



## An Overview of *Helicobacter pylori* Infection in Gastrointestinal Disease

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### INTRODUCTION

An unknown entity 10 years ago, *Helicobacter pylori* is now the center of interest in gastric pathology as the medical implications are becoming clearer and more significant. First isolated in 1982 by Warren and Marshall from the gastric antral epithelium of inflamed mucosa in patients with active chronic gastritis,<sup>1</sup> *H pylori* is a gram-negative spiral or curved microorganism with characteristics of a smooth cell surface, possession of polar tufts of four to six flagella, production of urease, and unique fatty acid profiles.<sup>2</sup>

The organism, which may live in the stomach for months or years, can be observed at three sites: the mucosal surface, and the upper and lower parts of the gastric pits. Persons with *H pylori* in the stomach also may harbor organisms in the metaplastic gastric epithelium of the esophagus (Barrett's esophagus) or duodenum.<sup>3</sup> It is now generally accepted that infection by the *H pylori* bacterium is the major cause of type B gastritis and peptic ulcer disease.<sup>1,2,3,4,5</sup> Previously, type B gastritis was believed to be caused by stress, ischemia, reflux of bile, chemical irritants (ie, ethanol, nonsteroidal anti-inflammatory agents) or infection by cytomegalovirus.

Recent data indicates that infection by this microorganism is a significant risk factor for development of gastric carcinoma and gastric non-Hodgkins lymphoma.<sup>6</sup> Additionally, a current report documents the elimination of recurrent benign hyperplastic gastric polyps by eradication of *H pylori*.<sup>7</sup>

### DETECTION

Patients with gastritis may be symptomatic or asymptomatic. Patients may present with nausea, vomiting, heartburn, anorexia, belching, and/or epigastric pain. In the absence of endoscopy, the physician is unable to determine if a patient has gastritis, peptic ulceration, or gastric carcinoma. Conclusive evidence has shown the bacterium to be a causative factor in gastrointestinal disease. Therefore, precise identification of the organism is important.<sup>2,3,4,5</sup>

*H pylori* organisms may be detected in stained smears by phase contrast microscopy, by the production of urease (a presumptive test), and by isolation through culture. Culture of *H pylori* in gastrointestinal biopsy material requires careful handling and an incubation time of 3 to 7 days.<sup>2</sup>

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A faster procedure tests for urease since the organisms are prolific producers of urease. With this procedure, biopsy specimens are macerated and placed into urea broth or Christensen urea agar. A change in color of the medium to alkalinity is a positive test for urease, and it is presumed indicative of the presence of *H pylori*.<sup>1</sup> Interest has arisen in serologic tests because the organism elicits an IgG and IgA antibody response. However, the tests are costly and complicated and are therefore unsuitable for screening large populations.

Histologically, special stains are used for the demonstration of *H pylori* in gastroduodenal biopsy specimens. The most widely used stains are: Giemsa, hematoxylin and eosin (H&E), the Warthin-Starry silver stain, and the Diff-Quik stain.<sup>2,3</sup> In our laboratory we have found the Modified Diff-Quik Stain for *H pylori*<sup>4</sup> the most rapid and easiest to perform and interpret on a routine basis (Figs 1 and 2).

#### INCIDENCE

Infections with *H pylori* are commonly acquired in childhood. Previous association between seropositivity, socioeconomic status, and overcrowding suggest that close person to person contact, such as sharing a bed in childhood, may increase the risk of infection.<sup>5</sup> In developing countries, including those in Africa, *H pylori* infection is acquired early in life and reaches high prevalence in early adulthood.<sup>10</sup> The prevalence of this bacterial infection increases with age to about 75% in persons 65 years of age and older.<sup>1</sup> *H pylori* is found in more than 90% of patients with duodenal ulcers and 70% of patients with gastric ulcers, always in association with chronic gastritis.<sup>1</sup> Factors associated with earlier and more frequent *H pylori* infection in healthy subjects include race and ethnicity (eg, Afro-American and Hispanic-American), socioeconomic deprivation, and residence in custodial institutions.<sup>2</sup>

