SAKURA

# Histo-Logic®

Technical Bulletin for Histotechnology • Managing Editor, Gilles Lefebvre • Scientific Editor, Vinnie Della Speranza, MS, HTL (ASCP) HT, MT
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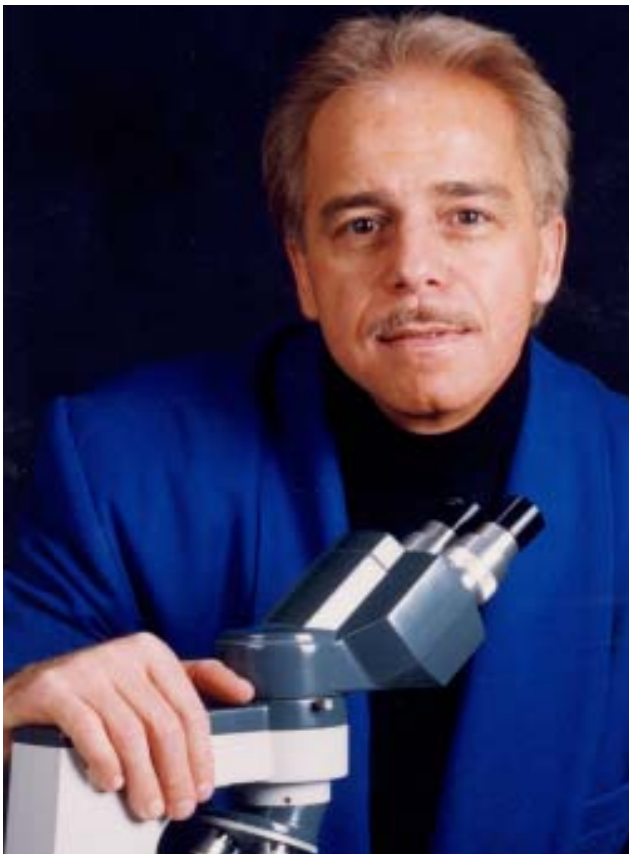
## Vinnie Della Speranza Named New Scientific Editor of *Histo-Logic*

The invitation to serve as Scientific Editor of *Histo-Logic* was an unexpected surprise to Vinnie Della Speranza, MS, HTL(ASCP)HT, MT. Although he has served a number of professional organizations in various capacities during his career, he'd never really imagined himself in this new role. Despite an already full schedule, he didn't hesitate to accept the challenge.



"When the invitation to serve *Histo-Logic* was extended to me, I found myself reflecting on the rich history this publication has enjoyed over the years. I recall with special affection the *Histo-Logic*s of yesterday and the valuable information they contained that I, for one, found so especially useful in my work at the bench. The opportunity to continue the rich legacy of those esteemed colleagues who preceded me as scientific editor is a treasured honor."

Vinnie is a familiar face to many at the NSH Symposium/Convention each year where he has presented workshops and participated in many committee, membership, or board meetings. Although he has long been active in histotechnology, he began his career as a medical technologist after earning his baccalaureate of science degree with a double major in medical technology and biology from the State University of New York at Stony Brook. It didn't take him long to realize that histology was his first love.

"Jules Elias was one of my professors in college. I vividly remember how his enthusiasm and passion for the discipline were so contagious for me. In fact, Jules introduced me at the time to a local pathologist

**IN THIS ISSUE**

who was looking for someone with my training to help bring immunohistochemical techniques into his lab at a nearby community hospital. Although I had other job offers at the time, I found myself drawn to the opportunity to become more involved with histology."

A year and a half later, Della Speranza was promoted to Histology Supervisor where he remained until late 1979, when he was invited to serve as Technical Director for the Anatomic Pathology Laboratories under Jules for the new, soon to open, University Hospital at Stony Brook. There he earned his master of science degree with an emphasis in immunology and pathology. Along the way, he also earned his HT and HTL credentials with the ASCP.

It was Jules Elias who encouraged him to begin his public speaking activities, first at local campus workshops and eventually at the NSH symposium/convention. Today, Della Speranza receives invitations to present workshops at national, regional, and local conferences. This year he is scheduled to give presentations to the Louisiana and New York societies, a reference laboratory in Florida, and the NSH in Providence, R.I. Della Speranza has authored a number of articles throughout his career. In the past year he wrote an invited chapter entitled "Safety Issues for the Clinical Laboratory" for the textbook *Saunders Manual of Clinical Laboratory Science*; an editorial for the *Journal of Histotechnology* entitled "Are We the Victims of Our Own Success?"; and an ASCP Tech Sample entitled "The H&E Stain, Concepts and Pitfalls."

Della Speranza is presently serving his third term as Region 1 Director for the National Society for Histotechnology. Despite a heavy regional and national agenda, he remains active with the New York State Histotechnological Society where he has served two terms as president and over 15 years on the board of directors. In 1998, he was the first recipient of the New York State Histotechnological Society's President's award for outstanding contributions and distinguished service.

It is as much Della Speranza's activities outside of work that make him an excellent choice to serve *Histo-Logic*. Two evenings a week he makes time in a hectic schedule to provide instruction to adults, some from disadvantaged backgrounds, who struggle to begin a career in the health field. He currently teaches phlebotomy and medical terminology.

*"I believe that we have the responsibility to pass on what we know. To do anything less would be a missed opportunity to help shape the future of the profession."*

"The opportunity to assist and encourage individuals looking for their start in a health career is incredibly rewarding for me. I am reminded of the many, like Jules, who freely extended a helping hand to me throughout my career. I feel compelled to do the same for the next generation of health care practitioners. I believe that we have a responsibility to pass on what we know. To do anything less would be a missed opportunity to help others and to shape the future of the profession."

His energy and an attitude like this leave little doubt that Della Speranza brings the right stuff to *Histo-Logic*.

## Something Old, Something New…

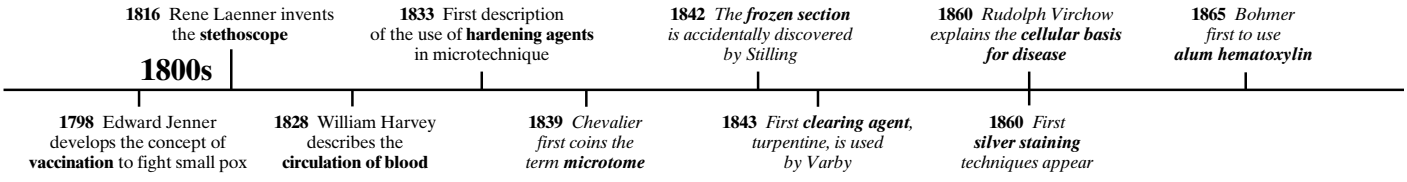
**Vinnie Della Speranza, Scientific Editor**

*Histo-Logic* began as the vision of Lee Luna who, in 1969, imagined the value of having a vehicle by which histotechs could communicate as a discipline. Predating even the *Journal of Histotechnology,* the first

## Countdown to the 21st Century

**Noteworthy Milestones Leading to the Present Day**

Compiled by Vinnie Della Speranza



issue of *Histo-Logic* appeared in July, 1971, sponsored by the Lab-Tek Division of Miles Laboratories.

Over the years, many of us have squirreled away those old issues of *Histo-Logic,* replete with stain modifications, processing tips, tricks, and trouble-shooting strategies. They saved my life more than once, always seeming to contain information that I needed to know in my daily work.

Today, the rich legacy of *Histo-Logic* continues with the guidance and commitment of Sakura Finetek. Together we are working hard to provide you, our readers, with a document that I hope you will continue to keep for years to come. If your collection is incomplete, by the way, you can view old issues of *Histo-Logic* at Sakura's web site at www.sakuraus.com.

Beginning with this issue, you will see some changes that I am very excited about. Be sure to read the section **All in a Day's Work!** where we will regularly feature a different workplace where you, our readers and colleagues, are often unsung heroes facing some rather interesting challenges. Who knows, maybe we'll see your group featured!

I know that you will enjoy our **Countdown to the 21st Century,** an interesting collection of facts and trivia looking back at the noteworthy events that have shaped society and our discipline as the century draws to a close.

To quote Lee Luna, "Reading *Histo-Logic* should be fun as well as educational." I couldn't agree more.

Won't you consider sharing your ideas, tricks, tips, shortcuts and yes, even your opinions with the rest of us? *Histo-Logic* is the forum that brings us all a bit closer. Histotechs too often believe, in error, that if they know something, everyone else must know it too. Someone will always benefit through our collective sharing. So if you have an idea or brainstorm but haven't written before, don't let that stop you. Simply contact me and I'll be happy to help you get it into print.

I hope that you will take a moment occasionally to tell us how we're doing. I'd like you to think of *Histo-Logic* as **your** publication, one that will chase away those workday blues and maybe even bring a few smiles along the way.

