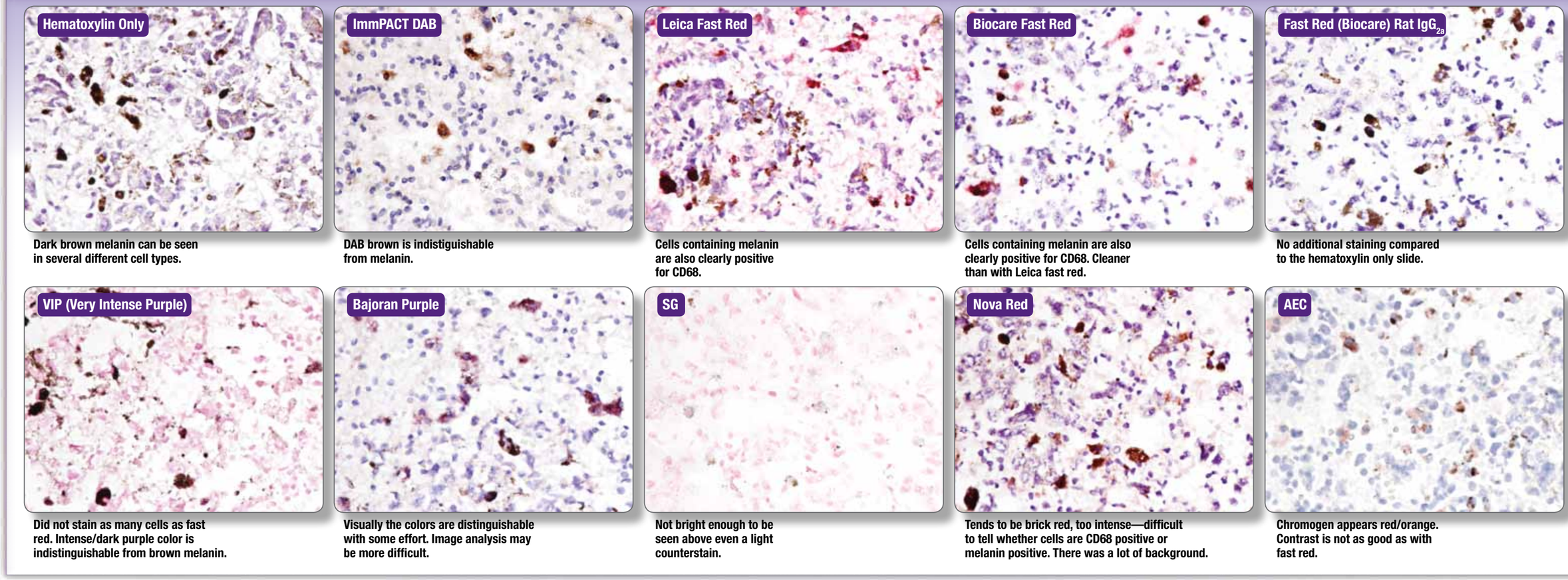


Fig. 2. CD68 staining in murine model of metastatic lung melanoma. Different chromogens were used to find alternatives to DAB for distinguishing it from melanin in highly pigmented necrotic tissues. 200X

in the metastatic murine model of lung melanoma. Our standard antibody staining method for demonstrating the CD68 (clone FA-11, AbD Serotec, Raleigh, NC) antigen in paraffin-embedded tissues utilizes DAB as a chromogen for detection. However, in this model of murine melanoma, the melanin appears a dark brown color, similar to that of the DAB, making distinctions between the two impossible for both the human eye and our computer software (MetaMorph, MDS Analytical Technologies, Downingtown, PA). Bleaching protocols such as hydrogen peroxide or potassium permanganate/oxalic acid in combination with the proteinase K pretreatment needed for staining could destroy the signal and lift the dense, somewhat poorly fixed tumors from the slides. It was therefore necessary to optimize a protocol in which the CD68 antigen could be localized and visualized above the embedded pigment.

This article will detail our efforts to locate a chromogen with the greatest contrast to melanin pigment in order to assess changes in the numbers of CD68 positive cells within murine lung melanomas. We will utilize the BondMax Immunostainer (Leica Microsystems, Bannockburn, IL) and image analysis software.

Materials and Methods

Female C57Bl/6 mice (The Jackson Laboratory, Bar Harbor, ME) 10 to 14 weeks of age were injected intravenously with 100,000 highly metastatic B16-F10 melanoma cells on study day 0. Mice were randomized and B16-F10 metastases were allowed to establish in the lungs. On day 21, animals were sacrificed by CO_2 asphyxiation and the lungs were removed. All animals were maintained according to the Genzyme Institute Animal Care and Use Committee guidelines, under approved animal study protocols.

The lungs were fixed in zinc buffered formalin (Electron Microscopy Sciences, Hatfield, PA) for 48 hours. After rinsing in PBS, lungs were dehydrated, cleared, and infiltrated into Paraplast X-tra Paraffin (Leica Microsystems) on a Tissue-Tek® VIP tissue processor (Sakura Finetek, Torrance, CA) using standard protocols. Five-micron sections were taken on a Leica 2255 microtome (Leica Microsystems) and placed onto charged slides (Erie Scientific, Portsmouth, NH). Using standard protocols, representative slides were stained with hematoxylin and eosin (Richard-Allan Scientific, Kalamazoo, MI) and Schmorl's reaction for melanin counterstained with nuclear fast red (Rowley Biochemical, Danvers, MA) to highlight the morphology and confirm the presence of melanin in the samples (not shown).

For each chromogen, a CD68 (clone FA-11) and a matched (rat) IgG control were stained. All buffer washes were performed with 0.05% Tween 20 in TBS pH 7.6. All slides were baked for 30 minutes at 60°C. They were placed onto the BondMax Immunostainer for all steps up to the addition of chromogen (Table 1). The DAB and Leica fast red were added by the machine. All other chromogens were applied manually and observed under a microscope to achieve the best staining possible (Table 2). After addition of the chromogen, slides were rinsed in running water and counterstained with either hematoxylin (Leica or Dako) for 1 minute, nuclear fast red for 30 seconds, or methyl green (Vector Laboratories) for 3 dips, followed by a rinse in water. All were air dried at 60°C for 1 hour and coverslipped with Acrytol (Leica Microsystems).

Conclusion

Poor fixation and abnormal morphology of slightly necrotic tumors added to the difficulty in interpreting the results. Bajoran purple, AEC, and the fast reds gave acceptable contrast against the melanin within the tumors. Overall, the fast reds were easier to mix and gave more consistent results in these mouse melanomas. In this model, other chromogens were more variable when repeated and harder to refine. Incubating slides with chromogen longer to get more intense staining either increased the background or overpowered the cells, making them too dark to read. The intense color of the fast red chromogen could still be seen in cells that also contained melanin, which gave us the overall best contrast and staining results (Fig. 2).