#### TREATMENT

Patients with gastritis or peptic ulcer disease are encouraged to eat a bland diet, stop smoking, eliminate intake of alcohol, limit use of nonsteroidal anti-inflammatory drugs, and reduce daily stress. Histamine receptor antagonists (Tagamet,<sup>®</sup> Zantac<sup>®</sup>), which reduce gastric acid; sucralfate (Carafate<sup>®</sup>), which is a gastroduodenal cytoprotective agent; and omeprazole (Prilosec<sup>®</sup>), which is a gastric enzyme inhibitor, have all been used worldwide to treat gastric and duodenal ulcers since the late 1970s but have not been effective in the treatment of *H pylori* infection.

Bismuth subsalicylate (Pepto-Bismol<sup>®</sup>) suppresses the organism but does not eradicate the organism

when used alone. Bismuth salts are known to increase secretion of mucus, absorb fluids, inhibit growth of microorganisms, coat gastric mucosa, decrease gastric and intestinal motility, and have an antacid effect. Metronidazole (Flagyl,<sup>®</sup> Protostat<sup>®</sup>), an antibacterial/antiprotozoal used in combination with bismuth, seems not only to prevent emergence of resistance but to also enable long-term eradication in about 75% of patients.<sup>1,4</sup>

Amoxicillin or tetracycline antibiotics can be used to clear the organism in 70% to 90% of patients. However, when used alone, the relapse rate is high and long-term eradication is achieved in only about 20% of patients. Results are better when bismuth is given with the antibiotics.<sup>1,4</sup> Triple therapy (involving the bismuth salt, metronidazole, and tetracycline or amoxicillin) results in 95% clearance and eradication of *H pylori*.<sup>4</sup> It is, therefore, the recommended choice of therapy.

#### RECURRENCE

Histamine receptor antagonists (Tagamet, Zantac) and omeprazole (Prilosec) do relieve symptoms associated with gastritis and nonulcer dyspepsia and heal ulcers, but they do not cure ulcer disease. The relapse rate is 80% at 1 year and 100% at 2 years.<sup>1</sup> It is likely that relapse and treatment failure observed are attributable to failure to treat the underlying cause of gastritis and peptic ulcer disease (PUD) which, in most cases, appears to be infection by *H pylori*.<sup>1,4</sup> The current approach of triple therapy results in eradication of *H pylori*, providing resolution of gastritis and healing of PUD without relapse.<sup>1,4</sup>

#### CONCLUSION

In less than a decade, *H pylori* has emerged from the unknown to be considered the main causative agent of gastritis and peptic ulcer disease. Recent data indicates that infection with the organism is a significant risk factor for development of gastric carcinoma and gastric non-Hodgkins lymphoma.

Previously, treatment for gastritis and PUD included histamine receptor antagonists, a gastroduodenal cytoprotective agent, and a gastric enzyme inhibitor. These relieve the symptoms and heal ulcers, but they do not cure ulcer disease because they do not eradicate *H pylori*. Presently, the recommended choice of treatment is triple therapy using a bismuth salt, an antibacterial agent, and an antibiotic. This treatment results in a cure of gastritis and peptic ulcer disease by eradication of the organism.

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## ACKNOWLEDGEMENTS

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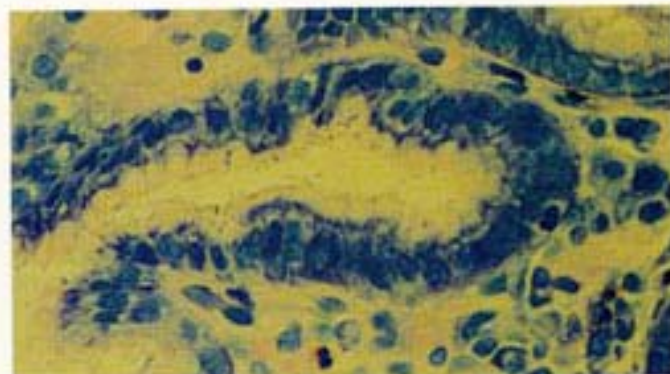


Fig 1.— Numerous bacilli consistent with *H pylori* in the gastric surface mucosa, adherent to the epithelium and in gastric glands (modified Diff-Quik stain, 600 $\times$ ).