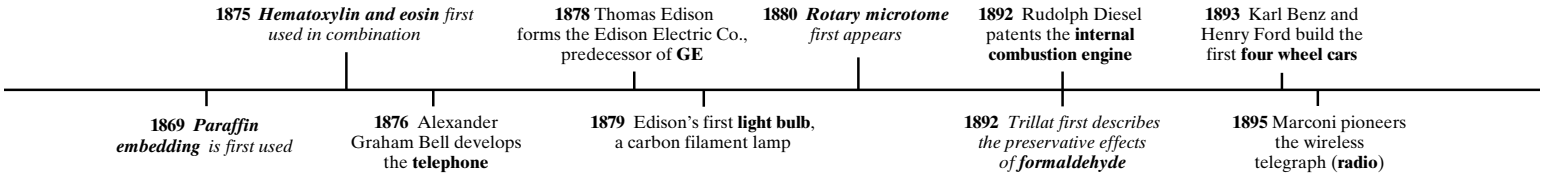
# A Comparison of Staining Methods for *Helicobacter pylori*

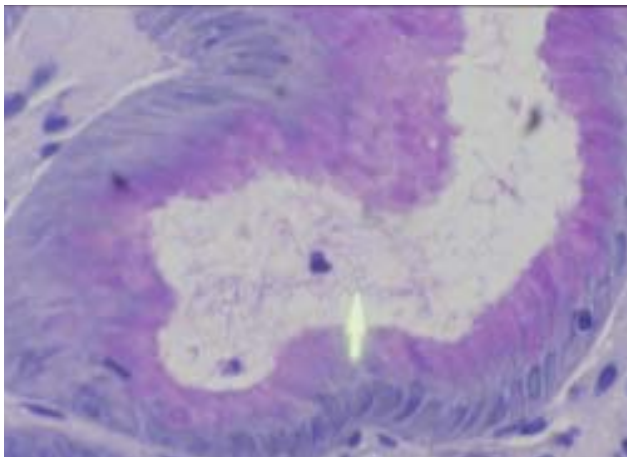
**Kim Rhatigan-Drexler MA, HTL(ASCP)**
**Histology Supervisor**
**University Hospital & Medical Center**
**Stony Brook, N.Y.**

**Abstract**
*Helicobacter pylori* is a spiral-shaped bacterium that has been found to be an important pathogen living in the stomach lining of patients suffering from chronic gastritis. It is a nonsporing, S-shaped, gram-negative rod measuring approximately 3.5 by 0.5 microns and is present in a high percentage of patients with chronic gastritis affecting the gastric antrum and corpus. *H. pylori* infection of the gastric mucosa is present in 90% to 100% of patients with duodenal ulcer and 70% of those with gastric ulcer.[1]

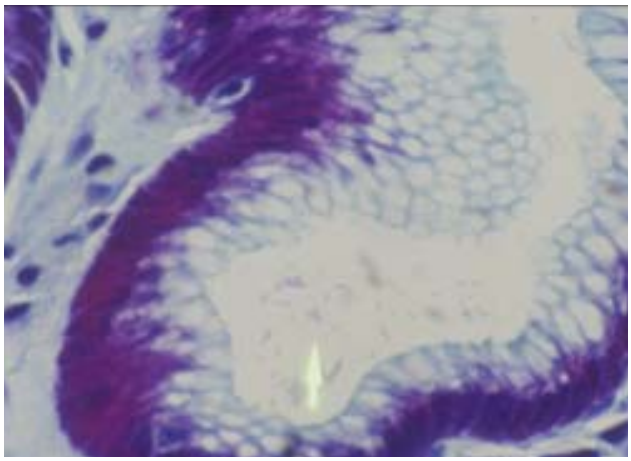
It has also been associated with many of the most important diseases involving gastroduodenal tissue.[2] Visualization of *H. pylori* may be seen with the standard Hematoxylin and Eosin stain in paraffin sections, however, the H & E can be unreliable because the organisms are often hard to distinguish from the mucosa since contrast between the organism and the intraluminal mucous layer is not distinct. A greater number of organisms can be identified through the use of the many special stains that are found to color this pathogen. This article compares 6 different methods for the visualization of this organism in gastric biopsies which are evaluated for effectiveness, ease of use, and reproducibility.

**Introduction**
*H. pylori* has been determined to be an important pathogen in man. It produces a number of products which damage the lining of the stomach. It was discovered in Perth, Australia by Robin Warren and Barry Marshall in 1982. The organism lives in the stomachs of most people throughout the world, although in countries like Australia, US, UK and Sweden less than 50% are infected and most children are *Helicobacter* free.[1] Once the infection is present, it may persist for many years, if not for life. Inflammation disappears if the infection is successfully treated with a regimen of tetracycline,

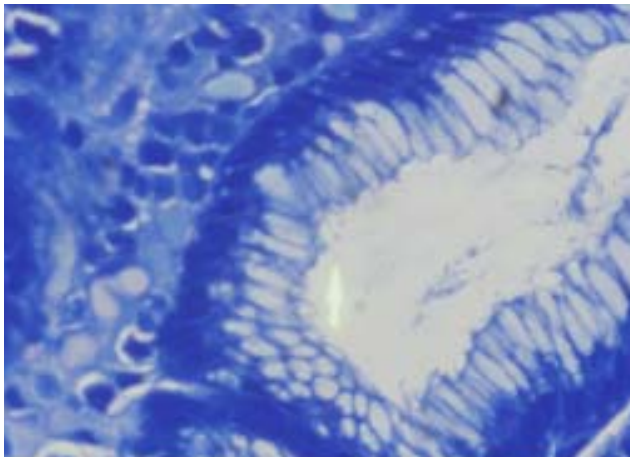




Sayeed method



Gimenez method



Giemsa stain

metronidazole, and Pepto-Bismol™(bismuth). *H. pylori* is now accepted as a cause of:

- inflammation of the lining of the stomach (gastritis)
- ulcers in the first part of the small bowel (duodenum)
- stomach (gastric) ulcers
- carcinoma of the stomach
- Mucosal Associated Lymphoid Tissue (MALT) lymphoma of the stomach

*H. pylori* has a unique way of adapting to the harsh environment of the stomach. The inside of the stomach is bathed in gastric juice which is rich in digestive enzymes and concentrated hydrochloric acid. The stomach is protected from its own gastric juice by a thick layer of mucus that covers the stomach lining. *H. pylori* takes advantage of this protection by living in the mucus lining. *H. pylori* has adapted to life in this harsh environment by using an enzyme it possesses called urease. Urease converts urea, of which there is an abundant supply in the stomach (from saliva and gastric juices), into bicarbonate and ammonia, which are strong bases. This creates a cloud of acid neutralizing chemicals around the organism, protecting it from the acid in the stomach.[1] This reaction offers an important test for the diagnosis of *H. pylori* called the breath test.

*H. pylori* has another advantage allowing it to reside in the mucus lining of the stomach. The immune system normally will respond by sending neutrophils, killer T lymphocytes, and other infection fighting agents to the site of a *H. pylori* infection. However, these potential *H. pylori* eradicators cannot reach the infection because they cannot easily get through stomach lining. They do not go away either, though, and the immune response continues to grow and grow. Polymorphs die and spill their destructive compounds (superoxide radicals) on stomach lining cells. Over time, gastritis and perhaps eventually a peptic ulcer results. It may not be the organism that causes peptic ulcer as much as the persistent inflammation of the patient's stomach lining in response to the organism's presence.[1]

Most duodenal ulcers are also caused by *H. pylori*. The pathogen can move into the duodenum and cause inflammation there as well. This may develop

**1900s**

**1895** German physician Ludwig Rehn is first to sew up a heart wound successfully

**1896** William Roentgen discovers **x-rays**

**1897** ***Coplin staining jar*** *is introduced*

**1898** First photographs taken with artificial light

**1903** Wright brothers first **airplane flight**

**1903** The EKG is developed

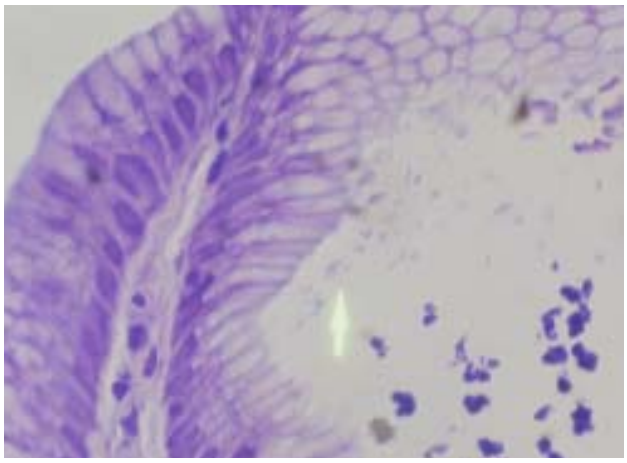
**1905** Einstein formulates his **theory of relativity**

