

Histo-Logic[®]

Technical Bulletin for Histotechnology - Scientific Editor, Lee G. Luna, D. Lit. (Hon.) H.T. (ASCP) - Managing Editor, Brent Riley Vol. XX, No. 2, Mar./Apr. 1990

An Economic Reticulum Stain

Osvaldo Jose Vilar Naval Hospital Buenos Aires 1405 Republica Argentina

Fixation	10% buffered neutral formalin.
Process	Paraffin.
Microtomy	Cut sections at 6-8 micrometers.

Solutions

Ammoniacal Silver Solution

To 5 ml of 20% silver nitrate solution and 2.5 ml of 10% aqueous solution of sodium hydroxide, add 2 ml of ammonia water (26-28%) stirring until precipitate is completely dissolved. Make up the solution with distilled water to twice its volume. Use acid-cleaned glassware. Make fresh solution each time.

1% Potassium Permanganate

Potassium permanganate .	 gm
Distilled water	ml

1% Sodium Metabisulfite

Sodium metabisulfite	 1.0	gm
Distilled water	 100.0	ml

2% Ferric Ammonium Sulfate

Ferric ammonium sulfate	gm
Distilled water	ml

Staining Procedure

- Deparaffinize slides in 2 changes of xylene, 2 minutes each; absolute alcohol 2 changes, 2 minutes each; and 95% alcohol, 2 changes, 2 minutes each.
- 2. Rinse slides well in distilled water.
- Oxidize in 1% potassium permanganate solution for 1 minute.
- 4. Rinse in distilled water for 1 minute.
- Differentiate with 1% sodium metabisulfite solution for 1 minute.

- 6. Rinse in distilled water for 1 minute.
- Sensitize with 2% ferric ammonium sulfate solution for 1 minute.
- 8. Wash in tap water and two dips in distilled water.
- Place in ammoniacal silver solution for 3 to 5 minutes, depending on intensity of reactions desired.
- 10. Dip twice in distilled water.
- 11. Wash in pyridine solution for 1 minute.
- 12. Two dips in distilled water.
- 13. Reduce in 10% formalin solution for 2 minutes.
- 14. Wash in tap water.
- Dehydrate slides in 95% alcohol, absolute, and clear in xylene, 3 changes each.
- 16. Mount coverglass with resinous media.

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No reader should utilize or undertake procedures in *Wino-Logic* articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

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Figure 1: This figure illustrates the intensity of the silver reaction on reticulum fibers. The degree of reaction can be reduced if desired by reducing the time of silver exposure at Step 8 test staining procedures.



Figure 2: A higher power of Figure 1 to show the quality of staining in greater detail.

Results

Reticulum fibers (Figs. 1 and 2) black Background gold and brown

References

Lunia, LG: Manual of Histologic Staining Methods of the AFIP New York, McGraw Bill, 1968, 92-93.

J. Vacca, LL: Laboratory Manual of Matschemiatry New York, Raven Press, 1983, 6-255.

Microwave Modification of Luna's Method for Melanin

Charles J. Churukian, B.A., HT, HTL (ASCP) Department of Pathology and Laboratory Medicine University of Rochester Medical Center Rochester, NY 14642

A microwave modification of Luna's' method for melanin is described. Like Luna's method, this procedure is useful for demonstrating melanin in amelanotic melanomas. Both of these methods produce staining results that are superior to and more specific than those obtained with the Fontana-Masson' procedure. This new method requires only half the amount of silver nitrate used in Luna's procedure, requires less than 15 minutes to perform, and produces consistent and reliable staining results (Fig. 1 and Fig. 2).

Fixation	10% buffered neutral formalin
Process	Paraffin.
Microtomy	Cut sections at 5 microns.

car sections at 5 milerous.

Solutions

Acidulated Water

0.5% Silver Nitrate Solution

Silver nitrate		0.2	gm
Acidulated water .		40.0	ml

2% Silver Nitrate Solution

Silver nitrate	0.2	gm
Acidulated water	10.0	ml

4% Gelatin Solution

Gelatin, BBL, or Fisher Type A	gm
Acidulated water	ml
Place in a beaker and place on a magnetic stirrer	and
apply centle heat until dissolved.	