Table 1: BondMax CD68 Stain Protocol		
Solution	**Temperature**	**Time**
Bond dewax solution[a]	72°C	30 seconds
Bond dewax solution[a]	RT	10 seconds
Bond dewax solution[a]	RT	10 seconds
100% reagent alcohol[b]	RT	3 x 10 seconds
TBS/Tween 20 wash solution[c]	RT	2 x 10 seconds
TBS/Tween 20 wash solution[c]	RT	5 minutes
Proteinase K[c]	RT	5 minutes
TBS/Tween 20 wash solution[c]	RT	3 x 2 minutes
Peroxidase block[c]	RT	5 minutes
TBS/Tween 20 wash solution[c]	RT	3 x 2 minutes
Protein block, free serum[c]	RT	10 minutes
TBS/Tween 20 wash solution[c]	RT	3 x 2 minutes
CD68 (Clone FA-11) or rat IgG_{2a} negative control[d]	RT	45 minutes
TBS/Tween 20 wash solution[c]	RT	3 x 2 minutes
Affinity purified rabbit anti-rat, mouse adsorbed secondary antibody[e]	RT	20 minutes
TBS/Tween 20 wash solution[c]	RT	3 x 2 minutes
Mach2 rabbit HRP3 OR Mach2 rabbit AP solution[f]	RT	20 minutes
TBS/Tween 20 wash solution[c]	RT	3 x 2 minutes
Application of chromogen	RT	Varied – see Table 2

Table 2: Summary of Staining Time and Results					
Chromogen	**Supplier**	**Application Method**	**Time in Chromogen**	**Counterstain**	**Contrast Rating**
DAB	Leica	Immunostainer	2 minutes	Hematoxylin[a]	Poor
ImmPACT DAB	Vector Labs	Manual	20 seconds	Hematoxylin[c]	Poor
Fast Red	Leica	Immunostainer	10 minutes	Hematoxylin[a]	Very good
Fast Red	Biocare Medical	Manual	10 minutes	Hematoxylin[c]	Excellent
Bajoran Purple	Biocare Medical	Manual	4 minutes	Nuclear fast red[g]	Fair
VIP (Very Intense Purple)	Vector Labs	Manual	3 minutes	Nuclear fast red[g]	Poor
Nova Red	Vector Labs	Manual	3 minutes	Hematoxylin[c]	Poor
SG	Vector Labs	Manual	10 minutes	Methyl green[e]	Poor
AEC	Vector Labs	Manual	10 minutes	Hematoxylin[c]	Fair

Supplier Key: a = Leica, b = Surgipath, c = Dako, d = AbD Serotec, e = Vector Labs, f = Biocare Medical, g = Rowley Biochemical.

References

1. Torisu H, Ono M, Kiryu H, et al. Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma: possible involvement of TNFalpha and IL-1alpha. *Int J Cancer.* 2000;85(2):182-188.
2. Kiss J, Tímár J, Somlai B, et al. Association of microvessel density with infiltrating cells in human cutaneous malignant melanoma. *Pathol Oncol Res.* 2007;13(1):21-31.
3. Mäkitie T, Summanen P, Tarkkanen A, Kivelä T. Tumor-infiltrating macrophages (CD68(+) cells) and prognosis in malignant uveal melanoma. *Invest Ophthalmol Vis Sci.* 2001;42(7):1414-1421.

Acknowledgments

The authors would like to thank the Department of Comparative Medicine and Tessa Green for their contributions with the animal model, and Biomedical Media Services for their expertise in image preparation.

Simple Rapid Method for Manufacturing Recipient Block for Tissue Microarray

Salah Deeb, PhD; Khalid El-Nesr, PhD; Emad Mahdi, PhD
Department of Pathology
Faculty of Veterinary Medicine
University of Beni-Sueif, Egypt

Salahdeeb40@yahoo.com

Introduction

In 1998, Kononen et al reported the technology of tissue microarray (TMA).[1] TMA is becoming an important tool for research and quality control when using immunohistochemistry (IHC) and in situ hybridization methods.[2-4] The technique is also useful in providing adequate material for pathology examination and for the preparation of reference slides for educational purposes in histology.[5] A TMA block is constructed by means of a commercial TMA instrument that punches tissue cores out of donor blocks. Then the tissue cores are transferred to a recipient block, producing blocks with a large number of different tissue cores.

TMA provides a link between molecular targets and tissue or cell morphology, as well as with the clinical data associated with the specimens.[6-9] The key TMA technology is the construction of its paraffin blocks. Although equipment such as the Manual Tissue Arrayer (Beecher Instruments, MTA-II, Silver Spring, MD) can be helpful,[10] the process of TMA paraffin block construction is dependent on manual manipulation. Using a pair of needles, researchers have to repeat sampling a tissue core (specimen cylinder) from the donor block and put it into holes in the recipient block that were punched in advance one by one.[10]

The efficiency of TMA paraffin block construction is dependent on the manual tasks that can bottleneck this technology. The recipient paraffin block should be made before TMA construction. A higher quality paraffin is required to avoid chipping. As stated by Pires et al,[11] the quality of a TMA paraffin block is affected directly by the performance level of the technician. Current equipment used in TMA paraffin block construction includes a precision location system, which is very expensive. Construction efficiency, technician proficiency, and equipment expense are some of the issues that have been limiting the popularization of TMA technology.

Meng et al[12] designed a tissue microarrayer (ZM-1 tissue microarrayer) manufactured of stainless steel and brass. As stated by the authors, the designed arrayer contains many features that make construction of TMA paraffin blocks faster and more convenient. With the ZM-1 tissue microarrayer, biopsy needles are used to punch the donor tissue specimens. All the needles with the punched specimen cylinders are placed into small holes dug into the array board to fit the needles. The recipient paraffin blocks need not be made in advance. The ZM-1 tissue microarrayer is easy to manufacture and does not need any precision location system, so it is more cost effective than other instruments currently in use. Because the ZM-1 tissue microarrayer technique of constructing TMA paraffin blocks is easier to perform and the instrumentation is more affordable, this method may facilitate popularization of TMA technology. A new technique described by Pires et al[11] does not use a recipient paraffin block for the tissue cores or a commercial TMA builder instrument. The technique is based on the construction of TMA needles by modifying conventional hypodermic needles to punch tissue cores from donor blocks. These are then attached to double-sided adhesive tape on a computer-generated paper grid used to align the cores on the block mold and are subsequently filled with liquid paraffin.

Materials and Methods

A new method developed in our laboratory requires only a short time to prepare the recipient paraffin block; the paraffin used to make the TMA block is the same as that used for routine pathological purposes. Needles are used manually to obtain tissue cylinders (tissue cores) of specific diameter to fit holes made in the recipient block with defined array coordinates. This method of making a recipient block is easy to do for trained technicians and is also very cost effective. The array holes in the recipient block were prepared using a template as seen in Fig. 1.

1. Use a block of modeling clay 2 x 4 x 1 cm thick.

2. Insert short plastic straws (1 cm long) (Fig. 2) aligned in a recommended array of 6 x 8 rows. Smear the surface of the block with a thin layer of jam (an aqueous-based layer that facilitates the separation of the modeling clay from the paraffin wax later on) (Fig. 3).

3. Transfer the block of modeling clay in a metal frame.

4. Pour melted paraffin wax on the mold and leave to cool at 4°C.

5. Remove the modeling clay (easily removable as it is separated from the upper layer of paraffin by the aqueous jam) (Fig. 4).

6. Remove the short plastic straws from the paraffin. This will leave empty holes to receive tissue cores from the donor paraffin blocks (Figs. 5 and 6).

7. To completely integrate the paraffin of the tissue cores into the recipient block, the array block made previously should be heated at 50°C for 10 minutes and flattened by pressing the surface of a hot clean slide against its surface.