Fig 2.— Gastric biopsy displaying numerous bacilli consistent with *H pylori*, some clinging to the cellular surface (modified Diff-Quik stain, 1000 $\times$ ).

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## A Fast Way to Prepare Paraffin Sections for H&E and Immunostains

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## INTRODUCTION

It takes about one to several hours to prepare a paraffin section for H&E or immunostains according to the conventional methods.<sup>1,2</sup> In order to accelerate this process for quicker diagnosis, our laboratory has developed a new method that allows us to finish the preparation in less than 13 minutes and to obtain the same quality of H&E and immunostains achieved by conventional methods.

## MATERIALS AND METHODS

The materials used in this study were obtained from the following sources:

**Tissue blocks:** Surgical Pathology laboratory of the Howard University Hospital

**Clear-Rite 3R (C3):** Richard-Allan, Richland, MI

**Poly-lysine:** Sigma Diagnostics, St. Louis, MO

**Primary antibodies:** DAKO Corporation, Carpinteria, CA

**Secondary antibodies:** BioGenex, San Ramon, CA

**Protease:** Sigma Diagnostics, St. Louis, MO

**Vega Red Chromagen:** Biomedica Corporation, Foster City, CA

Slides were coated with poly-lysine. Three to four  $\mu\text{m}$  sections were prepared, and pairs of two adjacent sections were placed on two different slides. One slide was treated with the conventional method, while the other slide was treated with the modified method.

## TECHNIQUE

1. 5 minutes in 80 $^{\circ}\text{C}$  oven.
2. 2 minutes in each of three C3 stations.
3. 15 dips in each of three 100% alcohol stations one 95% alcohol and one 80% alcohol station.
4. Rinse briefly in hot tap water, then proceed for H&E or immunostain.



To assess the possibility that high temperature (80°C) may have destructive effects on the tissues, pairs of slides were stained with H&E. Also, pairs of such slides were treated with 0.2% protease solution for 15 minutes at room temperature and were vigorously washed with tap water for 15 minutes. These slides were then processed for immunostains with various antibodies. The overall appearance of the morphology of the tissues in these slides was compared.

To examine the potential effect that high temperature may eliminate or even destroy the antigenicity of the tissues, pairs of slides were stained with different antibodies. Then, 15 randomly selected fields in each slide were screened, and the identical fields in its adjacent section were identified. The morphology, the number of positive cells, and the intensity of the immunoreaction in these slides were compared. All the stains for these slides were carried out under the same condition, according to the protocols provided by the manufacturers.

#### RESULTS

H&E stains reveal that the overall appearance of morphology of the tissues prepared by our new method and the conventional method is very similar (Figs 1A and 1B). In both sections, the nuclei are uni-

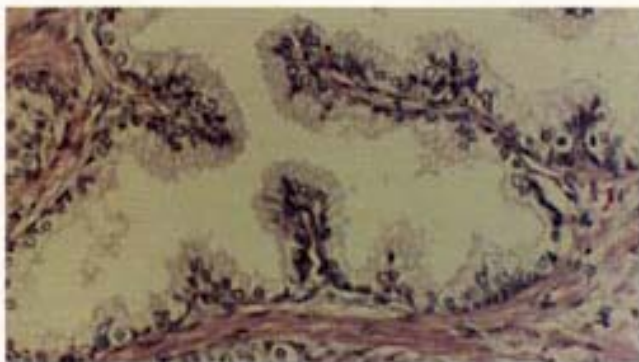


Fig 1A.—Section of prostate gland prepared with new method (H&E, 280×).

