



# The Kentucky Society for Histotechnology

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## *Slice of Life*

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### Special points of interest:

- Immunocytochemistry 2: JA Kiernan
- KSH March Symposium
- Teleconferences
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### Preservation and retrieval of antigens for immunohistochemistry – methods and mechanisms. 2. Retrieving masked antigens.

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The mechanisms of fixation by formaldehyde were described in a previous article (*The Cutting Edge*, January 2005, pp. 5-9), and it was noted that the cross-linking of protein molecules can render the epitopes (antibody-binding regions) of antigens inaccessible to large molecules, preventing detection by immunohistochemical methods. The lipoprotein membranes that enclose cells and organelles constitute another barrier to penetration of tissue by large molecules, especially when processing has not involved passage through organic solvents.

Despite having been available for many years (Coons et al, 1942) immunostaining was still a “new” method in the 1960s and 1970s. The deleterious effects of fixation on enzymatic activity were well known, and it was widely assumed that fixation also destroyed the chemical basis of antigenicity. Cryostat sections of unfixed or minimally fixed tissue were commonly used for immunohistochemistry (eg Nairn, 1976). Some fixatives, including alcohol-based mixtures, Bouin’s fluid and periodate-lysine-formaldehyde (McLean & Nakane, 1974), were said to “preserve” the antigenicity of certain peptides and proteins. Since about 1980 it has been recognized that distortion of macromolecular architecture by a fixative may either expose or conceal epitopes. Although antigens respond differently to fixatives it is generally true to say that epitopes are exposed by coagulation of proteins and masked by cross-linking. In terms of making antigens accessible to immunoreagents, neutral buffered formaldehyde was the worst of seven fixatives compared by Arnold et al (1996).

Nevertheless, neutral formaldehyde is most frequently used fixative. Fortunately there are several ways to improve the access of antibodies to tissue antigens that have been masked by formaldehyde fixation.

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**Be sure to attend the 2006 Annual Kentucky Society for Histotechnology Symposium. March 31-April 1 at the Louisville Marriot East. To secure symposium rates of \$79.00 you must contact the hotel directly at 800-627-7468. Indicate you are with the KSH. Information regarding the program on-line at [www.kyhistotechs.com](http://www.kyhistotechs.com) or call Lena Spencer at 502-629-7815.**

## President Ponders \* Lena Spencer

I hope everyone had a blessed and peaceful holiday season. It is difficult to believe that we are at the start of another New Year. I have decided that instead of New Year's Resolutions, I will make New Year's promises. My promise to the KSH and its! membership is to keep the lines of communication open so that we will have a flow of information within the Society.



The "Slice of Life", website and our bimonthly meetings are the best sources for information sharing. When the KSH receives information from the National Society for Histotechnology or from regional histology organizations we post the information on our website, [www.kyhistotechs.com](http://www.kyhistotechs.com). Please take time to visit this site, Cynthia Long has worked very hard to keep it up to date, providing valuable links to other histology professionals.

We are quickly finalizing the program for the KSH Symposium, to be held March 31-April 1st, in Louisville. We have chosen many diverse topics: immunohistochemistry, how water can present problems to most staining techniques, handling stress, various disease processes, looking at future trends and a humorous look at our lifestyles and how life has changed throughout the years.

We look forward to seeing all of you at the Annual Symposium, but we would like to see you at our bimonthly meetings. By attending the bimonthly meetings you will hear some very interesting lectures on relevant histology topics, receive CE's (1 hour), network with fellow histotechs, and participate in the business of the KSH. We would love to see you at one of these meetings. We usually rotate between Lexington and Louisville, so that the trip is not always too long for a particular group.

Again, I look forward to seeing each of you. If you have questions or concerns please call or e-mail me, I would love to talk with you.

May your New Year be wonderful! .

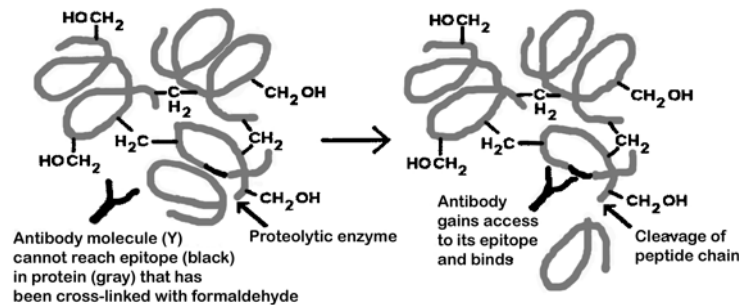
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### Proteolytic enzymes.

Probably the earliest way of unmasking formaldehyde-fixed antigens was to incubate the preparation, before exposing to the primary antibody, in a solution of a proteolytic enzyme (see Bullock & Petrusz, 1982). Usually an inexpensive grade of porcine trypsin (containing some chymotrypsin) is used at a concentration of 0.1% in 0.0.1% aqueous  $\text{CaCl}_2$ , adjusted to pH 7.8 with TRIS or a few drops of 0.1M NaOH. An optimum incubation (in the range 10 to 60 minutes, at room temperature or  $37^\circ\text{C}$ ) must be found for each tissue and antigen. The rationale of using a proteolytic enzyme is that breaking some peptide bonds will make holes in the matrix of cross-linked proteins, allowing the entry of antibody molecules (Fig. 1). Enzymes other than trypsin have been used in much the same way, including pronase and pepsin (Hume & Keat, 1990). Endogenous proteolytic enzymes, released from cells damaged by the microtome knife, have been shown by Mori et al (1992) to unmask certain epitopes of extracellular proteins.