Results

A recipient block is made containing circular holes ½ cm in depth and a diameter dependent upon the diameter of the plastic straws used. The advantages of this technique are that it is rapid to perform, cost effective, and creates uniform blocks with aligned cores that are adherent and easy to cut with negligible loss during cutting. The technique permits unrestricted use of the customized block, its distribution, and reproduction of sections after insertion of the tissue cores.

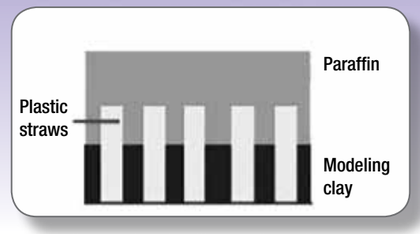


Fig. 1. Diagram showing different layers in constructed recipient block.

Fig. 2. Cut plastic straws into 1-cm lengths to insert in the modeling clay.

Fig. 3. Jam used to smear the surface of the modeling clay and straws.

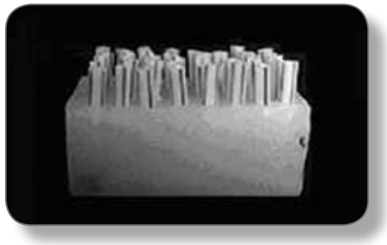


Fig. 4. Short plastic straws inserted into recipient paraffin block (side view).

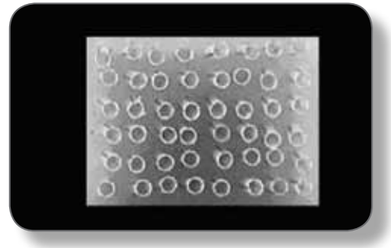


Fig. 5. Recipient paraffin block with short plastic straws inserted (view from above).

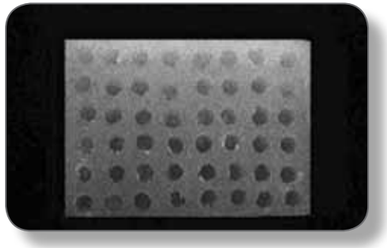


Fig. 6. Recipient paraffin block showing the holes after removal of the plastic straws.

References

1. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med.* 1998;4(7):844-847.

2. van de Rijn M, Gilks CB. Applications of microarrays to histopathology. *Histopathology.* 2004;44(2):97-108.

3. Packeisen J, Buerger H, Krech R, Boecker W. Tissue microarrays: a new approach for quality control in immunohistochemistry. *J Clin Pathol.* 2002;55(8):613-615.

4. Packeisen J, Korsching E, Herbst H, Boecker W, Buerger H. Demystified: tissue microarray technology. *J Clin Pathol.* 2003;56(4):198-204.

5. Deeb S, Badii M, El-Begawey M, Ali K, Mahdy M. Tissue microarrays and their use for preparation of reference slides for educational purposes in histology and histopathology. Paper presented at: 26th African Health Sciences Congress; November 27, 2005; Ismailia, Egypt.

6. Bubendorf L. High-throughput microarray technologies: from genomics to clinics. *Eur Urol.* 2001;40(2):231-238.

7. Fuller CE, Wang H, Zhang W, Fuller GN, Perry A. High-throughput molecular profiling of high-grade astrocytomas: the utility of fluorescence in situ hybridization on tissue microarrays (TMA-FISH). *J Neuropathol Exp Neurol.* 2002;61(12):1078-1084.

8. Mousses S, Kallioniemi A, Kauraniemi P, Elkahloun A, Kallioniemi OP. Clinical and functional target validation using tissue and cell microarrays. *Curr Opin Chem Biol.* 2002;6(1):97-101.

9. Simon R, Sauter G. Tissue microarrays for miniaturized high-throughput molecular profiling of tumors. *Exp Hematol.* 2002;30(12):1365-1372.

10. Gulmann C, O'Grady A. Tissue microarrays: an overview. *Curr Diagn Pathol.* 2003;9:149-154.

11. Pires AR, Andreiuolo FA, de Souza SR. TMA for all: a new method for the construction of tissue microarrays without recipient paraffin block using custom-built needles. *Diagn Pathol.* 2006;1:14.

12. Meng PQ, Hou G, Zhou GY, Peng JP, Dong Q, Zheng S. Application of new tissue microarrayer–ZM-1 without recipient paraffin block. *J Zhejiang Univ Sci B.* 2005;6(9):853-858.

Readers are invited to access **http://www.sakura-americas.com/products/tisstek-quickray.html** for online information about Tissue-Tek® Quick-Ray™, a microarray system that features a preformed paraffin recipient block.

March 10, 2010—A Day for Worldwide Celebration!

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

dellav@musc.edu

Earlier this year, the National Society for Histotechnology (NSH) announced that the first annual ***Histotechnology Professionals Day*** will be celebrated on March 10, 2010, to spotlight the essential contributions to society that are made by histotechnology professionals from around the globe. In a recent interview, NSH president Kerry Crabb stated that "We hope that Histotechnology Professionals Day will become an annual global event, a time when laboratories around the world celebrate the immense contributions that histologists make in a variety of laboratory settings every day. In medicine, accurate diagnosis ultimately rests on the secrets locked within a patient's tissue biopsy and it is only through the skills of the histotechnologist that those secrets become revealed. But the work done by histology professionals is also used to protect the world's food supply, for the development of new drugs, in research to better understand our bodies and disease, to identify how nature is affected by toxins and pollutants, and in forensics to determine the circumstances that contributed to an individual's death."

The discipline has its roots dating back to the 17th century when Robert Hooke, using one of the earliest microscopes, reported in his publication, *Micrographia* (1665),[1] his observations of the many structures he viewed with a magnifying device that he created. While many of his contemporaries had their sights set on the heavens, Hooke, a fellow of the Royal Society of London, became fascinated with the secrets held within the tiniest of structures. He is perhaps most remembered for slicing a piece of cork and examining it with his magnifying lens. There he observed and referred to what he called "cells" within the cork, a term that remains entrenched in microscopy to this day. In its infancy, microtechnique was, of course, not a discipline but rather a series of reported efforts by Hooke and many others to develop, through trial and error, ways to make tissues and other biologic samples more suitable for microscopic observation.

Advances in microscopy were limited at the time by two things: (1) the evolution of man's understanding of lens design and manufacture and (2) techniques for treating samples in such a way that the most minute details would be observable. An accidental discovery by a teenager in 1854 (William Henry Perkin), almost 200 years after Hooke's publication, led to the development of synthetic dyes, which remain essential tools in the modern histology laboratory.[2] A flurry of discoveries over the next 100 years led to the discipline we call histotechnology today.

"It would be impossible to imagine a world without histotechnology, yet the contributions of these laboratory professionals remain unknown to much of the world's population," President Crabb went on to say. "Modern medicine would simply not be what it is today. It would still be using herbs in place of modern day pharmaceuticals to treat disease and our understanding of cancer would be very limited. Without question, histotechnology professionals have contributed to our quality of life in almost unimaginable ways and without them, we would not have the health benefits available today. Through this annual celebration we would like the world to learn of the important work these dedicated professionals perform on our behalf."

"Touching Lives, One Slide at a Time" is the theme for the 2010 Histotechnology Professionals Day celebration. Wherever you may be, this seems like a wonderful opportunity for you to be recognized for the important work you do. The NSH has posted a toolkit at its web site to help you plan your celebration activities. The toolkit is available at no cost to anyone who has an interest in participating in this important celebration. It can be found at **www.nsh.org**. The society also has t-shirts available for sale with the logo that appears at the bottom of this page.