**1908** **Bakelite**, the first synthetic plastic, is developed

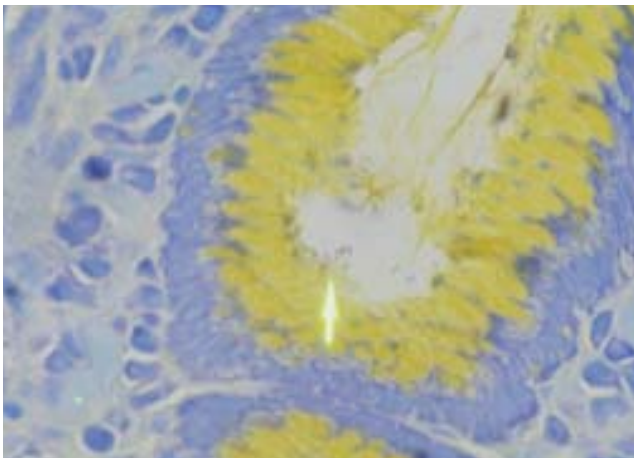
**1909** *First* ***automatic tissue processor***

**1912** Swiss chemist develops **cellophane**

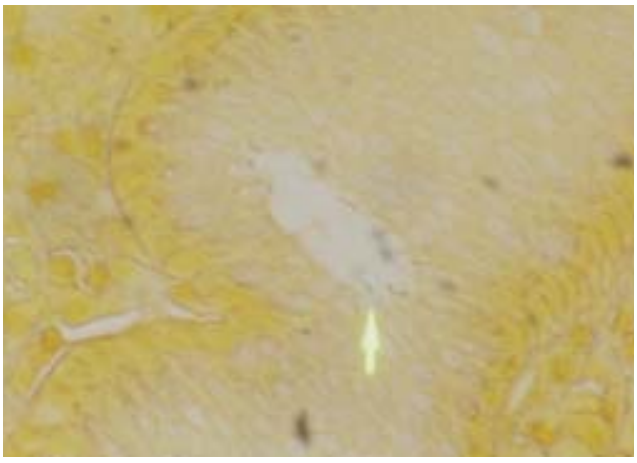
**1912** The **Titanic** sinks



Cresyl echt violet stain



Rapid method



Dieterle stain

into an ulcer and in some cases severe bleeding or perforation into the abdomen can result.

*H. pylori* is believed to be transmitted orally through the ingestion of waste-tainted food or water. In addition, it is possible that *H. pylori* could be transmitted from the stomach to the mouth through gastroesophageal reflux (in which a small amount of the stomach's contents is involuntarily forced up the esophagus) or belching, common symptoms of gastritis. The bacterium then could be transmitted person-to-person through oral contact.[1]

Despite the availability of simple screening tests, gastric biopsy is the most definitive method of detecting *H. pylori* infection. The histologist is often called upon to perform methods that can demonstrate the presence of this organism.

**Methods**

Six special stain methods were used on the same gastric biopsy specimen to stain for *H. pylori*. The biopsy was fixed in 10% neutral buffered formalin at room temperature and processed on a Sakura VIP tissue processor through ethanol and xylene into Paraplast paraffin wax. Sections were cut at 3 microns, placed onto clean glass slides and dried at 60°C for one hour prior to staining. The results of each method were assessed microscopically by three independent observers.

**1. Cresyl Echt Violet (CEV)**[3]

This method utilizes a metachromatic dye, cresyl echt violet (also called cresyl violet acetate) to stain the bacteria.[3] The bacteria stand out well because the mucous layer does not stain heavily with the CEV and provides a nice contrast for the organism. It is a very easy and quick stain utilizing a 0.1% aqueous working solution of CEV. The slides are deparaffinized, hydrated to water, and stained in the CEV for 3 minutes. They are then rinsed in distilled water, dehydrated, cleared, and mounted.

**2. May-Grünwald Giemsa**[4]

This stain uses solutions of both Jenner's and Giemsa to acquire the results. Slides are deparaffinized, hydrated to water, and treated with methanol. They are incubated in Jenner's working solution and then in Giemsa working solution for 10 minutes each. Slides are differentiated in 0.1% acetic

**1915** First **transcontinental telephone call**, from NYC to San Francisco

**1922** ***The American Society of Clinical Pathologists (ASCP)*** *is founded*

**1922** **Insulin** is isolated and used to save a dying baby

**1924** A Ford touring car costs $290

**1924** MGM and Columbia Pictures open

**1926** *ASCP begins plans for a technician registration program*

**1926** B.F. Goodrich pioneers **synthetic rubber**

**1927** Charles Lindbergh flies non-stop from New York City to Paris

**1927** Babe Ruth hits 60 home runs, a record that will stand for 33 years

acid and then dehydrated through tertiary butanol to xylene. This stain is relatively fast and easy, however care must be taken not to over-differentiate the slides.

**3. Dieterle-Microwave Modification**[4]
This silver method utilizes many solutions to stain the bacteria black. Slides are deparaffinized, hydrated to water, and put into uranyl nitrate for the first step for 2 minutes. The slides are then incubated in silver nitrate solution for 5 minutes. Exposure to a developer solution containing hydroquinone, sodium sulphite, acetone, formaldehyde, and pyridine turns the bacteria black. The background is yellowish-brown.

**4. Sayeed Staining Method**[2]
This kit, purchased from Poly Scientific, Bay Shore, New York (cat# K090), uses periodic acid as an oxidizer in the initial step. Slides are deparaffinized, hydrated to water, and oxidized in 0.5% periodic acid for 1 minute. They are then treated with Coleman's Feulgen solution for 2 minutes. From here they are stained with Mayer's Hematoxylin, rinsed in water to blue, and stained with 10.0% Methylene Blue working solution. Bacteria are stained blue and the mucin is stained magenta.

**5. Rapid Staining Method**[5]
This Poly Scientific kit (cat# K086) requires slides to be oxidized in 1.0% periodic acid for 10 minutes followed by treatment with 5.0% sodium metabisulfite for 5 minutes. After a rinse in water, slides are stained in 1.0% Alcian Yellow for 5 minutes. After another rinse in water, the slides are placed in 1.0% Toluidine Blue working solution for 3 minutes. The *Helicobacter pylori* are stained blue, the mucin yellow, and the background is pale blue.

**FIXATION:** 10% buffered formalin
**SECTIONS:** paraffin, 3-5 microns

**SOLUTIONS:**
1. Periodic acid 1% aqueous
2. Sodium metabisulfite 5% aqueous
3. Alcian Yellow(C.I. 12840) 1% (filter before use)
4. Toluidine Blue (C.I. 52040) stock (1% aqueous)
5. Sodium hydroxide 3%
6. Toluidine Blue working solution: PREPARE FRESH 0.5 ml of Toluidine Blue stock—
   50.0 mL distilled water
   2 drops 3% sodium hydroxide

**STAIN PROCEDURE:**
1. Deparaffinize and hydrate slides to deionized water
2. Oxidize in 1% periodic acid aq for 10 minutes at room temperature
3. Rinse well in tap water
4. Sodium metabisulfite 5% for 5 minutes
5. Tap water rinse for 2 minutes
6. Stain in Alcian Yellow 1% for 5 minutes
7. Rinse well in tap water
8. Stain in freshly prepared Toluidine Blue working solution for 3 minutes
9. Rinse well in tap water
10. Blot dry, dehydrate quickly, clear and mount

**RESULTS:**

| | |
|---|---|
| *Helicobacter pylori* | blue |
| Mucin | yellow |
| Background | pale blue |

**6. Gimenez Technique**
(Poly Scientific kit# K072) This method uses Ziehl Neelsen Carbol Fuchsin to stain the bacteria and malachite green as a counterstain. Slides are deparaffinized, hydrated to water, and stained in the working carbol fuchsin for 5 minutes. After a rinse in water, slides are counterstained in 0.8% malachite green and then air dried prior to mounting.

**Discussion of Results**
Out of the six methods we tried, three independent observers favored the staining achieved with the Rapid method. This method provided clear, crisp bacteria as well as good morphology and distinctive, yellow staining of mucin. The organisms stood out very well against the background and were easy to locate. The morphology of the biopsy was very clear and distinct. This method offers the technologist an easy technique that is quick and reproducible.

The CEV (cresyl echt violet) method was deemed second best in our trials. The bacteria were very easy to locate and the background was not distracting. It is a very simple and fast stain and it is inexpensive enough that it could be used routinely on all gastric and duodenal biopsies.