**Fig. 1.** Unmasking of an epitope by the action of a proteolytic enzyme.

The chief disadvantage of proteolytic enzymes is their propensity to digest the tissue, including the antigen one is attempting to demonstrate. A duration of exposure to the enzyme solution must be found that is just right for the job at hand (see Hayat, 2002). This requirement makes enzymatic treatment rather too labour-intensive for routine use.

### Heat induced antigen retrieval.

Most of the formaldehyde bound to a fixed tissue can be removed by prolonged washing (two to three weeks) in cold water (Helander, 1994; see also my previous article in *The Cutting Edge*). One may reasonably suspect that removal of formaldehyde would be accomplished more rapidly at high temperatures. Water alone, however, is seldom used for heat induced antigen retrieval. Other substances are nearly always dissolved in the water, and the reasons for trying the various solutes are not explained by the authors of most publications in this field. The first hot solutions to be used (Shi et al., 1991) contained either zinc sulfate or lead thiocyanate. The slides, bearing hydrated sections, were brought to  $100^\circ\text{C}$  in these solutions. The sensitivity of immunohistochemical staining was usefully increased for most of the 52 antibodies tested, and lead thiocyanate was generally more effective than zinc sulfate.

Subsequent studies of antigen retrieval in the 1990s focused especially on the pH of the hot water, and the type and duration of heating. The general consensus is that for most antigens pH 6 (nearly always obtained with a citrate buffer) is suitable. It is also generally agreed that a minority of antigens require either more acidic (pH 1) or more alkaline (pH 9) retrieval solutions. The source of heat may be a microwave oven, a boiling water bath or an autoclave. Some have argued that microwave heating may do more than simply raise the temperature, but the arguments are not convincing (see Hayat, 2002 for references and discussion). Effects of temperature have also been examined. It is evident that higher temperatures permit shorter times in an antigen retrieval solution. An overnight immersion in citrate buffer (pH 6) at  $80^\circ\text{C}$  is as effective as immersion for less than one hour in the same solution at  $100^\circ\text{C}$  (Koopal et al., 1998). With autoclaving (about  $120^\circ\text{C}$ ), antigen retrieval is accomplished in about 10 minutes (Bankfalvi et al., 1994; Hunt et al., 1996), but much additional time is taken up waiting for the autoclave to cool without releasing the pressure. The usual procedure of decompression followed by closing the air intake valve causes boiling of the water in the jar containing the slides and detachment of all

the sections (Kiernan – unpublished observation that should have been anticipated). A domestic pressure cooker is better suited to antigen retrieval than an institutional autoclave (Pileri et al., 1997). After 10 minutes at full steam remove it from the source of heat but do not release the pressure valve or cool the outside of the pressure cooker with cold water.

**Ingredients of antigen retrieval solutions**

No-one doubts the importance of pH (Shi et al., 1995; Boon, 1996), but ingredients other than buffer salts can also contribute to the efficacy of solutions for antigen retrieval. Before the advent of methods involving heating, techniques to improve immunostaining included treating sections at room temperature with 5M urea (Hausen & Dreyer, 1982) or with detergents (see Feldmann et al., 1983). Table 1 shows some of the substances that have been included in solutions for heat induced antigen retrieval.

**Table 1.** Possible functions of substances other than water in some solutions used for heat induced antigen retrieval. The formulations are listed in order of date of publication. The references should be consulted for exact details of composition of the solutions.

Solute	Possible function	Reference for formulation	Comments
Zinc sulfate [ZnSO <sub>4</sub> ], 1%	Protein coagulant cation	Shi et al., 1991, 1992	Shi et al. (1991) examined retrieval of 52 antigens. ZnSO <sub>4</sub> was generally less effective than Pb(SCN) <sub>2</sub>
Lead thiocyanate [Pb (SCN) <sub>2</sub> ], 1%	Protein coagulant cation with chaotropic anion	Shi et al., 1991; Takahashi et al., 1993	Takahashi et al. found that heating with Pb (SCN) <sub>2</sub> improved immunostaining of Bouin-fixed tissue. Methacarn (a non-aqueous fixative that does not contain formaldehyde) pro-
Citrate buffer, 0.1M, pH 6	pH control; chelation of Ca <sup>2+</sup>	Shi et al., 1993, 1994	Generally more effective than Pb(SCN) <sub>2</sub>
Urea, 0.8M (5%)	Non-ionic chaotrope	Shi et al., 1994	Concentration lower than those used by other investigators
Glycine-HCl buffer, pH 3.5	Acidic medium	Shi et al., 1994	Less background immunostaining than after 0.8M urea
Aluminum chloride <sub>3</sub> , 4% (pH2.5) or HCl, 0.1M (pH 1.0)	Acidic protein coagulants	Evers & Uylings, 1994	Vibratome sections of brain that had been in formaldehyde for more than a year.
Citrate buffers, pH 2.5, 4.5, 6.0	pH control; chelation of Ca <sup>2+</sup> (at higher pH)	Evers & Uylings, 1994	Vibratome sections of brain that had been in formaldehyde for more than a year.
Citrate buffer, 0.1M, pH 6.0	pH control; chelation of Ca <sup>2+</sup>	Beckstead, 1994	Applicable to most of the antigens tested