References

1. Hooke R. *Micrographia: Or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses.* Lincolnwood, IL: Science Heritage Ltd; 1987.

2. Bracegirdle B. *A History of Microtechnique.* 2nd ed. Lincolnwood, IL: Science Heritage Ltd; 1987:8-12.

Comparative Microscopic Anatomy of Human and Porcine Liver

Rashmil Saxena, BFA, HT(ASCP)CM;
Mary A. Maluccio, MD; A. Joseph Tector, MD, PhD
Division of Transplantation, Department of Surgery
Indiana University School of Medicine
Indianapolis, IN

rasaxena@iupui.edu

The pig serves as a large animal model for various human diseases. As such, the porcine model has become the most promising source of organs for xenotransplantation. A starting point for evaluating porcine livers with respect to human liver disease and transplantation is recognizing normal morphology of the porcine liver and comparing it with the human liver.

Differences Between Porcine and Human Liver by Hematoxylin and Eosin (H&E) Stain

The most striking difference between porcine and human livers is the clear definition of lobular architecture in the pig liver. The lobules appear as 5- or 6-sided polygons that are outlined by fibrous bands (Fig. 1). The fibrous bands can be seen on both H&E (Fig. 2A) and Masson's trichrome (Fig. 2B) stains. The central vein is at the center and the portal tracts are at the corners of the polygon. In contrast, the lobule in the human liver is not outlined by fibrous bands. Instead there is suggestion of a structure with a central vein and portal tracts at the periphery of what appears to be a hexagon or a pentagon (Fig. 2C, D).

Another difference is that Ito cells can be identified on an H&E stain in porcine liver but not in human liver. These are small cells that reside in the space of Disse (Fig. 3A). They are easily identified by the prominent fat vacuole that pushes the nucleus to one side (Fig. 3B). The function of Ito cells is to store lipids and fat-soluble vitamins, particularly vitamin A.

Similarities Between Porcine and Human Liver by H&E Stain

Hepatocytes and portal tracts have a similar appearance in both human (Fig. 4A) and porcine livers (Fig. 4B) when stained with either H&E or Masson's trichrome stains.

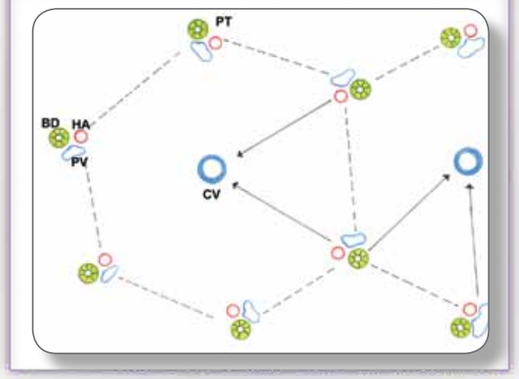


Fig. 1. Graphic showing the general architecture of the hepatic lobule. The hepatic lobule is a 5- or 6-sided polygon that has a central vein (CV) at the center and portal tracts (PT) at the peripheral points of the polygon. The portal tracts contain 3 structures: portal vein (PV), bile duct (BD), and hepatic artery (HA). Blood flows from the portal tracts through the sinusoids to the draining central vein (in the direction of the arrows).

Conclusion

In summary, the two major differences between porcine and human liver seen on routine staining are (1) presence of fibrous bands outlining the hepatic lobule in porcine liver but not seen in human liver and (2) prominent Ito cells in porcine liver but not human liver. Hepatocytes and portal tracts appear similar in both species on routine H&E stain.

Porcine tissues provide medical science with an optimal environment for the study of various human diseases. An understanding of the morphologic and structural differences between pig and human tissues is essential to achieving an accurate understanding of disease progression and treatment response in this animal model and to avoid possible misinterpretation resulting from the microscopic anatomical differences between these species.

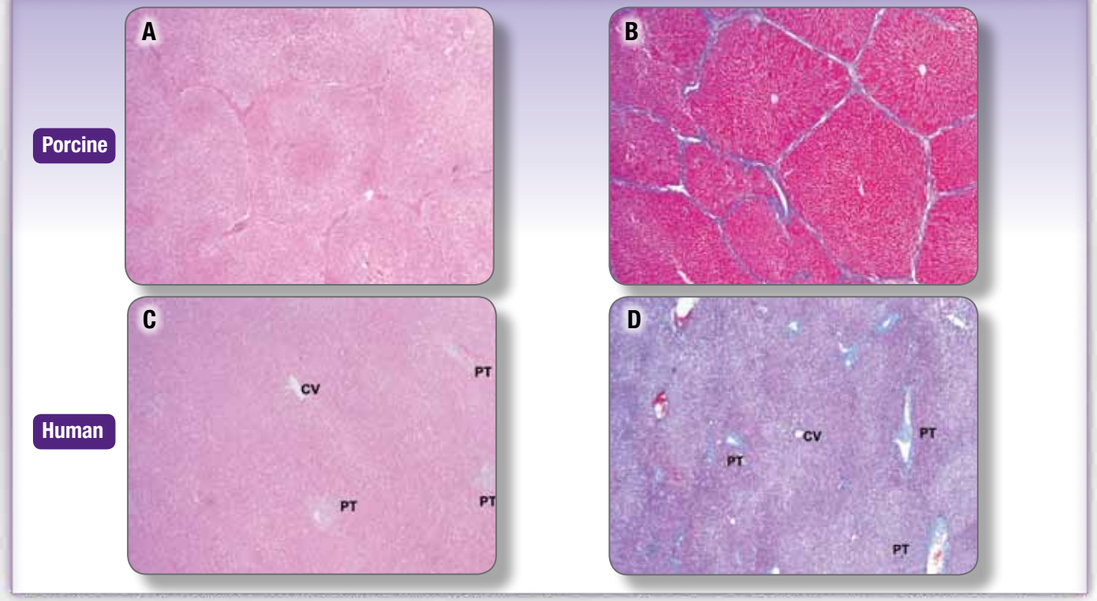


Fig. 2. Differences in comparative anatomy of porcine and human hepatic lobules. Lobules are well demarcated by fibrous tissue in porcine liver but not in human liver. A) Porcine liver, H&E, 20X; B) porcine liver, Masson's trichrome, 20X; C) human liver, H&E, 20X; D) human liver, Masson's trichrome, 20X. In human liver, note the central vein (CV) in the center and the portal tracts (PT) at the points of an imaginary polygon.

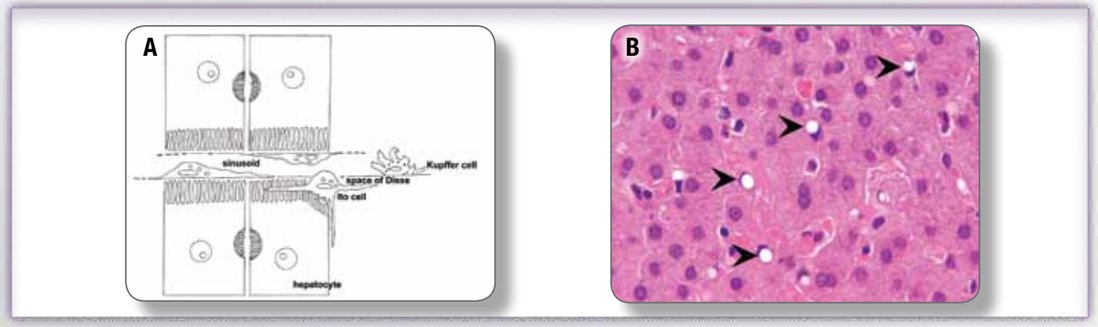


Fig. 3. Ito cells are prominent in porcine liver but not human liver. A) Graphic demonstrating Ito cells in the space of Disse; B) Ito cells in porcine liver, H&E, 200X. Note the fat vacuoles (arrows) that push the nucleus to one side.