0.1% Hydroquinone Solution

Hydroquinone 0.015 gm Acidulated water 15.0 ml Place the 2% silver nitrate, 4% gelatin, and the hydroquinone in separate flasks in a 56°-58°C oven. Allow the solutions to remain in the oven until thoroughly warmed before preparing the developer.

Silver Nitrate-Gelatin-Hydroquinone Developer

2% silver nitrate	ml
4% gelatin	ml
0.1% hydroquinone	ml
Combine in order given in a warm 125-ml flask m	aking
certain the solutions are mixed well after each add	ition.
Prepare immediately before use.	

Nuclear Fast Red (Kernechtrot) Solution

Dissolve 0.1 gm nuclear fast red in 100 ml or 5% solution of aluminum sulfate with the aid of heat. Cool, filter, and add a few grains of thymol as a preservative.

Staining Procedure: Use positive control slide.

- 1. Deparaffinize and hydrate to acidulated water pH 3.2.
- Place slide in 40 ml of 0.5% silver nitrate in a plastic coplin jar and microwave at power level 8 (480W) for 45 seconds. Dip the slides up and down several times and allow them to remain in the hot solution (85°C) for 5 minutes.
- Place slides in freshly prepared silver nitrate-gelatinhydroquinone developer in a plastic coplin jar and microwave at power level 1 (60W) for one minute. Dip the slides up and down several times and allow them to remain in the warm solution (63°) for 2-3 minutes.
- 4. Wash quickly and thoroughly in running hot water.
- 5. Rinse in two changes of distilled water.
- 6. Place in nuclear fast red solution for 3 minutes.
- 7. Rinse in two changes of distilled water.
- 8. Dehydrate in graded alcohols.
- 9. Clear in three or four changes of xylene.
- 10. Mount with Permount.

Results

Melanin	. black
Nuclei light pink or	orange
Cytoplasm	vellow

References

- Warket, RL, Luna LG: Helwig EB: A modified Warthin-Starry procedure at low pH for melanin. Amer J Clin Pathol 1980; 73:822-815.
 Masson, P: Carcincids and nerve hyperplasia of the appendicular macosa. Amer J
- Masson, P. Carcinolis and nerve hyperplasia of the appendicular macosa. Amer J Clin Pathol 1928; 4:181-212.
- Kerr DA: Improved Warthin-Starry method of staining spirochetes in tissue sections. Amer J Clin Pathol 1938; 8:63-67.



Figure 1: This section illustrates the staining qualities of the silver reaction (right) with those of the background (left), X400.



Figure 2: High power of the same section seen in Figure 1 to illustrate the well-defined melanin granules. X400.



Figure 3: This photograph demonstrates the staining of lipofuscin in the cytoplasm of hopstocytes, X400.

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Comments:

Luna's and this staining method are modifications of the Warthin-Starry' method for spirochetes and bacteria. The pH of the acidulated water in the Warthin-Starry is 4.0-4.4. In Luna's and our method, the pH of the acidulated water is 3.2. This lower pH results in less background staining allowing for better visualization of melanin.

We have found that this method is more sensitive and specific for melanin than the Fontana-Masson. The Fontana-Masson stains argentaffin, chromaffin, lipofuscin and ferric iron. None of these pigments, except for lipofuscin, will stain with the described method. (Fig. 3). Amelanotic melanomas that stain poorly or not at all with the Fontana-Masson are usually well stained with our method. The staining results obtained with the described method compare favorably to those obtained with the immunocytochemical method, using the monoclonal antibody HMB-45, in staining melanin in amelanotic melanoma. Obviously, this silver nitrate method is less specific than the immunocytochemical procedure for demonstrating melanin, but it appears to be nearly as sensitive.