The Giemsa method provided adequate staining of the bacteria. However the bacteria were not crisp and appeared muddy in some microscopic fields. The

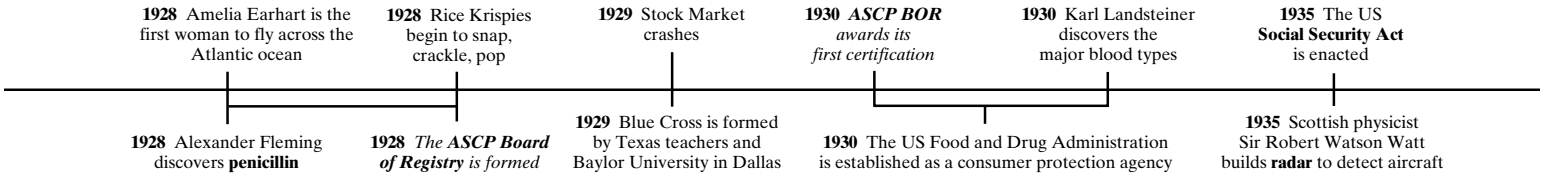


***Table 1.*** **Comparison of Staining Methods for Detection of *H. pylori***

| | ***Cresyl Echt Violet (CEV)*** | ***Giemsa (May-Grünwald)*** | ***Dieterle (Microwave Modification)*** | ***Sayeed Stain Kit (#K090)**** | ***Rapid Stain Kit (#K086)**** | ***Gimenez Kit (#K072)**** |
|---|---|---|---|---|---|---|
| **Time to complete (approx.)** | 15 minutes | 28 minutes | 45 minutes | 19 minutes | 30 minutes | 18 minutes |
| **Stain Results:** | | | | | | |
| **Color of *H. pylori*** | Purple | Blue | Black | Blue | Blue | Red |
| **Background Color** | Light Purple | Light Blue | Yellowish | Magenta (Mucin)/ Blue | Yellow (Mucin) | Green |
| **Remarks:** | Good results—Bacteria are easy to identify | Bacteria are visible but not crisp; tissue morphology less than optimal | Background is distracting—silver method can be capricious; toxic reagents | Bacteria are hard to distinguish in the mucin—variable results from run to run | Excellent results—great color contrast between bacteria and background—bacteria are crisp | Toxic reagent; carbol fuchsin precipitate may be problematic |

* stain kits purchased from Poly Scientific R&D Corp., Bay Shore, NY

Giemsa stain does not provide good tissue morphology which is a drawback to this method. The background was not as sharp as that achieved with the Rapid method. The technique requires differentiation, a potential pitfall for the inexperienced technologist and a potential source of inconsistency from run to run, especially when different individuals perform the stain from day to day.

The Gimenez method provided a nice color contrast of the bacteria and the background. It is a simple stain but we experienced a precipitate of the carbol fuchsin that could distract from the organisms. An advantage of the Gimenez method is that other acid-fast organisms such as Rickettsiae may be demonstrated. Methods offering a better staining result make working with the hazards of carbol fuchsin unnecessary.

The Sayeed method, like the Rapid technique, provided for the staining of the bacteria as well as the mucin. However, it was our experience that the color contrast is not distinct, making organism identification slow, tedious, and uncertain. We found the mucin staining to be distracting and felt the organisms were obscured with this method. We also experienced inconsistent results with this method that were not explored further.

The Dieterle is the least recommended stain for *H. pylori* for a number of reasons. The precipitate that is common among silver techniques in general may distract from, or be mistaken for the black staining *H. pylori*. There is not enough contrast between the bacteria and the background to locate the bacteria easily. It is also a labor intensive and time consuming technique as compared to the other methods. The reagents in the Dieterle stain are also quite toxic and costly. Silver stains can be capricious, with staining repeats more likely to be necessary with this method.

**Conclusion**

The Rapid method reported by Leung et al is a simple, reproducible method that provides great contrast between the bacteria and the background and was determined to be the method of choice at our facility. The *H. pylori* organisms may be located quickly and easily and the method also provides good tissue morphology and distinct mucin staining.

**1936** **Fluorescent light** is invented

**1937** First **jet engine** is built

**1938** **Teflon** makes its debut

**1939** **Television** is demonstrated at the New York World's Fair

**1940** RCA demonstrates the first **electron microscope**

**1941** First **aerosol spray cans**

**1941** *George Papanicolau develops the **Pap smear** to screen for cervical cancer, dramatically reducing the 26,000 annual deaths from this disease*

**1945** The first **Tupperware** product, the polyethylene cup

**1948** First **microwave oven**

**1948** **Velcro** makes its debut

The interpretation of the slide is less time consuming than the other methods. Our second choice, the cresyl echt violet method, is a simple, economical method that can easily be performed with good reproducibility in most laboratories where cost is the first concern.

The importance of detecting *H. pylori* and the ease with which it can be identified is of growing importance to pathologists and gastroenterologists today as the eradication of *H. pylori* is essential to the treatment of ulcer disease.

**Acknowledgments**
The author thanks Joel Israel and Linda Chen for technical assistance; Bernard Lane, MD for participation in stain evaluation, and Kathy DaSilva for the photography.

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## Welcome Tom Lieb

Meet Tom Lieb, new Area Manager for Washington, Northern Oregon, Idaho, Montana, and Alaska.

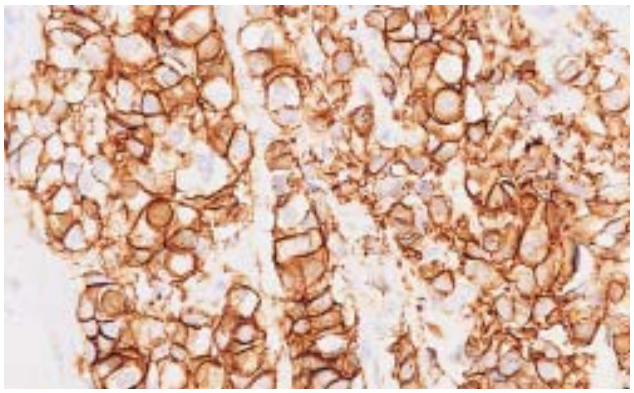
Tom was born in Oklahoma, and attended the University of Oklahoma where he received a B.S. in Zoology.

He has held two positions in laboratory-related companies, Scientific Products and Fisher/IL as a Diagnostic Specialist. His interests include scuba diving, skiing, and whitewater rafting.

## Breaking News… New Weapon in the Fight Against Breast Cancer

**Vinnie Della Speranza, Scientific Editor**

HERCEPTIN (Transtuzumab), the latest weapon in the fight against metastatic breast cancer, is a humanized monoclonal antibody that targets the HER2/neu protein in metastatic breast cancer patients. In approximately 30% of patients with breast cancer, the HER2 protein is over-expressed as part of the process of malignant transformation and tumor progression. Over-expression of the HER2 protein on the surface of breast cancer cells suggests that it could be a target for this therapeutic antibody (HERCEPTIN) which is a humanized monoclonal antibody that binds with high affinity to the HER2 protein and has been shown to inhibit the proliferation of human tumor cells that over-express HER2 protein in vitro and in vivo.



The HercepTest (available through Dako Corp.) is the first Class III PMA FDA-approved IHC test that aids in the assessment of patients for whom HERCEPTIN treatment is being considered. HercepTest is an IHC method for demonstrating Human Epidermal growth factor Receptor 2 (HER2). HER2 is a protein component of normal epithelial cells and is pathologically abundant in a variety of cancer cells, in particular, some forms of breast cancer. The discovery of HER2 (also called p185HER2) was prompted by studies showing that small quantities of the gene encoding for it, known as

**1951** UNIVAC, the first **electronic computer**, is used by the US Census Bureau

**1952** The British Comet is the first **jet airliner service**

**1952** F. John Lewis performs the first successful **open heart surgery**

**1952** Jonas E. Salk develops a successful **polio vaccine**

**1953** IBM's first computer, the 701, is introduced

**1953** Sony's **pocket transistor radio** makes its debut

**1954** ***Fluoroscein isothiocyanate (FITC)*** *becomes the first antibody-labeling agent*

**1956** **Fortran**, the first computer programming language, is developed

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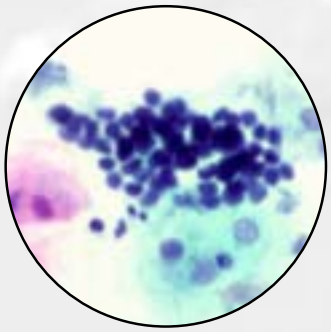

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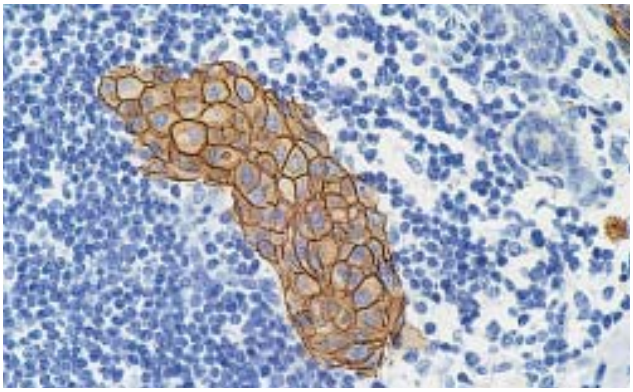
HER2 proto-oncogene, c-erbB-2 or HER2/neu, could transform normal cells into cancerous lines. The protein HER2 is thought to mediate these transformations by transmitting growth signals from the cell membrane to the nucleus, increasing cell division.