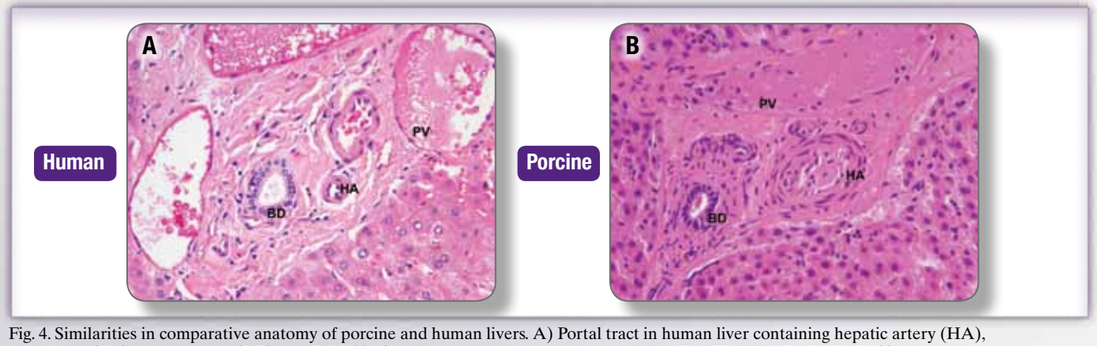


Fig. 4. Similarities in comparative anatomy of porcine and human livers. A) Portal tract in human liver containing hepatic artery (HA), portal vein (PV), and bile duct (BD); H&E, 200X; B) portal tract in porcine liver containing HA, PV, and BD; H&E, 200X.

Histology Lab Safety: Frequently Asked Questions

Jason Burrill, HT(ASCP)SLS
Manager, Global Environment Health and Safety
Charles River Laboratories
Wilmington, MA

Jason.Burrill@crl.com

Like most of you, I have spent a great deal of time in my histology career struggling with interpreting and applying government and regulatory standards that apply to the histology lab. In the text that follows, I have shared the questions most frequently asked of me and the relevant safety standards that apply.

The answers I've provided are based on the federal Occupational Safety & Health Administration (OSHA) and Environmental Protection Agency (EPA) regulations; the applicable Code of Federal Regulations (CFR) reference has been listed after most answers. Where appropriate, I have listed my recommendations for establishing best practices. Always remember that safety and compliance have to be balanced to ensure program success.

Chemical Hygiene Plan

Does my facility need a hard copy of the Chemical Hygiene Plan or can we maintain only an electronic version?

According to Occupational Exposure to Hazardous Chemicals in Laboratories, the Chemical Hygiene Plan must be readily available to employees and employee representatives.
29 CFR 1910.1450(e)(2)

Therefore, if all employees have access to a computer and know where this information can be found, you are in compliance. Since computers and networks are not always reliable, it is advisable to maintain a hard copy in the lab for quick reference, if needed.

Can I wear contact lenses in the lab?

OSHA standard General Requirement for Personal Protective Equipment (PPE) requires employers to assess the workplace to determine if hazards are present, or are likely to be present, which necessitate the use of PPE.
29 CFR 1910.132(d)(1)

If during the hazard assessment it is determined that wearing contact lenses would expose the employee to additional risk, then the employer has the responsibility of developing policies to prohibit the use of contact lenses in the lab.

Can my lab use web-based or video training for our initial and annual safety training?

This depends on what type of safety training it is. Web-based training is appropriate for the following types of safety training:

- EPA Resource Conservation and Recovery Act (Hazardous Waste)
- DOT Hazardous Materials/IATA Dangerous Goods (Shipping) 49 CFR 172

Most OSHA standards require employee training to be based on hazards encountered in an employee's specific job functions and, because the circumstances in labs may differ, it would be hard to justify that generic web-based training meets the required training curriculum. It is also required that an employee have the opportunity to ask questions during training. Training required by the Chemical Hygiene Plan must be specific for the hazards encountered in the employee's lab as this document/training is performance based, which means that policies, procedures, and training should be specific to the individual lab's hazards.

Is my employer required to monitor for a chemical if I think I have been overexposed?

According to Occupational Exposure to Hazardous Chemicals in Laboratories, if you are developing signs and symptoms of overexposure (consult the chemical material safety data sheet [MSDS] for this information), then your employer is required to provide you with the opportunity to receive a medical examination and must perform exposure monitoring if possible for the job tasks in question.
29 CFR 1910.1450(g)(1)

The method for this monitoring may vary based on the substance but typically there are several different techniques available to perform vapor exposure monitoring (passive air monitoring badges, Draeger-Tubes, etc).

Do I have to keep all the MSDSs that are sent to me (I have several for the same reagent)?

Occupational Exposure to Hazardous Chemicals in Laboratories requires employees to maintain all MSDSs that are sent to you by the manufacturer.
29 CFR 1910.1450 (h)(1)(ii)

With this said, you are only required to keep one copy of each of these MSDSs. If the chemical manufacturer sends an MSDS with each shipment, compare it against the one in your existing file. If it has the same revision date and no other information has changed, you are not required to keep it.

Formaldehyde

Do I need to have a written Formaldehyde Control Plan?

According to the formaldehyde standard, an employer must develop, implement, and maintain at the workplace a written hazard communication program for formaldehyde exposures in the workplace, which at a minimum describes container labeling, other forms of warning, material safety data sheets, and employee information and training.
29 CFR 1910.1048(m)(5)

How often do I have to perform formaldehyde exposure monitoring?

Exposure monitoring must be performed unless the employer documents, using objective data, that the presence of formaldehyde or formaldehyde-releasing products in the workplace cannot result in airborne concentrations of formaldehyde that would cause any employee to be exposed at or above the action level or the short-term exposure limit (STEL) under foreseeable conditions of use. If this can be demonstrated, the employer will not be required to measure employee exposure to formaldehyde.
29 CFR 1910.1048(d)(1)(ii)

Exposure monitoring is to be done for both 8-hour time-weighted average (TWA) and STEL. A representative sample for each job classification for each work shift must be done unless objective data show that each work shift has the same exposure level. 29 CFR 1910.1048(d)(1)(iii)&(iv)

Exposure monitoring must be done initially and must be repeated each time there is a change in production, equipment, process, personnel, or control measures that may result in new or additional exposure to formaldehyde. Also, if the employer receives reports of signs or symptoms of respiratory or dermal conditions associated with formaldehyde exposure, the employer shall promptly monitor the affected employee's exposure.
29 CFR 1910.1048(d)(2)(ii)&(iii)

Exposure monitoring may be discontinued for employees if results from 2 consecutive sampling periods taken at least 7 days apart show that employee exposure is below the action level and the STEL. The results must be statistically representative and consistent with the employer's knowledge of the job and work operation.
29 CFR 1910.1048(d)(4)

Most histology labs choose to perform exposure monitoring annually, at a minimum.

What personal protective equipment (PPE) is required when working with formaldehyde-based solutions (10% formalin)?