When solutions are heated with microwave irradiation, there can be up to a 15°C difference in temperature between the top and bottom portion of the solutions. Therefore, in order to equalize the temperature of the solutions, the slides are dipped up and down in Steps 2 and 3 of the staining procedure. The temperature of the 0.5% silver nitrate solution will be about 85°C and that of the developer about 63°C.

The microwave oven that we use is a General Electric Model JE 1027J with touch pad variable power supply up to a maximum of 600W. Other makes of microwave ovens can be used in performing this method, but it may be necessary to make changes in the selection of the power settings and times used in Steps 2 and 3 of the staining procedure. The shelf life of silver nitrate and hydroquinone can be greatly increased by storing the reagents in a refrigerator at 3-6°C.

Autoradiography and Correlative Imaging International Conference August 1-3, 1990, in Chapel Hill, NC.

International Conference and Workshop on In Vitro/In Vivo Autoradiography & Correlative Imaging (a satellite conference of the Joint Histochemical Meeting of the American and Japanese societies), University of North Carolina, and Merck Sharp & Dohme Research Laboratories. Topics include techniques, receptors, drugs, hormones, molecular probes, toxins, metabolism, and image analysis. The program will include submitted papers, invited speakers, workshops, and round-table discussions. There will be a workshop on the application of in vitro techniques to SPECT & PET imaging. Fees: scientists-\$145: students/fellows-\$35, contact Autoradiography Conference, University of North Carolina, P.O. Box 64, Chapel Hill, NC 27514, (919) 966-1144. Scientific Inquiries and Exhibitors, contact Howard Solomon, (215) 661-6834; or Walter Stumpf (919) 966-1144.

Reserve Your Space Now!

NSH Annual Symposium/Convention San Antonio, TX September 8-14, 1990

A reminder to register for workshops...

A schedule of events and scientific sessions can be obtained through the NSH office:

> NSH 5900 Princess Garden Parkway Suite 805 Lanham, MD 20706 (301) 577-4907

National Society for Histotechnology Benefits of Membership

Lena T. Spencer Membership Chairperson

Why do I want to become a member of the NSH? What are the benefits of being a member? What do I get for my money? We all want to know that our money is being spent wisely and that we will get a return on our investment. Members and nonmembers may have forgotten or be unaware of the opportunities afforded them by being involved in the National Society for Histotechnology.

With membership renewal time only a few months away and the desire to increase NSH membership. I thought this would be an opportune time to update and review the benefits with you.

I. Information, Communication and Networking

Journal of Histotechnology – What better way to stay abreast of the changes taking place in the field of histology. The journal is a highly respected professional publication specifically directed to the specialty of histotechnology. Articles consist of basic and highly technical information, book reviews, and literature abstracts. This is your place to publish! A subscription is included with membership.

NSH In Action – The Intra-Society Newsletter providing members with general information on Society affairs, official activities, and noteworthy news. The newsletter permits NSH to communicate with members on a regular basis.

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Extension Seminar – A traveling seminar provided to states with unorganized or inactive societies. This gives all members the opportunity to participate in continuing education.

Educational Aids – Available at a minimal cost are the Carbohydrate Staining Reference Manual and the Self-Assessment Examination Booklets: Fixation, Processing and Decalcification. Routine Staining, Embedding and Sectioning, Immunologic Staining, Electron Microscopy, and An Introduction to In-Situ Hybridization and Flow Cytometry.

Training Aids, Educational Resources, and Teleconferences-Providing quality programs for all levels of expertise in the field of histotechnology.

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III. Scholarships and Awards – Opportunities for educational advancement and recognition of excellence for members. Over \$10,000 (ten thousand dollars) is available to those who take the initiative to apply for the scholarships and awards that are presented yearly.

IV. Additional Benefits

Liability Insurance Policy Employment Opportunities Updates Promotional Materials-Vocational and Career Information

Volunteer Opportunities—Participation on committees and task forces to develop the programs and promote the growth of the Society.

What are the benefits of membership? Professional growth through formal and continuing education, maintaining knowledge and skills, opportunities for volunteerism, a means of networking and communication, and a voice for this laboratory discipline. The return on your investment will ultimately translate into better patient care.