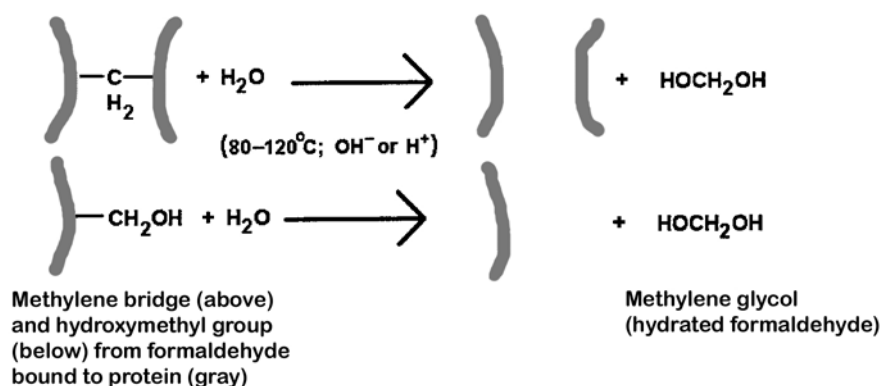
Urea, 3M (18%)	Chaotrope	Beckstead, 1994	Applicable to most of the antigens tested
Tris buffer, 0.05M, pH 10	Alkaline medium	Beckstead, 1994	Retrieved some antigens that could not be immunostained after pH 6 buffer or 3M urea
No solutes	Distilled water	Umemura et al., 1995	More effective than buffers for autoclave retrieval of Bcl-2 protein
EDTA, 0.001M, pH 8	Chelation of Ca <sup>2+</sup> ; pH control	Balaton et al., 1995	1.5 minutes in pressure cooker
Citrate buffer, 0.01M, pH 6	pH control; chelation of Ca <sup>2+</sup>	Man & Tavassoli, 1996	70-80°C (oven)
Urea, 0.8M (5%) in Tris buffer, pH 9.5	Non-ionic chaotrope at high pH	Shi et al., 1996	Effective with 32 of 34 antigens examined
EDTA, 0.001M, pH 8	Chelation of Ca <sup>2+</sup> ; pH control	Pileri et al., 1997	2 minutes in pressure cooker. Tested with 61 antibodies, and found generally superior to citrate pH 6, Tris pH 9.5 or a proteolytic en-
Tris buffer, pH9.0	Alkaline medium	Koopal et al., 1998	Used for 16 antigens; overnight at 80°C
Formic acid, 19M (88%) followed by guanidine thiocyanate, 4M	Acid followed by chaotropic cation and anion	Everbroek et al., 1999	For unmasking prion protein. The sections were autoclaved before exposure to the reagents.
EDTA, 0.01M, pH 8	Chelation of Ca <sup>2+</sup> ; pH control	Gown & Willingham, 2002	Higher concentration of EDTA than in earlier studies; 10 minutes at 100°C
Citraconic anhydride, 4.5mM, pH 7.4	May reverse formaldehyde fixation	Namimatsu et al., 2005	Equal or superior to two other retrieval solutions, for 62 antigens tested

### Mechanisms.

At least four features of antigen retrieval solutions may be involved in their actions on formaldehyde-fixed tissue: hydrolysis of bonds to bound formaldehyde, actions of metal cations, chaotropic effects, and chelation of calcium ions.

### **Hydrolysis of bonds to bound formaldehyde.**

The possible action of hot water in undoing some of the cross-linking of protein molecules has already been mentioned, but this putative mechanism (Fig. 2) had not, until recently, been tested experimentally. Montero (2003) noted that prolonged fixation in formaldehyde results in poor stainability of tissue proteins by eosin, and that eosinophilia was restored by hot solutions used to retrieve antigens. Stronger evidence supporting the breaking of cross-links comes from the work of Yamashita & Okada (2005), who used SDS gel electrophoresis to study proteins that had reacted in solution with formaldehyde. Cross-linking resulted in the formation of dimers, trimers and other polymers; subsequent heating restored the original monomers. Eosin anions are electrostatically attracted to the basic side chains of proteins – the ones to which formaldehyde molecules bind covalently. Hot water may also alter the conformations of protein molecules. The latter process (cooking) can be expected to expose antigenic sites in much the same way as a coagulant fixative.



**Fig. 2.** Removal of bound formaldehyde and undoing of cross-links by base- or acid-catalyzed hydrolysis.

The effects of pH on different formaldehyde-fixed epitopes may be due to their different constituent amino acids, with some linkages to formaldehyde being more easily broken by hydrolysis in an acidic or alkaline medium. Shi et al. (1997) noted that some antigens could be heat-retrieved over a wide range of pH whereas others required an alkaline medium and yet others were retrievable at low or high but not at neutral pH.

Recently heating in an aqueous solution of citraconic anhydride (0.05%, pH 7.4, 45 minutes) has been proposed as a “universal antigen retrieval method” (Namimatsu et al., 2005). The procedure was equal or superior to citrate (pH 6.0) or Tris-HCl-5% urea (pH 9.0) for all 62 antibodies tested. The authors suggest that citraconic anhydride, which can combine reversibly with amino groups, attacked the bonds between formaldehyde-derived carbon atoms and protein nitrogens, and protein nitrogens, thereby breaking the cross-links and giving antibodies access to epitopes.

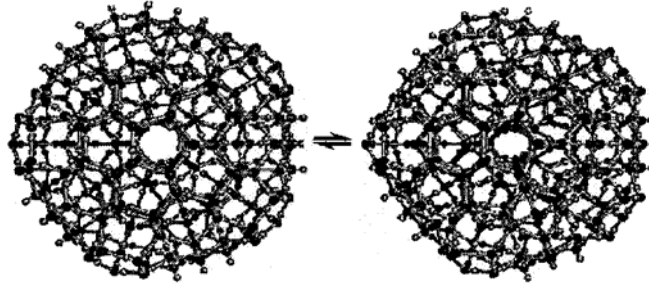
**Effects of metal cations.**

Solutions of the metal salts used in antigen retrieval solutions acidify the water. For aqueous solutions the pH is about 2 for 1% zinc sulfate, 5 for 1% lead thiocyanate, and 2.5 for 4% aluminum chloride. Zinc, lead and aluminum ions also coagulate proteins. The first are included in several modern fixatives, and the second in some older mixtures (see Gray, 1954). Solutions of zinc, lead and aluminum salts are traditional astringent lotions, which coagulate blood and proteinaceous exudates on inflamed skin or mucous membranes. Aluminum ions are not used in fixatives but they are used to harden the gelatin in photographic emulsions.