All contact of the eyes and skin with liquids containing 1% or more formaldehyde must be prevented by the use of

chemical protective clothing made of material impervious to formaldehyde and the use of other personal protective equipment, such as goggles and face shields, as appropriate to the operation.
29 CFR 1910.1048(h)(1)(i)

A job hazard analysis should be performed to determine appropriate engineering controls, work practices, and PPE to be used when working with formaldehyde solutions.

Emergency Equipment

Are eyewash bottles an acceptable alternative to plumbed eyewash stations?

OSHA standard Medical Services and First Aid requires that suitable facilities for quick drenching or flushing of the eyes and body be provided within the work area for immediate emergency use where the eyes or body of any person may be exposed to injurious corrosive materials.
29 CFR 1910.151(c)

OSHA may issue a citation based on having only eyewash bottles and no plumbed eyewash stations available in areas where corrosive materials are used. The reason for this potential citation is based on the eyewash bottles being unable to deliver 15 minutes of continuous flushing, which is typically required for first aid measures in most hazardous chemical MSDSs.

How often do plumbed eyewash stations need to be flushed?

OSHA does not enforce a specific frequency for plumbed eyewash station flushing. At a minimum, they should be flushed periodically; I would suggest monthly, but weekly would be a best practice.

Hazardous Waste

How do I know if waste is hazardous?

The federal EPA defines waste as hazardous if it has one of the following properties:

- Listed (F, K, P, U)
- Ignitability (liquid with flashpoint <140ºF or solid that readily sustains combustion)
- Corrosive (liquid with a pH <2 or >12.5)
- Reactive (reacts violently with air or water or forms toxic gases when exposed to neutral conditions)
- Toxic (one of 40 metals, solvents, or pesticides over a certain concentration)

State variations do occur, so state regulations should also be referenced in determining whether or not a chemical waste is hazardous. An additional resource is the waste vendor that picks up your waste. They should be able to help you properly classify the waste based on the constituents.

Where can I store hazardous waste in the lab?

Most hazardous waste stored in the lab is in a location called a satellite accumulation area (SAA). An SAA is an area at or near the source that generates the waste. This is where drums and other containers are stored while being filled until they are either transferred to a central accumulation area or picked up by the waste vendor for offsite treatment or disposal.
40 CFR 262.34(c)(1)

What information has to be on the label of the hazardous waste container in the satellite accumulation area?

The EPA only requires labels to list the container contents and the words "Hazardous Waste" or "with other words that identify the contents of the containers."
40 CFR 262.34(c)(1)(ii)

Many states and institutions require that the specific contents, actual words "Hazardous Waste," and the associated hazards (eg, ignitable, corrosive, reactive, or toxic) be listed on the container label.

After how many days must I remove a hazardous waste container from a satellite accumulation area after the container is full?

Variations can occur state by state, but the federal EPA requires the hazardous waste to be removed within 3 days if the 55 gallons of hazardous waste or 1 quart of acutely hazardous waste is exceeded.
40 CFR 262.34(c)(2)

A best practice would be to remove a full container as soon as possible to avoid any regulatory risk.

Conclusion

Navigating the regulatory maze of OSHA safety standards can be very challenging to laboratory practitioners. Even if one has access to the text of the specific standards, interpretation of the stated requirements is not always intuitive. Noncompliance may not only lead to severe penalties, but may also result in conditions that leave staff vulnerable to injury or health issues. When in doubt, it is best to consult with a safety expert who can guide you to address your particular circumstances. If such an individual is not available to you, questions may be addressed directly to the Occupational Safety & Health Administration by calling 1-800-321-OSHA (6742) or via email through their web site at **www.osha.gov**. There is no penalty for laboratories seeking advice from OSHA.

Proper Hand Hygiene Is the Key to Safeguarding the Nation's Lab Practitioners

Susan Della Speranza, RN, BSN
Infection Control Practitioner
Summerville Medical Center
Summerville, South Carolina

Susan.dellasperanza@hcahealthcare.com

Health authorities are cautioning the public to remain vigilant with the worldwide pandemic stemming from the H1N1 influenza virus, more commonly referred to as swine flu. The term "swine flu" is derived from the observation that the H1N1 virus contains genetic material similar to influenza viruses known to infect swine herds in North America and Europe. Although there have been a number of fatalities in the United States, the virus has thus far been less virulent than initially predicted, at least in some age groups. However, hospitalizations continue to rise as a vaccine that was slow to become available remains in short supply.

The Centers for Disease Control and Prevention (CDC) defines the start of flu season as week 40 of a calendar year, which is equivalent to early October. However, H1N1 influenza cases began appearing in the summer of 2009. The number of outpatient clinic visits by individuals reporting flu-like symptoms was double that for the national baseline for seasonal flu and, as of week 46, 32 states had reported widespread influenza activity (Fig. 1).[1,2]

As of this writing, there have been over 1 million confirmed cases of H1N1 illness worldwide with approximately 13,000 confirmed deaths.[3] Unconfirmed cases could easily be twice these numbers.

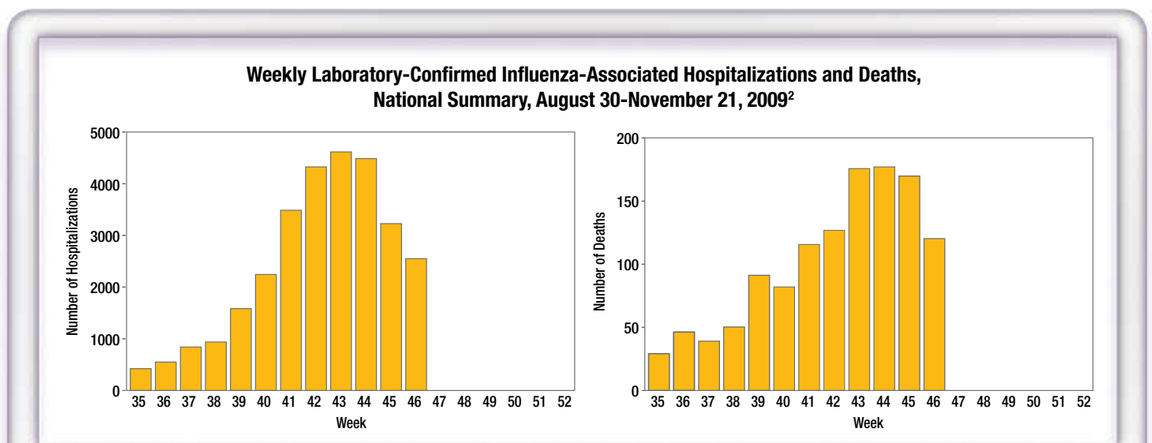


Fig. 1.

I began my healthcare career as a laboratory technician with the US Army so I know only too well how essential a role our nation's laboratory practitioners play in the diagnosis and treatment of disease. Keeping our nation's laboratory professionals healthy will be essential in our effort to stay ahead of this serious illness.

By all means, obtain the seasonal and H1N1 flu vaccines when they become available to you. This is your best defense. While those receiving the vaccines are believed to be protected, it is still possible for a vaccinated individual to be a vector for spreading the virus to others. Whether you are vaccinated or not, practicing good respiratory etiquette is key to limiting the spread of virus to others by droplet infection. A sneeze or cough can spread infectious particles over a fairly wide area (Fig. 2).