"The proto-oncogene c-erbB-2 (HER2/neu) is amplified in about 30% of human breast carcinomas. The transmembrane product of this gene has become a useful target for antibody localization and/or therapy. Detection of c-erbB-2 amplification in tissue therefore, is now an important part of overall breast cancer patient management."[1] Protein over-expression has also been demonstrated in the absence of gene amplification so that in target-directed therapies, detection of the protein product may be more relevant than analysis of gene copy number.[2] HercepTest directly visualizes the presence of the protein targeted by HERCEPTIN therapy, not its genetic determinants.



HercepTest is performed on two tissue sections (one for primary antibody and one for negative reagent control). The method uses an enhanced polymer system that avoids the use of avidin-biotin techniques. The polymer is labeled with multiple HRP enzymes and secondary anti-rabbit antibodies, thus enhancing sensitivity without the background staining that can occur from endogenous biotin. The detection antibody in the HercepTest is a polyclonal rabbit primary antibody that has a high affinity for the HER2 antigen in formalin-fixed, paraffin-embedded tissues. Binding to non-HER2 protein is claimed to be minimal by the manufacturer. The specificity of the HercepTest primary antibody for HER2 protein has been demonstrated by Western blot analysis using established cell lines and stable transfectants. The procedure takes about 3 hours to perform and is interpreted by a pathologist.

Stained sections are evaluated using a graded scale, which is interpreted as positive or negative. Strongly positive (+3 staining intensity) and weakly positive (+2 staining intensity) scores correlate with response to therapy, while patients with negative scores (+1 and 0 staining intensity) are not treated with HERCEPTIN. These positive or negative results, in combination with the patient's clinical history and other diagnostic tests, aid in classification of abnormal cells/tissues and provide a basis for HERCEPTIN treatment selection.

**HercepTest Staining: Interpretation of HER2 Overexpression**

| Staining Pattern | Score | HER2 Overexpression Assessment | Example Stain |
|---|---|---|---|
| No staining is observed or membrane staining is observed in less than 10% of the tumor cells. | 0 | Negative | |
| A faint/barely perceptible membrane staining is seen in more than 10% of the tumor cells. The cells are only stained in part of their membrane. | 1+ | Negative | |
| A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells. | 2+ | Weak Positive | |
| A moderate to strong complete membrane staining is observed in more than 10% of the tumor cells. | 3+ | Strong Positive | |

Anti-HER2 therapy has been described as a true breakthrough. Given the high prevalence of breast cancer, it is valuable to have a diagnostic test that can accurately identify those patients most likely to benefit from HERCEPTIN therapy. By reliably identifying HER2 positivity, the HercepTest enables women with metastatic breast cancer to be considered for a new therapeutic option that may significantly extend their lives and decrease morbidity.

*Editor's note:*
*Those interested in learning more about the HercepTest should contact their Dako representative or visit the HercepTest Information Center at www.dakousa.com*

**References:**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2. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER2/neu proto-oncogene in human breast and ovarian cancer. *Science*. 1989;244:707-712.
3. HercepTest product literature, Dako Corp. 1998.


**1957** The first satellites, **Sputnik I & II**, are launched by the Soviet Union

**1957 Erythromycin** is developed for penicillin-allergic patients

**1960** Dr. Albert Starr and engineer J.L. Edwards design the **artificial heart valve**

**1960** Frank Colton develops the first **oral contraceptive**

**1960** Roger Maris ties Babe Ruth's home run record (60)

**1961** Soviet cosmonaut Yuri Gagarin is first person to orbit the earth

**1961** *Watson and Crick decode the **DNA molecule***

**1961** Alan Shepard makes the first U.S. space flight

**1962** ***Tissue-Tek Embedding Rings*** *become available to histotechs in the lab*

**1962** John Glenn orbits the earth

# All in a Day's Work!

**By Vinnie Della Speranza, Scientific Editor**

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

"She was a big-eyed baby with a look that was described as an impish grin. Many were heartbroken when she died suddenly at the age of sixteen months after a brief and mysterious illness. Her name was Kumari. A short while later doctors realized that another baby, this time an eighteen-month-old male named Astor, had died years earlier of the same viral illness at another facility. Testing performed on stored fixed tissues revealed that the fatal virus belonged to the Herpes family of viruses known to cause disease in people and animals."

These cases appeared in a recent report by Denise Grady in the *New York Times*. In some ways, they resemble countless examples that each of us might share of lost heroic attempts to conquer disease at any one of our facilities. What keeps us all going in the face of defeat are our success stories, but few would disagree that we learn as much, and perhaps more, from our failures as we do from those cases with happy endings. What makes this story different is that Kumari and Astor were baby elephants.

Alfred Ngbokoli must prepare several hundred perfect slides each week.

I had an opportunity recently to visit a very unique pathology service located just north of New York City at the Bronx Zoo. There, my gracious hosts, **Alfred Ngbokoli**, histology supervisor, and **Dr. Tracey McNamara**, chief of the pathology service for the **Wildlife Conservation Society (WCS)**, took me on a tour that left me feeling that each of us needs to do more. They didn't give me a sales pitch. Frankly, they didn't need to. They are scientists with a passion that is contagious. They simply gave me the facts, which I share with you here. The facts speak for themselves.

Each work day begins with a morning strategy session. (Left to right, Alfred Ngbokoli, Dr. Tracey McNamara, and Dr. John Trupkiewicz)

What makes this facility so unique is that while there are 184 accredited zoos in North America, only ***four*** have pathology departments, and only ***three*** are equipped with histology labs! WCS services four NYC zoos, the New York Aquarium and the Wildlife Survival Center in Georgia, a refuge for endangered species, with a total animal population of approx. 18,000, representing almost 1500 different species.

Until my fateful visit to the WCS, I simply regarded zoos as a place for amusement, a fun place to take the kids. It turns out that modern zoos are so much more and that the public never gets to see the most impressive part of any zoo, the dedicated professionals working behind the scenes on behalf of conservation. "The global human population, now at almost six billion, is increasing by 85 million people each year and as they multiply, humans appropriate wildlife habitats. Calculations suggest that if tropical rain forests continue to be felled at the current rate, one-quarter of all wild species on earth will be lost in 50 years" according to the 1998 annual report of the

**1963** Dr. Michael DeBakey is first to use an **artificial heart** during surgery

**1963** **Cassette tape** makes its debut

**1963** Patent is issued for the first **computer mouse**

**1963** *Hepatitis B is discovered and isolated*

**1965** **Medicare** and **Medicaid** are enacted

**1965** First space walks by American and Russian astronauts

**1967** IBM builds the first **floppy disk**

**1967** Dr. Christian Barnard performs the first **human heart transplant**

**1967** *The Clinical Laboratory Improvement Act (CLIA'67) establishes minimum quality standards for clinical laboratories*

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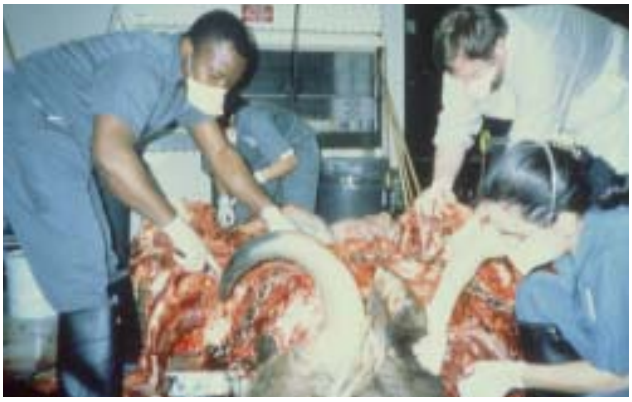
WCS. While zoo collections provide the only contact most of us will ever have with wild animals, their conservation efforts to save endangered species place a much greater importance upon their work than our simple entertainment.



Large animals may be necropsied on the floor.

Animals too large to transport are necropsied in the field.