Coagulant metal cations are not now considered major ingredients of antigen retrieval solutions, though they may be necessary for some particular antigens. The cations may cause changes in the conformations or protein molecules, especially in tissue that has not been adequately fixed by formaldehyde. Associated anions may have related effects; these will be discussed next.

### **Chaotropic effects.**

Water molecules occur in clusters of 280 molecules that can flip between an expanded and a collapsed structure (Fig. 3).



**Fig. 3.** Expanded (left) and collapsed (right) structures of water clusters. [Reproduced with permission from Chaplin (2004)]

Large molecules such as proteins dissolve by occupying the spaces between clusters. These spaces become wider when clusters change from the expanded to the collapsed form. The latter can therefore hold more macromolecules in solution. Smaller molecules or ions dissolved in water can alter the equilibrium between expanded and collapsed structures. Solutes that favor the collapsed structure are called chaotropes. Chaotropic ions, which include guanidinium, and thiocyanate, make the spaces bigger by inducing water clusters to flip to the collapsed state, making more room for dissolved macromolecules. Urea, a non-ionic compound, is a chaotrope when dissolved at high concentrations. Chaotropes are included in a number of solutions used for antigen retrieval (Table 1) but their modes of action have not been investigated. It is possible that these substances modify some proteins in fixed tissue to make them resemble proteins in solution, with more of their epitopes exposed.

### **Chelation of calcium.**

The most popular ingredient of antigen retrieval solutions is the citrate anion. This is a component of the buffer system that stabilizes the pH, but citrate ions can also form soluble complexes with calcium ions. Indeed, sodium or ammonium citrate is an ingredient of at least five solutions used for decalcifying bony specimens (see Lillie & Fullmer, 1976). A more powerful chelator of calcium is the EDTA anion, which is also widely used for decalcification. EDTA is included in several recently published antigen retrieval solutions (see Table 1 for a few examples), in which it serves to buffer the pH and to remove  $\text{Ca}^{2+}$  from the tissue. A chelating agent reacts with a metal ion, which becomes one of a ring of covalently bonded atoms in a stable, soluble, unreactive compound. Chelation reactions remove metal ions from liquid or solid materials.

A  $\text{Ca}^{2+}$  ion can form 4 coordinate bonds to other atoms such as oxygen or nitrogen. A coordinate bond is a covalent (strong) bond in which both electrons are donated by the O or N atom. Such a bond is often represented in a structural formula by an arrow; the head of the arrow pointing towards the metal atom indicates the electron donation. Some antigen-antibody reactions in solution are known to be inhibited in the presence of  $\text{Ca}^{2+}$  and facilitated by EDTA, presumably because the conformations of the proteins are changed by coordinate bonding between calcium and their amino, hydroxyl or carboxyl groups. Simple experiments have shown, for a few antigens, that addition of a calcium salt can impair immunostaining of sections of formaldehyde-fixed tissue. Heating in a  $\text{Na}_2\text{EDTA}$  buffer effectively retrieved these masked antigens, but a  $\text{CaEDTA}$  solution with the same pH was ineffective (Shi et al, 1999). It has been suggested that coordinate bonding of tissue-derived calcium occur with protein side-chains and also with bound hydroxymethylene groups derived from formaldehyde (Jasani et al., 1997; see also Hayat, 2002 for references and diagrams).

More recently, however, Yamashita & Okada (2005) have used SDS gel electrophoresis to examine the effects of some heat induced antigen retrieval procedures on five proteins in solution. This analytical procedure separates protein molecules according to size.

Treatment with formaldehyde caused aggregation of protein molecules into dimers and trimers. Heating restored four of the proteins to their unfixed, predominantly monomeric, conditions. (The fifth protein was degraded by the heating procedure, yielding molecules smaller than the original monomers.) No effects of added calcium ions or of EDTA were detected, indicating that cross linking and deformation of antigens by calcium ions is not a major mechanism of epitope masking in formaldehyde-fixed proteins.

### Conclusions.

The large variety of ingredients in solutions for high temperature antigen retrieval indicates that more than one mechanism is probably involved. There is experimental evidence for temperature-dependent chemical reactions of water with formaldehyde-protein linkages, with breaking of cross-links. Most antigens can be retrieved at near-neutral pH, but a more alkaline medium is needed for some. In a few cases bonds to tissue-bound calcium ions may mask epitopes, necessitating removal of the metal ions by chelation. Other ingredients of retrieval solutions include heavy metal ions, which may expose epitopes by a coagulation-like action on proteins, and chaotropic substances which may modify the shapes of proteins by changing the structures of clusters of water molecules. Most recently, a hot citraconic anhydride solution has been introduced as a reagent to undo the fixation of proteins by formaldehyde (Namimatsu et al., 2005). Further work will be needed to determine if this is truly a universal antigen retrieval method as claimed, and to clarify the mechanism of action.

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(This is an excerpt from an article “Kiernan Presents Culling Lecture” written by Janet Dapson, NSH in Action Editor, Volume 30, Number 2 September 2003) Permission was obtained from Janet to print this excerpt.

“Since 1972, Dr. John Kiernan has been a professor in the Department of Anatomy and Cell Biology at the University of Western Ontario, Canada. He teaches undergraduate and graduate courses in neuroanatomy, a graduate histochemistry course, and conducts research in neuroscience. He is also involved in developing histological and histochemical techniques.