Covering the mouth or nose when sneezing or coughing, preferably with a disposable facial tissue, is not only the polite thing to do, but also an essential respiratory infection prevention tool. While tradition dictates that a gentleman should always have a clean handkerchief available, avoid reusing contaminated handkerchiefs. Disposables are really the way to go. Reusing a handkerchief over and over recontaminates the fingers, which can contaminate objects in public areas, such as door handles.

Some advocate covering the nose or mouth with the inside of the elbow to protect those around you when facial tissues are not immediately available or when a sneeze or cough sneaks up on you (Fig. 3). However, this can be a source of recontamination of the hands because it is quite common for individuals to fold their arms during conversation with others, touching potentially contaminated areas of clothing (Fig. 4). This practice should be used as a last resort and not as your first defense strategy.

Mothers all over the world are frequently overheard admonishing their children to "wash your hands." It turns out that these mothers are really on to something. Hand washing is the single most effective way to reduce the spread of germs that cause respiratory disease.[4] Of course, frequent and effective hand washing is good

Fig. 2. A sneeze or cough can produce a cloud of infectious particles that may spread over a wide area.

routine practice for anyone working in a healthcare environment, especially in the laboratory. Do you know how to wash your hands properly? It may seem silly to ask about something you perform so regularly. The CDC recommends that you wash your hands with soap and water for 20 seconds. That's a fairly long time. When soap and water are not available, alcohol-based disposable hand wipes or gel sanitizers may be used,[1] although hand wipes are not as effective as gel sanitizers. If your hands are visibly soiled, you must use soap and water to clean them adequately.

We are finding that individuals using hand sanitizers tend to rub their palms together but focus little attention on the fingers, especially the fingertips, which are the parts most likely to come into contact with the nose, mouth, and eyes. Viruses love mucous membranes and transfer from contaminated fingertips should not be discounted as a possible source of transmission.

We already have most of the tools we need to stay healthy during flu season. With greater awareness of how to perform hygiene tasks, we can keep you working on the front lines where we need you most.

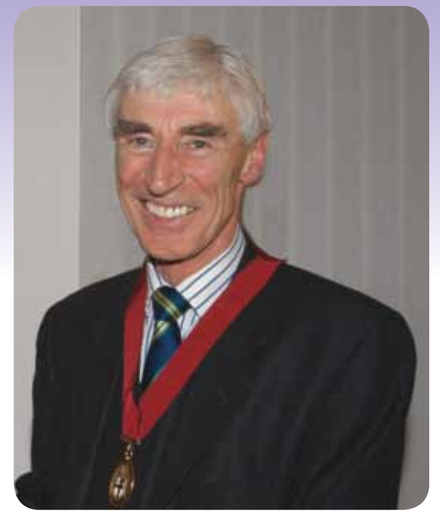
References

1. 2009 H1N1 flu ("swine flu") and you. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/H1N1flu/ga.htm. Accessed January 13, 2010.
2. Seasonal flu. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/flu/weekly/. Accessed January 13, 2010.
3. flucount.org Web site. http://www.flucount.org/. Accessed January 13, 2010.
4. Cough etiquette and respiratory hygiene. Australian Government Department of Health and Aging Web site. http://www.flupandemic.gov.au/internet/panflu/publishing.nsf/Content/infection-control-posters-1/$FILE/cough_etiquette.pdf. Accessed January 13, 2010.

Fig. 3. Sneezing into the inside of your elbow should be used only when a facial tissue is not available.

Fig. 4. Folding your arms can contaminate the hands if one has used the inside of the elbow to cover a cough or sneeze.

Russell Thomas Allison



The 100-year history of this Institute is decorated with the names of those who repeatedly gave more back to the profession than they received. Given the length of time involved, this list is now quite long, but, given the number of members who have come and gone, in reality it is quite short.

One of those names is Russell Thomas Allison, known universally as 'Russ'. There are few people in life who are recognisable by only one name, but Russ came close to achieving that distinction within biomedical science. It was with great sadness that Council noted recently the untimely death of Russ Allison. Russ was President of the Institute from 2002 to 2004 and during this period oversaw many of the changes that resulted in the reinstatement of our professional examinations. However, his main contribution now affects the professional life of all our members (including the President and Chief Executive!). It was due to Russ' foresight more than 20 years ago that the Institute became one of the first in the UK to introduce a Continuing Professional Development scheme.

Men of vision and commitment are not particularly common, but Russ ticked both boxes. His work at the University of Wales College of Medicine in Cardiff included developing practical facilities, supervising the training of undergraduates, and publishing research into histological methodology – a previously neglected area. In fact, his publications are legion. This led to invitations to lecture at various levels, from degree courses to international specialist meetings around the world. He was a senior research fellow on the academic staff of the University of Wales College of Medicine Dental School.

Russ joined the Institute in 1959 and quickly became a stalwart of his local IBMS branch committee (Cardiff), which he joined in 1963. He was at various times chairman, secretary and vice chairman of the branch, and was a member of the South West region committee for 36 years. It was as a regional member that he was elected to Council in 1984 and he served continuously in this capacity until 2005. His main interest and passion was for science and he will remain the Institute's longest-serving chair of the Science Committee. He served on the editorial board of the *British Journal of Biomedical Science*, a publication that he supported passionately and to which he contributed significantly during its development.

Russ had an easy-going manner, which made him many friends, and it was his ability to network that made him a key figure in the development of the IBMS Congress programme. Those who knew him personally will be aware of his passion for Welsh rugby, and the local amateur team that he championed was a major part of his life. Those who knew him really well will also have seen a softer side. He loved animals of all kind, but in particular dogs, and his daily 'walkies' were part of a routine that he loved.

Like others, I counted Russ as one of my friends, but within this profession there are many more who owe him thanks. The life of Russell Thomas Allison, within his chosen profession, really did make a difference. ■

Alan R Potter
Chief Executive

This obituary first appeared in *The Biomedical Scientist* (2009;[11]:911) and is reproduced here by kind permission of the Institute of Biomedical Science.

✔ Mark Your Calendar!
Educational Opportunities in 2010

JANUARY

15 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Cancer and Angiogenesis: The Role of Histology in Drug Discovery**
Speaker: Anne C. Lewin, HT(ASCP), QIHC(ASCP)
Department of Oncology Drug Discovery
Bristol-Meyers Squibb
Princeton, NJ

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Heat Induced Epitope Retrieval: A Review of Methods, Reagents and Devices**
Speaker: Joseph D. Myers, MS, CT(ASCP)
Biocare Medical
Clearwater, FL
Phone: (443) 535-4060
Email: histo@nsh.org

FEBRUARY

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Brain Tumor Histology**
Speaker: Jennifer L. Hofecker, HT(ASCP)
Department of Neuropathology
Vanderbilt University Medical Center
Nashville, TN

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Managing Stress in Times of Change**
Speaker: Elaine Tarassian, MS
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060
Email: histo@nsh.org

MARCH

4-6 **Indiana Society for Histotechnology**
Contact: Larry Fields
Phone: (317) 894-5359
Email: lfields@ameripath.com

10 **Histotechnology Professionals Day**
First annual *worldwide* commemoration of Histotechnology

13-14 **Mississippi Society of Histotechnology**
Site: Beau-Rivage Casino-Hotel
Biloxi, MS
Contact: Jerry Santiago, Region III Director
Phone: (904) 244-6149
Email: jerry.santiago@jax.ufl.edu

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Dermatopathology for the Histologist**
Speaker: Clifford M. Chapman, MS, HTL(ASCP), QIHC
Caris Cohen Dx
Wakefield, MA