Let's make this The Decade of Growth. Encourage and invite one new person to join the NSH.

Lena T. Spencer, HT (ASCP) HTL Membership Chairperson

For additional information on the 1990 Symposium/ Convention Program, or membership application, contact:

National Society for Histotechnology 5900 Princess Garden Pkwy, Suite 805 Lanham, MD 20706 (301) 577-4907

Sociedade Brasileira De Histotecnologia (Brasilian Society of Histotechnology)

15 Years Old

The Brasilian Histotechnology Society celebrated its 15th anniversary in May of 1990, during its annual meeting. The Society was founded in Brasilia, the capital of Brasil, with the assistance and encouragement of Lee G. Luna during his conduct of the Second, two-week International Training Course in 1975. The Society has been very active since its inception, and has conducted semi-annual meetings in conjunction with the Brasilian Society of Pathologists. The formation of this Society in 1975 was directly related to the interest stimulated for the National Society for Histotechnology being formed just two years prior. The technologists in Brasil were aware of the progress made by NSH, and it was their desire to model their Society after our own. The initial membership numbered 163 histotechnologists, all of whom were in attendance at the training course cited above. The number of members has fluctuated between this figure and 200, making it an active paramedical group in a country sparsely populated with histopathologic laboratories.



Application for NSH Membership

Editor's Note: The National Society for Histotechnology is a professional society representing all of those involved in histology. We at Mifes Inc. encourage aff histotechnologists to consider joining this highly respected and worthwhile organization.

If you are interested in becoming a member of the NSH, please fill out this application and return it to:

> National Society for Histotechnology 5900 Princess Garden Parkway Suite 805 Lanham, MD 20706

Name	
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	and the second second
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Place of Employment	1
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	- The state of the
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Signature	Dute

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□ MT (ASCP)	□ MA/M\$	Private Lab
CT (ASCP)	D PhD	□ Veterinary
C RT (CSLT)	GMD	□ Marine
ART (CSLT)	DVM	Botany
Other	Other	_ 🗆 EM
□ Not Certified		C Research
		Industrial

Annual dues \$30.00 United States lunds.

\$10 of dues applied to Journal of Histotechnology subscription, PROFESSIONAL SOCIETY DUES ARE TAX DEDUCTIBLE.

Questions in Search of an Answer

Editor's Note: Please send responses to Lee G. Luna, American HistoLabs, Inc., Kolb Center, 7605-F Airpark Road, Gaithersburg, MD 20879

Figures 1, 2, 3, and 4 demonstrate an occasionally occurring artifact in our laboratory. We would be interested in receiving information as to the possible reason for the deposition/development of this artifact. The slides have been stained with a conventional hematoxylin and eosin procedure. The artifact is not evident during the staining process but becomes evident after mounting of the coverglass. As seen throughout these photographs, the problem can be extremely troublesome and, more importantly, can affect the diagnostic process.

Fred Argilan Pathology Associates, Inc. Frederick, MD 21701



Figure 1: The boson pigmentike material sent throughout this phonograph is a tradienter occurrence in out laboratory. Now These does not seem to be a specific pattern of deposition development.



Figure 2: Higher power of section to better illustrate the pigmentifile material. Notice that there is less pigmentlike material in the nonlocal plane of the section.



Figure 3: Illustrates the same problem but with a dightly different pattern of pigmentilat deposition: development.



Figure 4: High power of Figure 3 to illustrate that the pigmentikle deposition/ development is more dense and limited to the glandular portion of the tissue.

Figure 1 illustrates a brown funguslike growth. The slide was stained with routine hematoxylin and eosin. The chromophoric-stained organism is not stained with the hematoxylin or eosin.



Figure 1: Figure illustrates chromophoric tangastike organisms stated with H & E.



Figure 2: This section demonstrated the chromophoric organisms in its natural color throws i and blue after alclass blue examing.

Figure 2 demonstrates the same funguslike growth on the surface of the epidermis of a section of skin. This slide was stained with H & E followed by the alcian-blue counterstain. Notice that some of the smaller organisms are stained positive. Any ideas on the cause of this artifact would be welcomed.