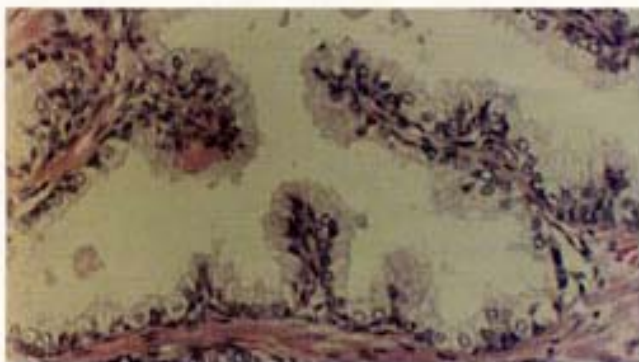


Fig 1B.—Adjacent section of prostate gland prepared with conventional method (H&E, 280×).

formly stained, and neither shrinkage nor cracks are found. Also, although protease digestion and intensive water washes are used, no tissues come off the slides and there is no noticeable difference in the immunoreactions or in the number of reactive cells detected in these pairs of slides (Figs 2A and 2B). These results suggest that the temperature we used has no significant destructive effects on the tissues.

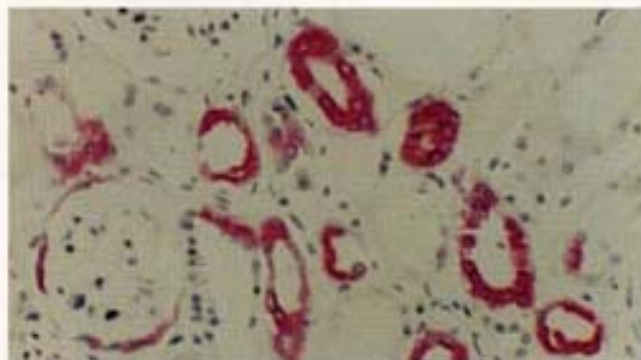


Fig 2A.—Section of kidney prepared with new method, treated with protease, and immunostained with anti-cytokeratin (AE1/AE3, 280×).

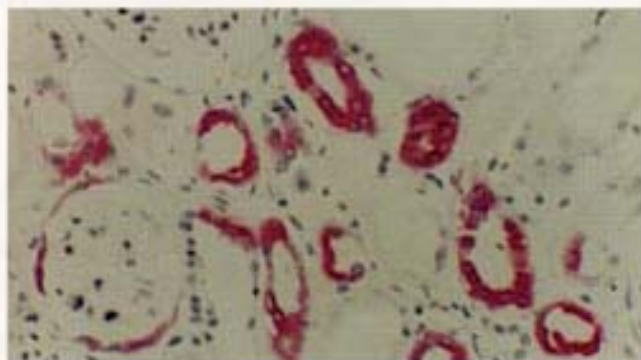


Fig 2B.—Adjacent section of kidney prepared with conventional method, treated with protease, and immunostained with anti-cytokeratin (AE1/AE3, 280×).

Immunostains with antibodies to both cellular constituents and secretory products in a variety of specimens, such as skin, organs of the digestive system, organs of the endocrine system, lungs, prostate gland, and kidneys, show that the patterns of the immunoreactions are almost identical in these pairs of slides (Figs 3A, 3B, 4A, 4B). These results indicate that our new method has no significant effects to eliminate the antigenicity of the tissues.

#### DISCUSSION

It has been well documented that long-term exposures to high temperatures have destructive effects on both the cellular structures and antigenicities of the tissues. However, short-term treatments of tissues with high temperatures seem to produce no significant damage



to the tissues. Recently, microwave oven and hot plate methods in which the tissue sections are boiled or heated to 100°C for 5 to 10 minutes have been widely used for immunostains and in situ hybridization. Although the mechanism is not yet known, these methods have been reported to produce better results. As the temperature used in our procedure was only 80°C for 5 minutes, it is unlikely that any destructive effects could be produced under this condition. Instead, the high temperature appears to be able to more effectively remove the paraffin from the tissues and to eliminate the air bubbles that may have formed underneath the sections.