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Getting Started with IHC**
Speaker: Jennifer Freeland, HTL(ASCP)
ThermoFisher Scientific
Kalamazoo, MI
Phone: (443) 535-4060
Email: histo@nsh.org

26-28 **Georgia Society for Histotechnology**
Site: Evergreen Marriott® Conference Resort
Stone Mountain, GA
Contact: Mike Ayers
Email: mike.ayers@piedmontnewnan.org

APRIL

10 **Nebraska Society for Histotechnology**
Spring Symposium
Contact: Janice Mahoney
Email: jmahoney@alegent.com

16 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Tagged Antibodies in Scanned Histology Slides**
Speaker: Dean Troyer, MD, Adjunct Professor
Department of Pathology
University of Texas Health Science Center
San Antonio, TX

22-24 **Region III Meeting**
Site: Doubletree Resort
Orlando, FL
Contact: Sue Clark
Phone: (954) 265-5371
Email: sclark@fshgroup.org

22-25 **Texas Society for Histotechnology**
Site: Omni Westside
Houston, TX
Contact: Veronica Davis or Kathy Dwyer
Phone: (972) 579-8291
Email: veronida@baylorhealth.edu
kdwyer@questdiagnostics.com

23-24 **Colorado Society for Histotechnology**
Site: YMCA of the Rockies
Estes Park, CO
Contact: Stacey Langenberg
Email: stacey.langenberg@comcast.net

23-24 **Histology Society of Ohio**
Site: Holiday Inn
Dayton/Fairborn, OH
Contact: Amy Aulthouse
Email: a-aulthouse@onu.edu

23-24 **Region I Symposium**
Site: Hilton Hotel
Mystic, CT
Contact: Nancy Troiano
Phone: (203) 785-5136
Email: nancy.troiano@yale.edu

24 **Washington State Histology Society**
Contact: Martha Pope
Email: popem@battelle.org

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Principles of Fixation and Staining in Cytopreparation**
Speaker: Beth Cox, SCT/HTL(ASCP)
Histology and Cytology Consultant
Branch, MI
Phone: (443) 535-4060
Email: histo@nsh.org

28-30 **Tri-State Meeting (Wisconsin, Minnesota, Iowa)**
Site: Des Moines, IA
Contact: Dave Cavanaugh
Email: dcavanau@iastate.edu

MAY

8 **Massachusetts Society for Histotechnology**
Site: Larz Anderson Museum
Chestnut Hill, MA
Contact: Jim Pepoon
Email: jpepoon@partners.org

14-16 **California Society for Histotechnology**
Site: Riverside, California
Contact: Lydia Figueroa
Email: lefigueroa90501@yahoo.com

14-15 **New York State Histotechnological Society**
Site: Ramada Hotel & Conference Center
Buffalo, NY
Contact: Mary Georger
Email: mary.georger@rochester.edu

15-16 **Michigan Society for Histotechnologists**
Site: Radisson Hotel
Lansing, Michigan
Contact: Paula Bober
Phone: (586) 596-0311
Email: p_bober@comcast.net

✔ Mark Your Calendar!
Educational Opportunities in 2010

MAY (Cont.)

21 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **HER2/neu and Breast Cancer**
Speaker: Prashant Jani, MD, FRCP(C)
Thunder Bay Regional Health Science Center
Northern Ontario School of Medicine
Thunder Bay, Ontario, Canada

26 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Troubleshooting H&E, Before, During and After**
Speaker: Peggy A. Wenk, HTL(ASCP)SLS
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060
Email: histo@nsh.org

JUNE

4-6 **Region VII Meeting**
Site: Phoenix, AZ
Contact: Andrea Grantham
Email: algranth@u.arizona.edu

10-12 **Region II Meeting**
Site: Atlantic City, NJ
Contact: Carole Barone
Email: cbarone@nemours.org

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Competency and Process Improvement in Paraffin Embedding**
Speaker: Joelle Weaver, BA, HTL(ASCP)
Columbus State Community College
Columbus, OH

23 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Basics of FISH/CISH for the Histotech**
Speaker: Traci DeGeer, HTL(ASCP)HT, QIHC
Ventana Medical Systems
Tucson, AZ
Phone: (443) 535-4060
Email: histo@nsh.org

JULY

16 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Hematoxylin and Eosin: The Most Common Special Stain**
Speaker: Elizabeth Sheppard, MBA, HT(ASCP)
Ventana Medical Systems, Inc.
Tucson, AZ

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Job Assessment Including Best Practices for the Histology Laboratory**
Speaker: Jason D. Burrill, HT(ASCP)SLS
Charles River Laboratories
Wilmington, MA
Phone: (443) 535-4060
Email: histo@nsh.org

AUGUST

20 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Innovations in Histology**
Speaker: Azorides R. Morales, MD
Department of Pathology
University of Miami
Miller School of Medicine
Miami, FL

25 **NSH Teleconference 1:00 pm Eastern Time**
Title: **The Association of Cancer and Infectious Agents**
Speakers: Sheron C. Lear, HTL(ASCP)HT, QIHC and
Alvin W. Martin, MD
CPA Laboratory
Louisville, KY
Phone: (443) 535-4060
Email: histo@nsh.org

SEPTEMBER

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **The Paradox of Change in Histotechnology**
Speaker: Herbert Skip Brown IV, MD, HT(ASCP)
Clinical Applications Manager
Leica BioSystems LLC
St. Louis, MO

18 **Nebraska Society for Histotechnology**
Autumn Symposium
Contact: Janice Mahoney
Email: jmahoney@alegent.com

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

24-29 **National Society for Histotechnology Symposium/Convention**
Site: Seattle, Washington
Contact: Aubrey Wanner, NSH Office
Phone: (443) 535-4060
Fax: (443) 535-4055
Email: aubrey@nsh.org

OCTOBER

15 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Carbohydrate Histochemistry**
Speaker: John A. Kiernan, MB, ChB, PhD, DSc
Department of Anatomy and Cell Biology
The University of Western Ontario
London, Ontario, Canada

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Connective Tissue Special Stains**
Speaker: Kimberly Feaster, HTL(ASCP), QIHC
West Virginia University
Morgantown, WV
Phone: (443) 535-4060
Email: histo@nsh.org

NOVEMBER

17 **NSH Teleconference 1:00 pm Eastern Time**
Title: **From LEAN to Green: A Partnership for Making an Environmentally Friendly Histology Laboratory**
Speaker: Carole Barone, HT(ASCP)
Nemours-A.I. Dupont Hospital for Children
Wilmington, DE
Phone: (443) 535-4060
Email: histo@nsh.org

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Understanding Fixation**
Speaker: Ada Feldman, MS, HTL(ASCP), HT
Anatech Ltd
Battle Creek, MI

DECEMBER

15 **NSH Teleconference 1:00 pm Eastern Time**
Title: **TB or not TB: That Is the AFB Question**
Speaker: Joshua Fink, HTL(ASCP)
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060
Email: histo@nsh.org

Sakura Finetek U.S.A., Inc.
1750 West 214th Street
Torrance, CA 90501

No varnish in this area.

HISTOLOGIC®

Technical Bulletin for Histotechnology

To receive your own copy of ***HistoLogic,***® or to have someone added to the mailing list, submit home address to: Sakura Finetek U.S.A., Inc., 1750 West 214th Street, Torrance, CA 90501.

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