Lee G. Luna American HistoLabs, Inc. Gaithersburg, MD 20879 In the neuropathology laboratory, 1 have encountered a problem with the ATPase stain. 1 use the calcium method for demonstrating adenosine triphosphatase in muscle biopsies. We have noticed that the stain begins to fade after one year, and in three years time, some cases are barely readable. Xylene is used as a clearing agent to clear the slides, and Permount as the mounting medium. Does anyone know of a way to prevent the stain from fading?

Cathy Housman, HTL (ASCP) Neuromuscular Pathology Laboratory University Hospital Penn State University Hershey, PA 17033



Brent Riley Managing Editor

Editor's Note:

From time to time, *Histo-Logic* will publish articles that provide management tips to those in supervisory positions and to those who have management as a career goal. If you supervise a lab and have questions or suggestions about the best way to perform responsibilities, please let us know.

With the rising demand for skilled histotechnologists, it is becoming increasingly difficult to fill vacant positions in the histology lab. But, as any good manager knows, the best way to avoid a shortage of help is to hold on to the people you have. That means taking advantage of every opportunity to improve the motivation and skill level of existing employees.

One of these opportunities is the employee performance review. Although most institutions require an annual or semiannual review, it often turns into a negative experience for both the employee and the manager. Sometimes, both actually fear the event; and respect, trust, friendship, and motivation can be destroyed. Usually it

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is the manager, not the employee, who is at fault. As a manager, it is imperative that you be familiar with the "Do's and Don'ts" of the employee performance review.

 Be sure to carefully plan and schedule the review process to meet institutional deadlines without rushing through, or giving the appearance of rushing, through the review process.

 Give the employees plenty of notice of the upcoming reviews, so they have adequate time to think about their own evaluations.

 Be sure your employees know what is expected of them. Write down the standards of performance you and the institution expect from them. Give them a copy of these performance criteria.

 Always conduct performance reviews in private and keep them confidential. Never discuss one worker's evaluation with another.

 Be positive. Don't say, "The hospital has insisted that I do this performance review." Make the employee realize that the review is for his or her benefit, too.

 Select no more than two or three areas for suggested improvement and provide suggestions as to how the employee can reach specific goals. The average individual can only effectively focus on two or three goals at one time.

 Ask employees for feedback. How do they feel about their performance? How do they think they can improve? What goals have they set?

 Don't make promises you can't keep. Don't entice an employee with a raise or promotion as a reward, unless you can fulfill that promise.

 Be certain to identify the positive aspects of an employee's performance, as well as the negative.

 Don't nitpick. Discuss only those points that significantly impact the employee's performance.

 Offer to help. Ask your employees how you and the institution can help them perform better or reach their goals.

A properly conducted employee performance review can be an effective tool in maintaining good employeemanagement relations. In the long run, it can help to significantly reduce the employee turnover rate in your lab.

Ammoniacal Silver Method for Fungi in Undecalcified Bone Marrow Sections

Charles J. Churukian, HTL (ASCP) University of Rochester Medical Center Rochester, NY 14642

Fixation 10% buffered neutral formalin.

Process Undecalcified glycol methacrylate embedded bone marrow.

Microtomy Cut sections at 4 micrometers.

Solutions

I

4% Periodic Acid

Periodic acid, H,I06	4.0	gm
Distilled water		ml

Ammoniacal Silver Solution

To 10 ml of 10% silver nitrate, add 5.0 ml of 4% lithium hydroxide, monohydrate. Then add concentrated ammonium hydroxide, drop by drop with constant shaking, until the precipitate just dissolves. Make up the solution to 1000 ml with distilled water and store in a refrigerator at 3-6°C. This solution is stable for about one month.