In conjunction with teaching and research, writing is another of Dr. Kiernan’s activities. He has written, co-authored and contributed chapters to numerous books, published 100 papers, and submitted articles on a variety of topics to less formal publications. His scientific article, “Silver Staining for Spirochetes in Tissues” appeared in *Laboratory Medicine*, September 2002. At the top of the list of publications are “Histological and Histochemical Methods (three editions) and Conn’s Biological Stains, 10th edition (co-authored with Richard Horobin, PhD). *Histological and Histochemical Methods* is thought by some to be the best reference available for explaining the mechanisms of fixation and staining. *Conn’s Biological Stains, 10th edition* published 2002 is a completely revised version of the classic text.

Visit Dr. John Kiernan’s website: <http://publish.uwo.ca/~jkiernan/> . The histochemistry segment of the website is filled with FAQ (Frequently Asked Questions), inspired by the Histonet. You will find topics from fixation to photomicrography, techniques, recipes, chemistry and even history”.

**The *Slice of Life* appreciates being able to reprint two articles in a series on immunohistochemistry written by Dr. J.A. Kiernan. We have the permission of both Dr. Kiernan and *The Cutting Edge*, newsletter of Region IX.**

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## Charter Member KSH \* John W. Starrs Sr.

**STARRS John W. Sr.**, died Jan. 12, 2006, after a short illness, in Tucson, AZ. Mr. Starrs moved with his family to Colony Blvd., Lexington, KY, in 1962, to join the staff of the Department of Anatomy at the University of Kentucky Medical School, retiring in 1982. He came to the university upon retiring from 20 years in the US Army. While serving in the Army, Master Sergeant Starrs was stationed in Europe during the occupation following WWII. Upon returning to the States, he was assigned to the Armed Forces Institute of Pathology at Walter Reed. In 1952, he was sent to a MASH unit in Korea. Completing this tour, he returned to AFIP, where he retired from the Army in 1962. In Lexington, Mr. Starrs was a member of Christ the King Parish. He was a devoted father to his surviving son, John W. Starrs Jr.; daughters, Iris Starrs (Philip) Powell, Maryland, Mary Starrs (Bill) Reynolds, Lexington, Catherine Starrs (Carlos) Rangel, Arizona; grandchildren, Kelly Powell (Zach) Walker, Christopher William Powell, Nalish Starrs Rangel, Sean Peter (Andrea) Rangel, Stacey Allene (Brent Oliver) Reynolds; and great-grandchildren, Lucy Starrs Walker, Gavin Christopher Powell. Family and friends are invited to attend a funeral mass at Christ the King Catholic Church on Fri., Feb. 24, at 10:30 am. Mr. Starrs will then be buried next to his beloved wife, Allene Iris Starrs, during a private burial at Camp Nelson. The family asks that all memorial donations be made to the Duval Presbyterian Home, 3395 Grand Ave., Glenwood, FL 32722 Published in the Lexington Herald-Leader from 2/18/2006 - 2/19/2006.



### Program Overview of the 2006 Annual Symposium. For details [www.kyhistotechs.com](http://www.kyhistotechs.com)

#### Friday March 31, 2006

**7:00 am–8:00 am** *Registration*. Exhibits open. Continental breakfast will be served

**AM Sessions** – 8:00 am to 11:30 am

#### Workshop 1

*Stress Relief in the Workplace*. John Price, RN. Louisville, KY

#### Workshop 2

*What Every Histotech Should Know About Water*. Ethel Macrea, HT(ASCP, QIHC), Tucson, AZ

**Lunch** 11:30 am–1:00 pm (*Lunch will be provided*). Visit the Exhibitors

**PM Sessions** 1:00 pm–4:30 pm

#### Workshop 3

*Strategies for Success with Immunohistochemistry*. Ada Feldman, MS HTL(ASCP), Dee Wolfe, HT(ASCP), Anatech, Ltd., Battle Creek, MI

#### Workshop 4

*Rapid, High Through-put Tissue Processing - Implementation of Microwave Processing*. Patty Erickson, HT(ASCP). Sakura Finetek

**Evening Social Event** 5:00 pm-8:00 pm – “A Call to Post”. Visit the Exhibitor Hall

The Kentucky Derby is not far away. Come to the Exhibitor Hall to start off the season. Join us for a little wagering, while enjoying Derby fare. This should be an exciting fun filled evening.

#### Saturday, April 1, 2006

**7:00-8:00 am** *Continental Breakfast will be served*. Exhibits open

**8:00-8:10 am** Welcome by Lena Spencer, KSH President

**8:10-9:00 am** *Quality Issues in Anatomic Pathology in 2006*. William Betsill, MD, LabCorp, Louisville, KY

**9:00-10:00 am** *Essential Cost Savings for Anatomical Pathology*. Patti Erickson, HT(ASCP), Sakura Finetek

**10:00-10:30 am** *Break*. Visit with Exhibitors

**10:30-11:30 am** *What You Need to Know to Take the QIHC Exam*. Ethel Macrea, HT(ASCP)(QIHC), Tucson, AZ

**11:30-1:00 pm** *Lunch* (provided)

**1:00-2:00 pm** *Porching*. John J. Buchino, MD (Kosair Children's Hospital, Louisville, KY) and Joseph J. Buchino, MD (Virginia Hospital Center, Arlington, VA)

**2:00-3:00 pm** *Endometrial Dating*. Dennis O'Conner, MD, Clinical Pathology Associates, Louisville, KY

**3:00-4:00 pm** *Immunohistochemistry: A Potpourri Including New Antibodies And Their Use In Diagnostic Pathology!!* Sheron Lear, HTL(ASCP)QIHC, University of Louisville, Louisville, KY