At first glance, the histology laboratory looks much like yours or mine. A clean, well-equipped facility. Cubes of formalin, bottles of stains and reagents, and the expected mountains of well-organized paraffin blocks and H&E stained slides, as well as the requisite jars of various sizes containing formalin-fixed organs of past studied cases are all within view. But as I look more closely, I begin to notice small details that remind me that I am in no ordinary histology lab. A window looks out into a meticulously clean necropsy area, much larger than any autopsy facility I have seen. The stainless steel cooler runs almost to the twenty-five-foot high ceiling, strong evidence that hints at the clientele that are served here. Its second inner door opens out onto a loading dock where trucks may pull up to deliver their cargo. A large electric hoist is suspended from the ceiling. I am told however that it is not uncommon for necropsies to be performed on the floor when the patient is too large to do otherwise. Those too large to bring to the facility are necropsied in the field.

I glance closer at the jars of stored organs, each labeled with the appropriate case number as yours or mine would be. My mouth drops as I look more closely and the labels reveal that I am peering at organs of siberian tiger, panda, crocodile heart, harbor seal kidney, elephant, giraffe, cottonmouth snake, egyptian tortoise, penguin, and surinam toad, to name just a few. I laugh out loud as I come across a jar containing a stuffed toy monkey wearing sunglasses, a taste of Alfred's humor poking fun at us mere mortals who only work with human specimens, I suspect.

A typical workday begins with a morning strategy session that first identifies any new cases that may have come in. Here the day's priorities are established. Large animal necropsies require the participation of many. Barring this, Alfred's day will likely be filled with the sectioning and staining of a high volume of paraffin sections from formalin fixed tissues, all to be stained with at least an H&E, but a rather large volume of special stains, including PAS and silvers for fungi, Kinyoun's AFB, mucicarmine for cryptococcosis and silvers for spirochetes, as well as iron stains by the hundreds may be needed. Working alongside the medical staff, the slides that Alfred prepares each day can lead to discoveries that are important to animal health and be of benefit to wildlife worldwide.

**1969** Neil Armstrong walks on the moon

**1969** **Videocassette tape** is developed

**1969** Boeing **747 Jumbo Jet** enters service

**1969** *Scanning electron microscope is built*

**1970** The **compact disk (CD)** is introduced

**1970** *The **Tissue-Tek II Cassette** becomes available (in clear only!)*

**1970** *Ludwig Sternberger develops the **peroxidase-anti-peroxidase (PAP)** method, revolutionizing immunohistochemistry*

**1971** Intel introduces the first **microprocessor** chip

**1971** ***Histo-Logic*** *is created by Lee Luna*

**1972** **Medical CAT scanning** developed

Dr. McNamara standing near the oversized refrigerator and electric hoist needed for large animals.

It is not unusual to find zoo veterinarians using advanced technologies like magnetic resonance imaging, computerized tomography, ultrasonography, endoscopy, and mammography on zoo patients, according to Dr. McNamara. But the lack of rapid diagnostic services at the vast majority of zoos is troubling. "The death of an animal in a collection is regrettable, but not to learn from it when there are still so many unknowns in the field of comparative medicine is truly wasteful," she said. "The best hope of avoiding losses may rest in rapid detection, necropsy and histopathology, of an initial or 'index' case of what could easily develop into an epidemic involving many animals."

In truth, there continues to remain large gaps in the knowledge base of the health problems of non-domestic species. Whenever there is an unexplained die-off of animals in the wild, such as the death of seals in the North Sea discovered to be due to a previously unknown virus, or the death of large numbers of lions in the Serengeti caused by, of all things, canine distemper, scientists like McNamara are reminded of just how little is known about exotic species.

This brings us back to Kumari and Astor. In modern zoos it has become common practice to create mixed species exhibits where the risk of interspecies disease transmission is becoming a serious concern. The deaths of Kumari and Astor, the first Asian elephants born at the National Zoo in Washington D.C., and the Bronx Zoo, respectively, led to the discovery that African elephants commonly carry a herpes strain that is relatively harmless in them but is deadly in their Asian counterparts. It is this very virus that is believed to be the major cause of death worldwide among young Asian elephants in captivity. The practice followed by modern zoos of keeping rare or endangered species in flocks or herds creates a large element of risk since illness in one may rapidly spread through an entire group. Indigenous wildlife including rodents, raccoons, and migratory waterfowl pose another threat of disease introduction into zoo animals. The tools that veterinarians employ in pets and farm animals, such as antibiotics and vaccines, are simply not available for the treatment of exotic species simply because their effects in these populations have not been sufficiently studied and they could actually do more harm than good.

Alfred preparing a leopard for necropsy.

McNamara is working hard to change this. She is actively engaged in collecting diagnostic and necropsy information and images that are shared electronically via telepathology to veterinarians in remote locations without the facilities found at WCS. Each month, Alfred is asked to prepare several hundred 'perfect' slides that are shared with zoos around the world or incorporated into teaching sets. This past year,

**1973** **MRI** medical imaging introduced

**1973** ***The Tissue-Tek II*** *opaque* ***Cassette*** *appears (in white only!)*

**1975** The first **personal computer** debuts

**1975** **Microsoft** is founded by Bill Gates & Paul Allen

**1976** Concorde supersonic airline makes first passenger flight

**1976** Steve Wozniak and Steve Jobs build computer circuit board and form Apple Computer Co.

**1977** First test tube baby born in England

**1978** ***Tissue-Tek II Cassettes*** *first appear with* ***colors*** *(yellow, blue, green, white)*

**1978** ***Tissue-Tek III Uni-Cassette*** *appears (in white only!)*

McNamara obtained a large collection of kodachromes and glass slides from the AFIP which contains a wealth of information that she intends to share with her colleagues. I learned that the AFIP's interest in animal health and disease traces back to the civil war when the study of pathology in horses was seen to have strategic importance to government soldiers.

McNamara feels that "a strong argument can be made for the need for diagnostic pathology services for all zoological collections. Perhaps even more so than their domestic counterparts, zoo animals are in urgent need of accurate and rapid pathology support. Information gained from review and interpretation of surgical biopsy, cytologic, and necropsy material by a pathologist empowers zoo staff to take management steps to halt or prevent disease problems. Only then will it be possible to ensure that these high risk animals do not succumb to a preventable disease for simple want of a diagnosis."

*(editor's note: readers may find additional information about issues confronting the world's wildlife at* www.wcs.org **,** *homepage of the Wildlife Conservation Society)*

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# A Closer Look at Xylene Substitutes to Reduce the Hazardous Waste Stream


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**North Shore University Hospital**
**Manhasset, NY**

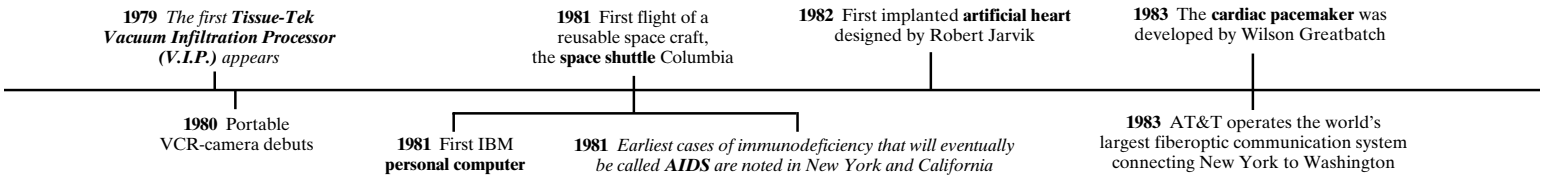


**Abstract**

Xylene has been known for many years to be a hazardous chemical used in large quantities in histology laboratories. This solvent has a toxic effect on human health and the environment that is well documented. However, its excellent reputation as a clearing agent has made it difficult for alternatives to be considered in the laboratory. One key feature of xylene that has made research into alternatives more popular is the high expense associated with its disposal. Reducing the xylene waste stream may be accomplished through recycling or the use of substitute clearing agents. Recycling systems require a significant capital outlay but are considered cost effective and payback of capital expenditure is quick–approximately one year, making recycling an attractive proposition to hospital administrators. More importantly, it reduces the laboratory generated waste stream for this hazardous material. Replacing xylene with substitute clearing agents may also be cost effective, but only if disposal costs can be eliminated. Substitutes however, may provide a safer work environment for staff. Some substitutes, specifically the short chain aliphatic hydrocarbons, may also be recycled. In this study, the performance of popular xylene substitutes available today is compared to xylene.