0.2% Gold Chloride

Gold chloride	 gm
Distilled water	 ml

2% Sodium Thiosulfate

Sodium thiosulfate	gm
Distilled water	ml

Fast Green, Stock Solution

Fast green, FCF, C.I. 420530.2	gm
Distilled water	ml
Acetic acid	ml

Fast Green, Working Solution

Fast green, stock solution	. 10.0	ml
Distilled water	. 40.0	ml

Staining Procedure

- 1. Place section in 4% periodic acid for 5 minutes.
- 2. Rinse in five changes of distilled water.
- 3. Pour 40 ml of ammoniacal silver in a glass coplin jar and allow to warm to near room temperature. Place slides in the solution and set the coplin jar in a 43°C waterbath for 3 minutes, then transfer to a 58°C waterbath for 25 minutes.
- 4. Rinse in five changes of distilled water.
- Place slides on a hot plate at 60-65°C for 5 minutes. This step is necessary to prevent the loss of sections in the proceeding steps.
- 6. Tone in 0.2% gold chloride for 30 seconds.
- 7. Rinse in two changes of distilled water.
- 8. Place in 2% sodium thiosulfate for 30 seconds.
- 9. Rinse in four changes of distilled water.
- Counterstain with working fast green solution for 2 minutes.
- 11. Rinse in three changes of distilled water.
- Dehydrate slides in 95% alcohol, absolute, and clear in xylene, 3 changes each.
- 13. Mount coverglass with resinous media.

Results

Fungi		. 5	hi	IT	ph	y ı	de	:li	nk	3	te	d	in	8	ra	y	to	blac	3k
Background																		gree	m

Remarks

In the staining method of Castro and Maynard for plastic embedded sections, chromic acid is used as the oxidizing agent. We found that this causes the plastic to stain varying shades of gray and brown but, even so, the fungi stain a rather intense grayish brown. However, because the plastic is stained, the fungi are somewhat difficult to locate. Oxidizing the sections with periodic acid prevents the plastic from staining, but the fungi are less intensely stained than when they are oxidized with chromic acid. Even so, the fungi are stained well enough to be easily located and identified.

We tried using methenamine silver in place of ammoniacal silver in the described method after oxidizing the sections with chromic acid and periodic acid but found that the fungi would not stain. This may be due to a difference in the pH of the two solutions. The working methenamine silver has a pH of about 8.9 and our dilute ammoniacal silver a pH of about 11.4.

References

- Churukian CJ, Schenk EA: Dilute Ammoniaeal Silver as a Substitute for Methenamine Silver to Demonstrate Pneumocyatic carinii and Fungi. Lab Med 1986; 17:87-90.
- Churukian, CJ: A More Stable Ammoniacal Silver Using Lithium Hydroxide. Eab Mod 1986; 17:260.
 Churukian CJ, Schenk EA: Staining Pneumocycritic corinii and Fungi in Unfraed
- Specimens with Ammoniacal Silver Using a Microwave Oven. J. Histotechnol 1988;11:19-21.
- Casto MD. Maward JH: Routine and Special Staining Techniques in Object Michaerylane. Presented at the 1988 NSH Symposium Convention in Louissille, Ky.

The Use of Hematoxylin in the Microwave Oven

Cheryl L. Crowder, HTL (ASCP) Department of Pathology School of Veterinary Medicine Louisiana State University Baton Rouge, LA 70803

Although heat is used to make some hematoxylins, excessive heat will overoxidize the stain and render it useless. The stain, in essence, "dies." Hematoxylins can be heated in the microwave oven to decrease staining time. However, a few precautions must be taken.

- Heat the hematoxylin solution first, check the color to make sure the stain has not broken down (see Step #5), and then add the slides. If slides and hematoxylin are heated together and these slides can be decolorized somewhat in acid alcohol, the decolorization is not complete and the slides do not restain well. Generally 50 ml of hematoxylin can be heated to 55°C in approximately 20 seconds on "high" with no deleterious effects.
- Different formulae of hematoxylins heat differently, depending on the solvent of the formula. The greater the percentage of alcohol in the formula, the more rapidly the solution will heat (e.g., Delafield's). Formulae with ethylene glycol (e.g., Gill's) appear to heat at the same rate as water.
- Hematoxylin solutions containing heavy metals (chrome alum, iron, mercuric chloride) heat more quickly than other formulae. Care should be taken not to overheat these solutions.
- 4. Weigert's iron hematoxylin is an exception to the rule. The hematoxylin is heated to 55°C for stains such as the Verhoeff-Van Gieson. The stain can be heated and slides added, or both can be heated together. The

slides are allowed to set on the counter for 3-5 minutes. They can be differentiated. If over-differentiation occurs, the stain can be reheated and the slides restained.