**4:00-4:30 pm** *Awards Presentation and Door Prizes*



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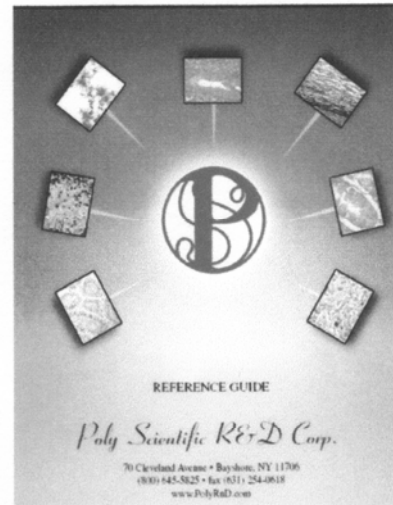
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January 2	Brenda Ice	Jewish Hospital, Louisville
February 6	Shanna Brotzge-Pollock	Suburban Hospital, Louisville
February 12	Lou Coronel	Louisville
February 27	Martha Davenport	University of Kentucky, Lexington
March 15	Vicki Noe	Baptist East, Louisville
March 29	Teresa Kaenzig	University of Kentucky, Lexington
March 31	Sherrie Nuehauser	University of Louisville
March 31	Barbara Beckman	LabCorp, Louisville
April 2	MaryBeth Knight	Norton Healthcare, Louisville
April 6	Denise Kelly	University of Louisville, Louisville
April 15	Phyllis Hubbs	University of Louisville, Louisville
April 18	Mary Martin	LabCorp, Louisville
April 23	Shauna Cameron	Jewish Hospital, Louisville
April 24	Mary Brandy	University of Louisville, Louisville

### Join us for the 2006 Annual KSH Symposium, March 31-April

The Marriott East  
**1903 Embassy Square Blvd**  
 Louisville, Kentucky 40299  
 (502) 499-6220

You should have already received a copy of the program for the spring symposium in the mail. If you have not, please contact Lena Spencer at [lenaspencer@insightbb.com](mailto:lenaspencer@insightbb.com)

**\* \* Please note street address change for the Symposium venue \* \***

## Teleconferences

**March 15, 2006 – Peggy A. Wenk, HTL(ASCP)SLS, William Beaumont Hospital, Royal Oak, MI**

### **SPILL KITS AND FORMALDEHYDE NEUTRALIZATION**

The Histology Laboratory has a myriad of chemicals, which at any time can be accidentally knocked over or dropped, resulting in a spill. Diluting with water is not the correct method to clean up a spill. But what is the correct technique? What reagents should be used to clean up these spills? Which products neutralize and which absorb? What are the by-products of neutralizing the spills – is there a new disposal concern? How do I dispose of the neutralized/absorbed waste? If the EPA or the water treatment plant says your lab can no longer dispose of formaldehyde/formalin down the drain, what are some of the options? This teleconference will discuss the chemistry of neutralizing and absorbing of spills, to equip the participants in selecting the type of spill kit needed for their facility.

**April 12, 2006 (note: 1 week early, due to religious holidays) – Sandy Wilkins, HT(ASCP), Michigan Institute of Urology, St. Clair Shores, MI**

### **STAINING FOR HELICOBACTER PYLORII**

Helicobacter pylorii is a bacteria associated with gastric and duodenal ulcers, and is one of the more commonly requested procedures in the histology laboratory. This teleconference will discuss advantages and disadvantages of various techniques, along with simple tips on techniques. Reproducibility of results, cost, ease of use, and disposal factors will also be discussed. Procedures to be covered include: Giemsa, Diff-Quik, Gram, silver techniques, Genta, Giemeniz, Toluidine blue-alcian yellow (Leung), and IHC.

**May 17, 2006 – Amy Dixon, William Beaumont Hospital, Royal Oak, MI**

### **COMMUNICATION STYLES/WORKING WITH DIFFICULT PEOPLE**

Histotechs work under stressful conditions – fast paced, hazardous chemicals, small space, variety of people and personalities. In this teleconference, participants will explore the concepts of communications and behavioral style. After identifying their own style. Participants will discover how each style assess the world, and therefore what drives their behaviors. Once participants understand all four styles, they will consider how the different styles can cause conflict and misunderstandings, then will learn how to adapt (or flex) their style for better communication.

**June 15, 2006 – Jerome Jasso,**

### **DILUTIONS, RATIOS, PERCENTAGES, TITERS AND THEIR MATHEMATICS**

In the field of immunohistochemistry, primary and secondary antibodies are “titered” to some mysterious number, such as 1:20, 1:400, or 1:1600. This teleconference will discuss what titering is, what is seen on the slide when the antibodies are improperly titered, and the cost saving factor of correctly titering. The difference between a dilution and a ratio will also be explained, along with their relationship to percent solutions. Finally, the mathematics of diluting will be demonstrated.

#### **Participate in the Newsletter”**

We would like you to participate in the newsletter. There are things going on in your lab that your co-workers would like to hear about. Do you have a specialty that incorporates new or different techniques from a standard histology/pathology lab. Has your company allowed you to attend workshops for continuing education.

Can you submit a photo or slide for the front page article. All photos can be returned. Supply a brief description and reference material for it and we can publish it in the newsletter.

**Contact:** Cynthia Long (Editor)

3500 Onyx Court, Lexington KY 40503-4387

## Around the Region \* Rae Ann Staskiewicz

Spring is just over the horizon, and I am sure all of you are looking forward to your state's annual spring meeting. I know I am anxious to get out of the lab for short periods of time to visit several of your meetings and get to meet more of the members of NSH in Region IV.

The last time I wrote that I would report the results of the Post HOD Board of Directors meeting. President Della Speranza nominated Dr. Frieda Carson as the NSH representative to the Biological Stain Commission, this motion was seconded and passed.

A discussion on holding a Career Day for local high school and junior college students to include a "mock" laboratory during the S/C in Phoenix was held. The idea is to promote Histotechnology as a career and also to make our profession "visible" to the public.