**Introduction**

Xylene continues to be the solvent of choice for carrying out the clearing and deparaffinizing of tissues during processing and staining in histology labs.[4] It is also used in the staining schemes employed in both histology and cytology laboratories. In 1971, the total xylene production in the U.S. was 612,325,000 gallons, up 14% from the previous year.[5] It has been estimated that today histology laboratories alone consume more than 1.5 million gallons of xylene each year.[4] This occurs despite the known hazards of this material which have been well documented. In 1975, NIOSH (National Institute for Occupational Safety & Health) issued its criteria recommending a standard for occupational exposure to xylene. This was done to develop a program to reduce the occupational diseases arising from chronic exposure to this compound. At that time, NIOSH found xylene toxicity to have a narcotic effect on workers causing muscular weakness, reduced muscle coordination, and mental confusion, as well as irritation to skin and mucous membranes including the conjunctiva and respiratory tract. OSHA has stated that xylene may have a target organ effect on fetuses. Repeated exposure can produce neurotoxic effects leading to irreversible CNS damage, as well as damage to the liver, kidneys,



and gastrointestinal tract.[4] NIOSH recommended that worker exposure levels not be permitted to exceed 100 ppm time weighted average over a ten hour period, which continues to be the OSHA enforced standard today. The problems associated with working with xylene are compounded by the fact that it may take 72 hours or more to clear from the body.[4] This is especially problematic for technologists who may be required to work more than a five day work week which will effectively prevent the solvent from being fully cleared from the body in such individuals.

Its toxicity and flammability characteristics make xylene a hazardous chemical waste whose disposal is regulated by the US Environmental Protection Agency. In addition, accredited medical laboratories are required by the CAP to have a hazardous waste minimization plan in place. Although relatively inexpensive to purchase, the disposal costs of xylene can be high since it is not permissible to discharge xylene into the environment via wastewater (drain disposal). The EPA requires that hazardous waste be collected by the generator of the waste and disposed of by a waste hauler licensed by that agency. Federal legislation (RCRA) holds the waste generator (the laboratory) responsible from 'cradle to grave' for any harm that comes to the environment caused by a facility's hazardous chemical waste.[1] Disposal costs are rapidly consuming a tremendous chunk of a laboratory's budget which is especially burdensome in this era of managed care.

The best waste minimization strategy is to replace highly hazardous chemicals with less hazardous or non-hazardous products.[2] A number of xylene substitute products have been offered to laboratories by chemical suppliers to provide a safer alternative for laboratory professionals. These may be categorized as limonene-based clearants, aliphatic hydrocarbons and hydrocarbon blends. The blends are hazardous in their own right and so are not considered further here. Once thought to be quite safe, limonene has been found to be a sensitizer, predisposing laboratory professionals to chronic allergic reactions after prolonged exposure, posing a serious health risk.

Substitutes classified as aliphatic hydrocarbons have received the greatest attention. They appear to be low in reactivity and toxicity. Manufacturers claim that these clearing agents are non-irritating and non-sensitizing. Their PEL of 300 ppm per 8 hours, which is well above that of xylene, would be difficult to achieve in a well ventilated room without heating the compound.[1] Substitutes falling into this group may be further divided into long-chain and short-chain aliphatic hydrocarbons. Short-chain aliphatics are more volatile and have a flash point below 110°F (those with a flash point below 100°F are classified as 'flammable' and have special storage requirements), while the long-chain varieties have flashpoints above 140°F. The short-chain substitutes are lighter in weight and will penetrate tissues faster than their heavier cousins.[1] They may also remove lipids more effectively but are relatively intolerant of water contamination, requiring the laboratory professional to be vigilant in adhering to a strict solution rotation schedule. Long-chain aliphatics approach the consistency of a light oil.

**Materials and Methods**

Two short-chain aliphatic hydrocarbon and two long-chain aliphatic hydrocarbon xylene substitutes were evaluated in this study. The long-chain aliphatic hydrocarbons selected were Slide-Brite Clearant (S & S Company, Albany, GA) and Micro-Clear (Micron, Fairfax, VA). Both of these clearants are considered non-toxic and non-flammable, which simplifies storage requirements. They also offer the possibility of recycling or drain disposal with permission from local waste water authorities. The short-chain aliphatic hydrocarbons selected were Pro-Par Clearant (Anatech Ltd., Battle Creek, MI) and Shandon Xylene Substitute (Shandon-Lipshaw, Pittsburgh, PA). These also are non-toxic but their low flash points require special storage as flammable liquids which makes permission for drain disposal unlikely.

Five parallel pieces of human tissue were cut from eight surgical specimens. Representative sections of skin, colon, lung, placenta, prostate, spleen, and two cases of uterus were selected. Tissues submitted for processing were approximately 3 mm thick. Tissues were fixed in 10% neutral buffered formalin (Fisher Scientific) and processed overnight on a Sakura Vacuum Infiltration Processor (VIP) tissue processor with a fourteen hour processing cycle (Table 1) utilizing 10% NBF, graded ethanols and cleared in xylene or one of the substitute clearants studied. Tissues were infiltrated and embedded in Tissue-Prep embedding medium (Fisher Scientific).

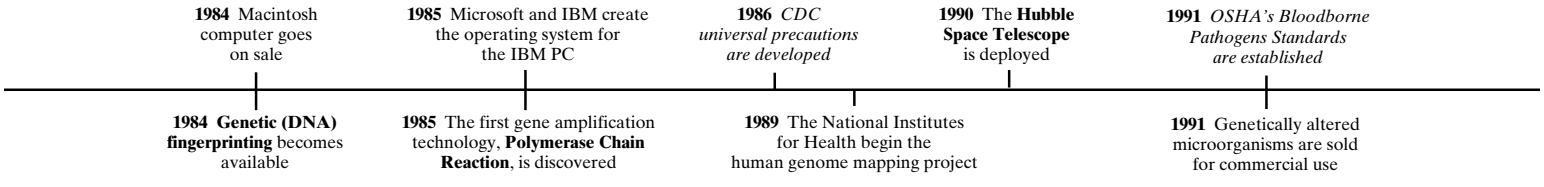


**Table 1**
**Tissue Processing Scheme—Sakura VIP**
• note: all stations utilized pressure/vacuum

| Station | Solution | Time | Temp |
|---|---|---|---|
| 1 | 10% neutral formalin | 90 min | 42°C |
| 2 | 10% neutral formalin | 90 min | 42°C |
| 3 | 60% ethanol | 30 min | 42°C |
| 4 | 95% ethanol | 60 min | 42°C |
| 5 | 95% ethanol | 60 min | 42°C |
| 6 | 100% ethanol | 60 min | 42°C |
| 7 | 100% ethanol | 60 min | 42°C |
| 8 | 100% ethanol | 60 min | 42°C |
| 9 | clearant | 60 min | 42°C |
| 10 | clearant | 60 min | 42°C |
| 11 | paraffin | 40 min | 60°C |
| 12 | paraffin | 40 min | 60°C |
| 13 | paraffin | 40 min | 60°C |
| 14 | paraffin | 40 min | 60°C |

Four-micron sections were cut from each of the blocks, dried at 60°C for one hour and stained together H&E on a Leica Autostainer. Respective slides were deparaffinized and cleared on the slide stainer with xylene or the appropriate xylene substitute before being mounted. The stained slides were evaluated blindly by eight pathologists whose scores were averaged. Sections were assessed for nuclear detail, cytoplasmic membrane, cytoplasmic clarity, hematoxylin staining, eosin staining, and overall appearance of the section for each clearing agent and graded with a score ranging from 1 (unacceptable) to 5 (best) (results summarized in table 2). Scores from one pathologist who found all slides to be unacceptable were deleted from the study.

## Results

Overall, the performance of the xylene substitutes studied was found to be inferior to xylene by the evaluators. The performance of each substitute is summarized in table 2. Numerical scores represent the average score among the observers for each criteria studied. Bias arising from the familiarity of the reviewers with tissues processed and stained with xylene as the clearant cannot be ruled out. Two, Pro-Par (Anatech, Ltd. Battle Creek, MI) and Slide Brite (S&S, Georgia) were found to more closely approximate the microscopic appearance of tissues prepared with xylene and warrant further study to see if results can be improved. They were also among the least expensive.