Clear-Rite 3R (C3) has been recently introduced as a substitute for xylene. According to the manufacturer, C3 has many advantages over xylene, and one of them is that C3 does not leave an oil residue and it does not have a citrus base. This advantage apparently has a positive impact that facilitates the penetration of the antibody solution and other reagents. We have noticed, on many occasions, that the preparation treated with C3 has a much cleaner background and has more intensive immunoreactions than the preparation treated with xylene.

#### ACKNOWLEDGEMENT

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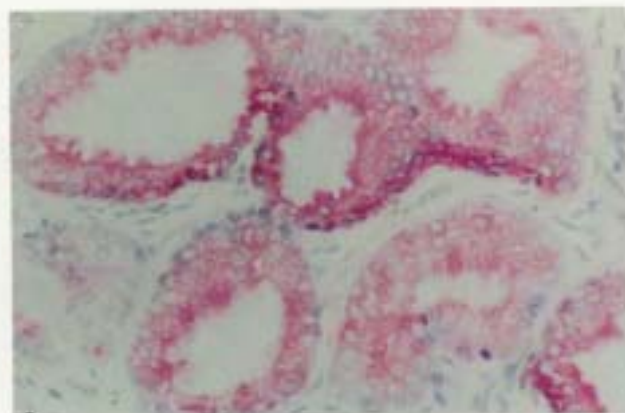


Fig 3A.—Section of prostate gland prepared with new method and immunostained with anti-cytokeratin (AE1/AE3, 280 $\times$ ).

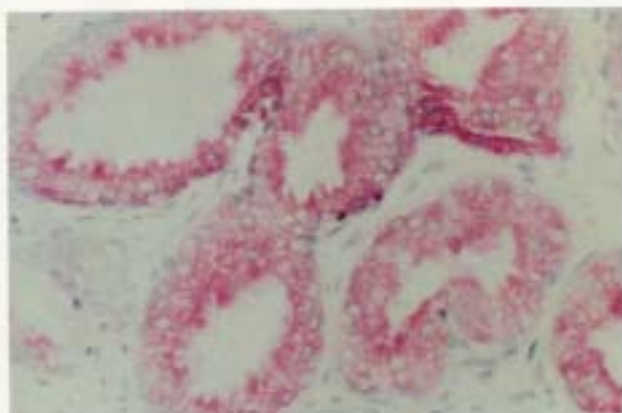


Fig 3B.—Adjacent section of prostate gland prepared with conventional method and immunostained with anti-cytokeratin (AE1/AE3, 280 $\times$ ).

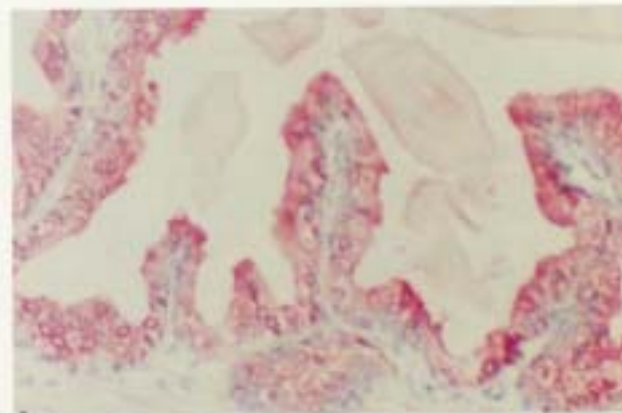


Fig 4A.—Section of prostate gland prepared with new method and immunostained with prostate-specific antigen (PSA, 280 $\times$ ).