A motion to form a task force to investigate the listing of Histology Technician and Technologist as professionals by the Federal government was presented, seconded and passed. President Della Speranza was to send invitations to suggested NSH members to serve on this task force.

The Executive Director was instructed to investigate and obtain 3 quotes as to cost for NSH and individuals for Vision and/or Short Term Disability insurance for the NSH office staff.

A motion for the acceptance as modified in the Post HOD Board of Directors meeting of the 2006 NSH Budget was made, seconded, and passed.

At the S/C in Ft. Lauderdale, I was asked by Region IV members to submit a bylaws change affecting the qualifications for officers and region directors of the NSH. At present the bylaws forbid anyone whose primary income results from the sale of scientific equipment or commodities from serving in these positions. It was felt that this was a very "grey" area as to who was qualified. I consulted with all of the Presidents of Region IV as to the feelings of their societies and the following was submitted to the Bylaw Committee chair. That the statement be removed, opening the positions to all NSH members, or that ALL employees of "vendor" companies be prohibited from holding these offices.

A statement from the bank holding our Region IV CD was received indicating that we received \$107.84 in interest for 2005.



Rae Ann Staskiewicz

The following is a listing of the Region's State Spring meetings:

### **March 3-4, 2006**

Indiana Society for Histotechnology  
Indianapolis, IN

Contact: LaDonna Elpers  
Tel: 812-985-5900 ext 128

Email: [lelpers@bioanalytical.com](mailto:lelpers@bioanalytical.com)

### **March 31-April 1, 2006**

Kentucky Society for Histotechnology  
Marriott East  
Louisville, KY

Contact: Lena Spencer, President  
502-629-7815

[lenaspencer@insightbb.com](mailto:lenaspencer@insightbb.com)

### **April 20-21, 2006**

Tri-State Meeting - Wisconsin, Iowa, Minnesota  
The Concourse, Madison, WI

Wisconsin: Contact: Maureen Decorah  
[decorah@rarc.wisc.edu](mailto:decorah@rarc.wisc.edu)

### **May 5-6, 2006**

Michigan Society for Histotechnology  
Doubletree Hotel, Novi, MI

Contact: Paula Bober, [pbober@dmc.org](mailto:pbober@dmc.org)

### **May 18-19, 2006**

Illinois Society for Histotechnology  
Lisle, IL

Contact: Jane Chladny, [jchladny@cvm.uiuc.edu](mailto:jchladny@cvm.uiuc.edu)

### **Contact information for NSH Region IV Director**

**Rae Staskiewicz**

1450 Bridge Ave.  
Galesburg, IL 61401

W(309)344-2451

Fax: 309-344-7358

[rstaskiewicz@agr.state.il.us](mailto:rstaskiewicz@agr.state.il.us)



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- #s172D EDTA Disodium Salt Formalin
- #s2593 Formic Acid-Formalin
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Country \_\_\_\_\_ Home Phone ( ) \_\_\_\_\_

Place of Employment \_\_\_\_\_

Department \_\_\_\_\_

Work Address \_\_\_\_\_

Country \_\_\_\_\_ Work Phone ( ) \_\_\_\_\_ Ext \_\_\_\_\_

State Histology License No. (if applicable) \_\_\_\_\_

Student Membership \_\_\_\_\_  
(Program Director Signature)

**Check all applicable boxes:**      **1=primary 2=secondary**

<input type="checkbox"/> HT (ASCP)	<input type="checkbox"/> AA	<input type="checkbox"/> University
<input type="checkbox"/> HTL (ASCP)	<input type="checkbox"/> BA/BS	<input type="checkbox"/> Hospital
<input type="checkbox"/> MT (ASCP)	<input type="checkbox"/> MA/MS	<input type="checkbox"/> Private Lab
<input type="checkbox"/> CT (ASCP)	<input type="checkbox"/> PhD	<input type="checkbox"/> Veterinary
<input type="checkbox"/> RT (CSLT)	<input type="checkbox"/> MD	<input type="checkbox"/> Marine
<input type="checkbox"/> ART (CSLT)	<input type="checkbox"/> DVM	<input type="checkbox"/> Botany
<input type="checkbox"/> Other _____	<input type="checkbox"/> Other _____	<input type="checkbox"/> Research
		<input type="checkbox"/> Industrial

**Annual Dues: \$40.00 Student Dues: \$20.00**

Mail information to      Home Address      Work Address

**Membership Year**

**June 1 - May 31 (renewal each May)**

Half year dues (\$20.00 New member; \$18.00 Student) applies from January to May only, with renewal June 1<sup>st</sup> at the yearly rate.

NSH membership includes a subscription to the Journal of Histotechnology published March, June September, and December. NSH will apply \$10.00 of your dues to the Journal subscription.

Remit fee with application, in US currency only.

**Mail to:** NSH, 4201 Northview Drive, Suite 502, Bowie, MD 20716-2604

Referred by NSH member: (Name) \_\_\_\_\_  
(Optional)

\*\*\*\*\*

# Kentucky Society for Histotechnology Application for Membership

Name: \_\_\_\_\_

Home Address \_\_\_\_\_

Business Address \_\_\_\_\_

Telephone (Home) \_\_\_\_\_ Work \_\_\_\_\_

Email \_\_\_\_\_

**Membership fees:**

**New member:**      \$10.00 per year

**Renew**                \$6.00 per year

**Membership year: June 1 to May 31**

**Checks payable to :**

***Kentucky Society for Histotechnology***

**Mail to: Sherrie Drake (Treasurer)**

**78 Elk Creek Court**

**Taylorsville KY 40071**

**Check out an abbreviated version of the program for the Annual Symposium of the Society in this newsletter. Page 12**

**The Marriott East  
1903 Embassy Square Blvd  
Louisville, Kentucky 40299  
(502) 499-6220**

**More info directly on our website [www.kyhistotechs.com](http://www.kyhistotechs.com)**

**\*\* Note address change \*\***

## **2006 KSH Executive**

**Lena Spencer** (President)

3206 Crestview Road

Prospect KY 40059

[lenaspencer@insightbb.com](mailto:lenaspencer@insightbb.com)