**Table 2**
**Averaged Scores of Clearant Performance Assessed Blindly by Seven Observers Utilizing A Scale of 1-5 (1 = poor, 5 = excellent)**

| Category | Micro-Clear | Xylene | Slide-Brite | Shandon Xylene Sub. | Pro-Par |
|---|---|---|---|---|---|
| Nuclear Detail | 3.71 | 4.0 | 3.86 | 3.29 | 4.00 |
| Cytoplasmic Detail | 3.71 | 4.0 | 3.86 | 3.43 | 3.29 |
| Cytoplasmic Clarity | 3.43 | 4.14 | 3.71 | 3.43 | 3.57 |
| Hematoxylin Staining | 3.57 | 4.14 | 3.71 | 3.71 | 3.43 |
| Eosin Staining | 3.43 | 4.0 | 3.57 | 3.43 | 3.57 |
| Overall appearance | 3.60 | 4.2 | 3.83 | 3.42 | 3.80 |
| **Aver. Score** | **3.6** | **4.1** | **3.8** | **3.45** | **3.6** |

## Discussion

This study suggests that xylene substitutes may offer a viable alternative to the hazards associated with xylene for laboratory staff. It is important to note that substitutes rarely perform identically to the material being replaced. The user must be prepared to adjust procedures to accommodate such differences. Substitutes may only offer economic advantage if disposal costs can be eliminated, either through recycling, burning as a fuel supplement, or drain disposal. Local water authorities must be contacted for approval to discharge a substitute into the waste water, despite any manufacturer's claims that this is acceptable for their product. Due to the limited nature of this study, the frequency with which substitutes may have to be removed from the processor or staining instruments relative to xylene could not be assessed. The economics of xylene substitute use require a closer look since it is well documented that the aliphatic hydrocarbons are less tolerant of water contamination than xylene, posing potential difficulties especially in more humid climates.

**1992** *The final CLIA'88 regulations are published*
**1992** The **Internet** becomes available to the public
**1993** Intel introduces the Pentium microprocessor chip
**1994** The Hubble telescope discovers the **Black Hole**
**1995** Microsoft releases Windows '95 operating system
**1995** DVD is introduced
**1996** World Chess champion Gary Kasparov loses first game to IBM computer program called Deep Blue
**1997** Dolly the sheep is cloned by Scottish scientists
**1997** The first septuplets are born
**1998** Mark McGwire hits 70 home runs
**1998** Viagra is marketed to correct male impotence

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# Histotechs to Invade the Smallest State in the Union

**By Vinnie Della Speranza**

This fall, histotechs from all over the nation and some from distant points around the globe, will descend on Providence, Rhode Island as the National Society for Histotechnology hosts its annual Symposium/Convention, October 16–21. Those who may be visiting the state of Rhode Island and Providence Plantations (the full name for the state) for the very first time will find that they are in for a real treat. Only 50 miles long by 40 miles wide, (some say an hour long and a half hour wide), every part of the state is accessible without big city delays. Only one hour from Boston and three hours from New York City, techs will be able to learn by day and play by night.

Providence is one of the last undiscovered great cities in the United States. *Newsweek* Magazine has named Providence one of the "Hottest Cities" in America. And *Parade* Magazine listed Providence as the "safest city in the continental U.S." Why all of the accolades? With a wealth of art galleries and loft studios, a Tony Award-winning theater company, a series of Broadway shows at the Performing Arts Center, a state orchestra, two ballets, fine museums, college theatre, choral groups and a nationally-esteemed school of design, Providence packs an artistic punch.

The state's capital, Providence has a long and rich history. Founded in 1636 as a bastion of religious freedom by a preacher from Salem, Massachusetts who fell into disfavor with the morally conservative puritans, Roger Williams thanked God for his "merciful providence." Located at the confluence of three rivers at the head of Naragansett Bay, Providence has a long history of sea trade with all parts of the world, including the near and far east. The sea made Providence wealthy in the 18th century as witnessed by the number of grand Victorian homes flanking the city's river banks. One family in particular, the Browns, ascended quickly to the highest ranks of Providence business and society, endowing Brown University and bringing back lovely treasures and artwork from China (on exhibit at the John Brown House). The Industrial Revolution of the 1800s shifted the focus of the city's economy to manufacturing, counting textiles, jewelry and silverware manufacturing among its largest industries. Providence was once deemed one of the "Five Industrial Wonders of the World".

One of Providence's charms is that you can see virtually the entire city on foot. A brief stroll from the Convention Center will allow visitors to take in most of the historic and architectural sites, including the new Providence Riverwalk's beautiful promenade that stretches from the capitol area to the head of the Bay. A short hike up College Hill brings you to Brown University and the Rhode Island School of Design, as well as several interesting museums and libraries. One can stroll up Smith Hill to the historic State House, cruise the shops, art galleries, and restaurants of Downcity or walk to Federal Hill, one of the city's great ethnic neighborhoods. Unlike big cities, Providence has the charm of a place where natives rarely walk more than just a few blocks before meeting someone they know.

Do you enjoy good food? You'll be delighted to find that Providence is a first class restaurant city with bargain prices. According to the *Boston Globe*, when Boston's finest chefs dine out they go to Providence. Frequent appearances on the Top Ten lists of such magazines as *Gourmet*, *Food & Wine,* and *Bon Appetit* keep the city's dining experience in the public eye. After departing from a local eatery, Julia Child was heard to remark, "I wouldn't change a thing." Seafood is a specialty in Providence but some of the finest Italian food in the world is also found here, as well as French, Indian, Thai, Japanese, Greek, and

**Sources for Countdown to the 21st Century:**

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Portuguese. A major international culinary institute (Johnson & Wales University) keeps the kitchens of the city supplied with its talented chefs. If casual is more your style, try the bevy of cafes and coffeehouses around the city that welcome you for a cup of java, the daily newspaper, and plenty of people-watching.

And after a full day of meetings, the city is full of nightlife. There are dozens of clubs, theater performances, and concerts every weekend in the Downcity area lasting well into the early hours of the morning. For anyone interested in venturing further out, Newport, located on the state's 32 miles of coast, is a 40 minute drive. Autumn is a wonderful time to consider an excursion to the orchards of western Rhode Island or to see the fall foliage. You might consider bringing the family along to enjoy all that the area has to offer.

Just before press time, Kerry Crabb, NSH Convention Chair told *Histo-Logic* that this year's convention program will include 98 workshops, almost half of which will be presented for the first time in Providence. In keeping with the meeting's new format, workshops and seminar presentations will be offered each day. On Saturday morning, a special plenary session will be sponsored by the Society for Applied Immunohistochemistry entitled "Selected Topics in Immunohistochemistry." A change from past meetings, the poster exhibits this year will be located in the center of the vendor exhibit hall to make them more accessible to the attendees. Those traveling to Providence by auto may be interested to learn that some of the convention hotels will offer free parking, a substantial savings for those on a budget. Be sure to check the conference program for details.

It isn't too early to plan to join the NSH in Providence, October 16-21. Convention week is certain to be educational and memorable. Programs will be mailed in late Spring to all NSH members. If you aren't a member, you will surely want to take advantage of the member rates for registration and workshop fees. Visit the NSH website at www.nsh.org for membership information or call (301)262-6221.

**Sources:**


Greater Providence Visitors and Convention Bureau, http://www.providencecvb.com/

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## Meet Dennis Keast

Sakura Finetek U.S.A., Inc., is proud to announce the appointment of Dennis Keast as the new Area Manager for Ohio and Michigan.

Dennis attended Oakland University in Rochester, Michigan where he earned a B.A. degree in Psychology. He has been in sales for five years.

Dennis's interests include football, hockey, chess, and reading.

## High School Eligibility Route for HT(ASCP) May End by 2005

On February 18, 1999 the NSH board of directors approved a motion to formally request the ASCP Board of Registry to discontinue the Route III eligibility option for histotechnician (HT) certification by January 1, 2005. Route III eligibility presently allows individuals with a high school diploma to sit for the H.T. exam after receiving on-the-job training and two years of work experience. The NSH board has taken this position believing that the complexity of new technologies available now and on the horizon will require the histotechnician of the future to possess a greater academic foundation in order to perform successfully on the job.

This request to the ASCP Board of Registry will culminate a 10-year effort by NSH to increase the educational standards for histology technicians. If approved, it will put the histology technician on the same level as the medical laboratory technician (MLT), with the same educational standards.

NSH president Sumiko Sumida said, "Histology technicians today are expected to do more than just produce microscopic slides for the pathologists. Many laboratories are expecting histology technicians to "gross-in" small specimens. CLIA requires an AA degree or 60 semester hours of college for anyone who starts performing this task after April 25, 1995. NSH would like to ensure that all future HT(ASCP) certified technicians can meet the CLIA regulations to perform gross description."

Technicians who have not yet taken the HT certification examination and who do not possess academic training beyond high school are urged to make plans to do so now. Their eligibility to take the exam after January 1, 2005 may be lost.

## Get to Know Ross Lindmeyer

Meet Ross Lindmeyer, new Area Manager for Iowa, Upper Peninsula Michigan, Minnesota, Nebraska, the Dakotas, and Wisconsin.

Ross comes to us with a sales career of over 15 years with Cardinal Health, Inc. as a Hospital/ Managed Care consultant in pharmaceutical distribution.

His interests include golf, gardening, fishing, and other outdoor activities.

To receive your own copy of *Histo-Logic*,® 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 , ***Histo-Logic*** Editor, L2, 766 University Hospital, Stony Brook, NY 11794-7025. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.

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