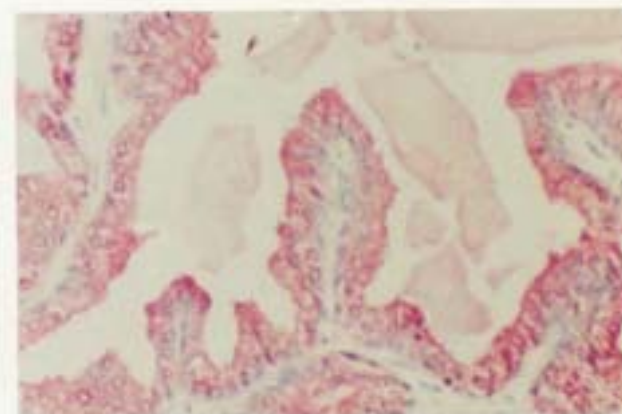


Fig 4B.—Adjacent section of prostate gland prepared with conventional method and immunostained with prostate-specific antigen (PSA, 280 $\times$ ).

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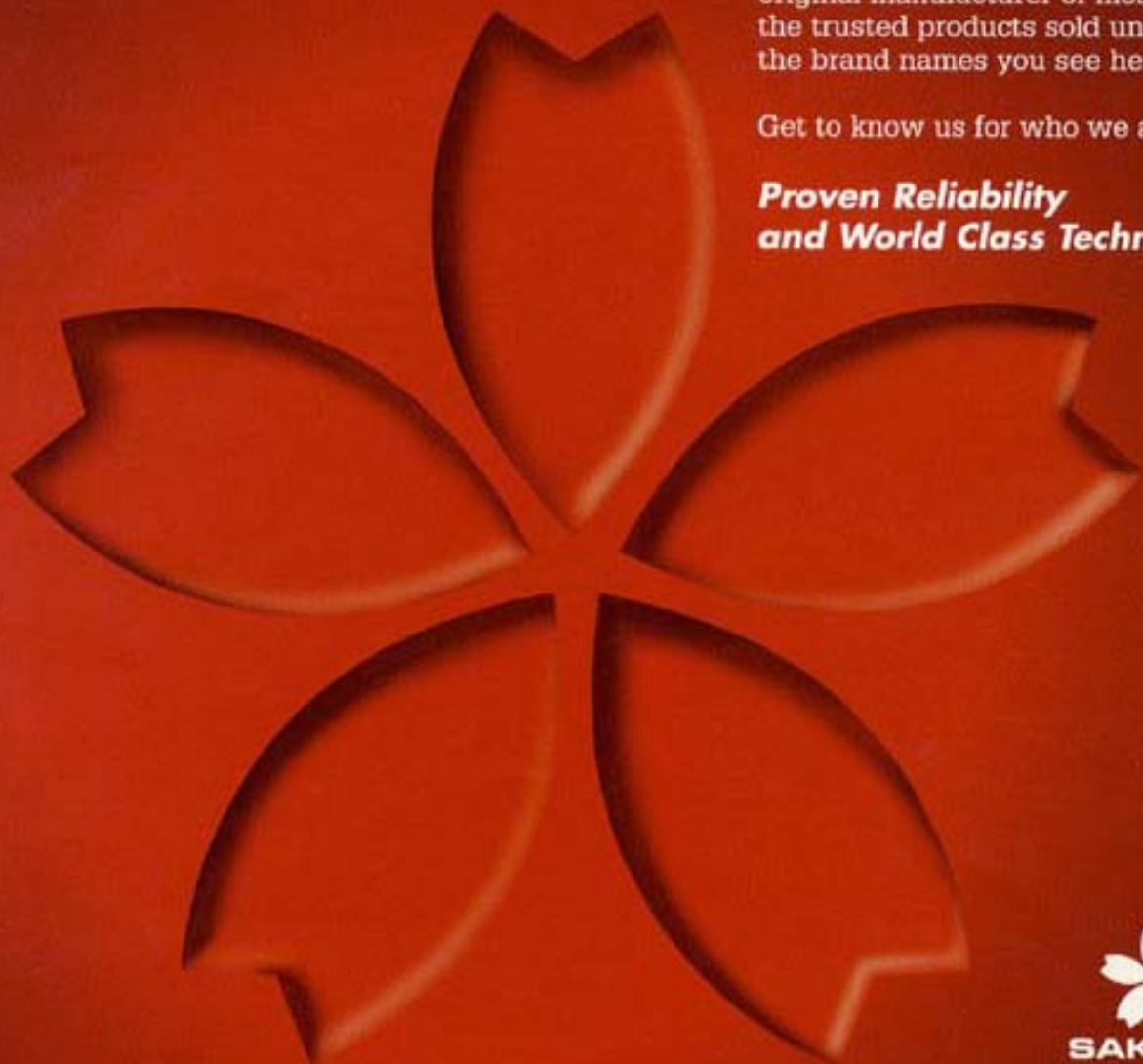
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This first issue of the new *Histo-Logic* presents the perfect opportunity to introduce you to Sakura Finetek U.S.A., Inc. — the 120-year-old company that acquired the histology and cytology product lines of Miles Inc.