**Sherrie Drake** (Treasurer)

78 Elk Creek Court

Taylorsville KY 40071

[sherrie.drake@nortonhealthcare.org](mailto:sherrie.drake@nortonhealthcare.org)

**Audra Rasmussen** (Vice President)

9716 Turnpike View

Louisville KY 40229

(w) 502-584-2070

[smiley1368@yahoo.com](mailto:smiley1368@yahoo.com)

**Barbara Bishop** (Secretary)

7410 Rome Beauty Place

Louisville KY 40228

(w) 502-852-6015

[bjbenno1@louisville.edu](mailto:bjbenno1@louisville.edu)

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Do you work in a research lab? clinical lab? teaching?, are you involved with immunocytochemistry? histochemical staining? slide preparation?, or do you just have an interest in histology? If you've answered yes to any of the above you should consider becoming involved in the state society for histotechnology.

The Kentucky Society for Histotechnology (KSH) is a non-profit organization established to foster communication and education among individuals interested in histotechnology and located at various institutions around the state of Kentucky. Bimonthly meetings held regionally are designed to provide an ongoing educational program. A major state symposium, including workshops, is held annually. Our objective is to improve standards in histopathology/research laboratories with up-to-date methodology and scientific advances in the field of histotechnology. Our state meetings supplement the educational effort of the National Society for Histotechnology (NSH) that holds an annual national symposium. The KSH has a listserv, website, newsletter and will be bringing a synopsis of the bimonthly seminars to the web for members unable to attend the regional meeting.

The Society represents your interests (certification issues, etc.) at the national level. Become a member and voice your concerns. By increasing our numbers we have a stronger voice in the House of Delegates at the National Symposium. Why not join today? Our annual fees are modest: new member \$10, renewal - only \$6.

**Contact: Sherrie Drake (Treasurer), 78 Elk Creek Court, Taylorsville KY 40071**

**Please post in your work area**

**The Kentucky Society  
for Histotechnology**

Anatomy & Neurobiology  
MN-225 Chandler Medical Ctr.  
University of Kentucky  
800 Rose Street,  
Lexington KY 40536-0298

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Fax: (859) 323-5946  
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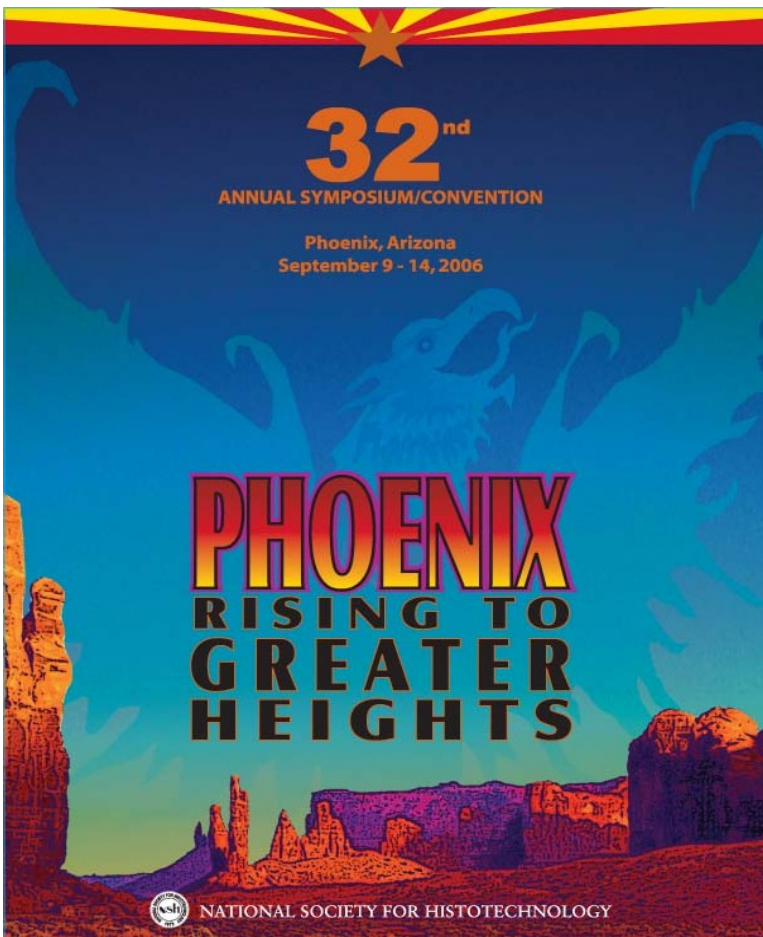
We're on the web  
[www.kyhistotechs.com](http://www.kyhistotechs.com)

**The Kentucky Society for Histotechnology** is a non-profit organization established to foster communication and education among the histotechnologists located at various institutions around the state of Kentucky. Our bimonthly meetings held regionally are designed to provide an ongoing educational program. In addition a major symposium, including workshops, is held annually. The program provides continuing education to histologists and pathologists from hospitals and research laboratories. Our objective is to improve standards in histopathology laboratories with up to date methods and scientific advances in the field of histotechnology. Our meetings supplement the educational effort of the *National Society for Histotechnology* (NSH) that holds an annual national meeting.

**Kentucky Society for Histotechnology Symposium**

**March 31-April 1, 2006**

**Louisville, Kentucky**



**NSH 32nd Annual  
Symposium/  
Convention**

Phoenix AZ

September 8-13, 2006

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