It may come as a surprise to many of our readers, but Sakura is the original manufacturer of most of the Tissue-Tek,<sup>®</sup> Cyto-Tek,<sup>®</sup> and VIP<sup>™</sup> products that you've become familiar with over the years. The acquisition also includes the rights to Accu-Edge<sup>®</sup> Blade Systems, Uni-Cassette<sup>®</sup> Systems, Histo-Tek,<sup>®</sup> and Cryomold<sup>®</sup> branded products.

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Mr. Matsumoto continues: "Sakura's business lines of medical care and health equipment are closely related to

growing industries such as biotechnology, fine chemicals, and computers. Innovative application of fresh ideas will lead to the development of new hardware and software and high-level engineering activity." The true test of expertise is to keep the present keenly in focus and under control, while guiding the company into the unknown realms of the future.

"Traditionally, medicine has focused almost exclusively on the diagnosis and treatment of physical illnesses. With a world population that continues to grow older, an integrated approach that covers both mental and physical illnesses is essential to promote health in such a society. Only then will the aged truly enjoy their longevity. Sakura aims today to become that type of health care industry — to grow to fulfill the many needs of the elderly, while keeping the current needs of the histotechnologist in the forefront of our research and development schedule."

As a truly diversified manufacturer with proven expertise in designing and manufacturing histology and cytology products, Sakura also produces high-volume sterilization equipment, water processing systems, ultrasonic cleaning equipment, electron microscopy processors, an automatic smear/stainer system for hematology, and more. Sales, distribution, and manufacturing facilities for Sakura are located throughout Europe, Africa, the Middle East, Asia, Oceania, Latin America, and the United States and Canada.

The agreement reached between Sakura and Miles, from the perspective of both companies, is destined to benefit Sakura's many customers. By consolidating product lines with the manufacturer and then streamlining marketing, sales, and customer support, Sakura officials are sure that cost consolidation will only positively affect product cost from the customer's point of view.

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## “Histoids”: Helpful Hints for Histology Hackers

- When inking specimens for margins, use a solution of 10% acetic acid to fix the ink. Not only does it work as well as Bouin's solution, it is less costly and environmentally safe. (submitted by Beth Rohland, HT, Monongalia General Hospital, Morgantown, WV)
- Histoplasmosis will not stain with the GMS method if oxidized with periodic acid; chromic acid must be used as the oxidizing agent.
- If you can see, smell, or taste your tap water, don't use it for staining.
- Acetone can be used in the place of Carnoy's to help visualize lymph nodes in fatty tissue.
- Compatibility with xylene substitutes and mounting medium depends upon the solvent used in the mounting medium.
- Try using a hair dryer to dry slides quickly that have to be mailed.
- Agar is great for use in preparation of cell blocks, but be aware that the agar will bind with the second layer in avidin-biotin immunoprocures.
- Stain the first and last slide when you are cutting controls for your special stains. If both are positive, then you can be pretty sure all the others in between will also be positive.

## Technical Note: A Cost-effective Masson's Trichrome Stain

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Pullman, WA 99164

Recently I did a cost analysis of all of our staining procedures. I was surprised to see how much it cost to perform a Masson's Trichrome Stain. I remembered a footnote at the bottom of Gomori's One-Step Trichrome procedure that substituted aniline blue for light green, if blue stained collagen was preferred.

I stained two sections of the same tissue, one with Masson's Trichrome procedure and the other with Gomori's Trichrome procedure using the aniline blue. Our pathologists saw no distinguishable differences between the two stains. When I told them the Gomori's stain cost about a third of the Masson's, they were impressed with the results versus the cost. My technicians also prefer Gomori's procedure for its simplicity. So now, when anyone requests a trichrome stain, it's just a matter of asking them whether they want blue or green collagen!

## The BLANK CONTROL Method: A Handy Tip for Using and Filing Control Slides for Special Stains

**Terri L. Braud, HT(ASCP)**  
St. Frances Cabrini Hospital  
Department of Pathology  
Alexandria, LA

A single batch of stain requires only one control to insure control for the entire batch of stain. Although multiple cases can be batched together in a single stain, the College of American Pathologists (CAP) requires a special stain control for every *case* and that control must be retrievable from a file. This can often result in having to use multiple control slides per batch (one to be filed with each case), or in having to maintain a separate file system to keep track of controls per batch of stain.

Our department has developed a unique, time-saving method that meets with CAP requirements and saves on control material as well. The **BLANK CONTROL** method calls for one control per batch of stain. That control is labeled with the first case number of the batch, the type of stain, "control" and is filed with the case. All other cases in the batch have a *blank slide* labeled with the case number, the type of stain, "control," and, in parenthesis, the case number where the batch control is filed. A labeled blank slide is filed with each case in the batch. This provides a quick method for maintaining stain control and meeting CAP requirements for filing.



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## See You in Buffalo!

**NSH Symposium/Convention Set for  
October 7 - 13, 1995**

The National Society for Histotechnology Annual Symposium/Convention is shaping up nicely for this fall in Buffalo, New York. A quick look at the program shows that over 75 separate presentations have been scheduled for this year's event — with 14 concurrent clinical and V.I.R. (veterinary industrial research) sessions scheduled for Thursday morning and afternoon, October 12.

The NSH Symposium/Convention will be held in the Buffalo Convention Center, which connects directly with the Hyatt Regency Buffalo — a spectacular complex located right in the heart of the theatre and financial districts.

NSH members should have received all registration information and materials by now. If you haven't, you should call the NSH office: 1-301-262-6221 (FAX: 1-301-262-9188) or write to:

**National Society for Histotechnology  
4201 Northview Dr., Suite 502  
Bowie, MD 20716-2604**

And be sure to reserve Tuesday evening, October 10, for a special dining event cosponsored by Sakura Finetek U.S.A., Inc.; Baxter Healthcare Corporation;

and Stephens Scientific for all members of the NSH. Join us for a well-deserved, relaxing evening with old friends and a chance to meet new acquaintances at the exclusive Starlight Café for a light dinner, hors d'oeuvres, and refreshments. That's Tuesday evening from 7:00 PM to 10:00 PM for light dinner, light music, a light evening at the **Starlight Café**.

Sakura will also be hosting a last-night cocktail party just prior to the Awards Banquet Thursday night, October 12.

We extend a warm invitation to all.  
See you in Buffalo!



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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., 18700 Crenshaw Boulevard, Torrance, CA 90504.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Terri Staples, *Histo-Logic* Editor, 1127 Caribbean Circle, Alabaster, AL 35007. Articles, photographs, etc. will not be returned unless requested in writing when they are submitted.

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