- 5. To check the hematoxylin's activity, add 1-2 drops of the hematoxylin solution in question to 25-50 ml of tap water. If the water changes color to blue, bluepurple, or black, the stain is good. If the water turns brown, the stain has lost its staining ability and must be thrown away. Once heated, the hematoxylin can be reused if the color is checked.
- 6. The most important factor in staining with heated hematoxylin is not to allow the solution to become too hot. Temperatures should not exceed 55-60°C. Above these temperatures some damage can be seen in the tissue itself, and the hematoxylin (depending on the formula) may begin to overoxidize. Several hematoxylin solutions have been tested in this laboratory. We have found that the average hematoxylin solution reached 55°C in a microwave oven set on "high" in 20 seconds. With shortened heating times (5-10 seconds) and/or lower oven temperatures, the stain should not break down, and staining should be accomplished successfully with no deleterious effects to the tissue.

Terri Staples ...48 Hours a Day

Brent Riley Managing Editor

"If I had 48 hours in every day and didn't have to sleep, I could probably get everything done I have to get done," explained Terri Staples. But Staples, winner of the 1989 Golden Forceps Award, can already accomplish more in a single day than most people can accomplish in 48 hours.

Terri Staples is a histotechnologist, a lab supervisor, a teacher, a student, a published writer, president of her state society, a region director for the NSH, a member of three NSH committees, a mother, a wife, and a singer in a rock-and-roll band.

"My mother says I'm the most disorganized-organized person she knows," Staples said. "I try to be efficient, but I'm not always neat. I have a list to keep my lists on." Staples is the histology supervisor at Montclair Baptist Medical Center in Birmingham, Alabama. She supervises four full-time and three part-time histotechnologists, as well as assists the education coordinator with the hospital's accredited school of histotechnology.

"All through high school, I was fascinated by biology," Staples recalled. "In fact, I wanted to be a marine biologist, until I found out how much chemistry was involved. I didn't care for chemistry."

After graduation, she became a clerk in the lab office at DePaul Hospital in Norfolk, Virginia. While there, she developed an interest in lab work, especially histology. "I knew I wanted to do more than be a lab clerk," she explained, "but I couldn't be a nurse because I couldn't deal with the patient. I would get too involved and too emotional. In the lab you can help without getting too involved with the patient. You can feel like you've done your part in helping someone, but you don't have to see their pain."

In 1974, Staples enrolled in the hospital's certified school of histotechnology. She passed her HT registry in 1975 and, five years later, returned to DePaul Hospital as supervisor of the histology lab.

After only six months in that position, her husband was transferred to Birmingham, and she started working as a bench tech at Montclair Baptist Medical Center. After three years, Staples went into research at the University of Alabama at Birmingham. While there, she was involved with tissue procurement for cancer research.

She had an opportunity to work with Dr. William Grizzle, whose specialty is endocrinology. The challenges of that work led her to try to find better ways to stain the pancreatic and adrenal tissues that were used in the research. "Dr. Grizzle believed in letting me grow and develop in my profession, and had faith in my abilities."

It was the result of these efforts that led to her first published article, "The Effect of Temperature on Argyrophil Impregnation—Development of a High Temperature Rapid Argyrophil Procedure." The paper was published in *Stain Technology*.

The procedure she discovered is quick and doesn't require expensive antibodies. "It's a rapid way of determining if these [argyrophil] granules are present," she explained. "It's a good procedure for screening. You can then go on to use the immuno procedures to determine what is in the granules."

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At about the same time, she had another article published in *Laboratory Medicine*. It, too, was about argyrophil impregnation. She and Dr. Grizzle determined that a methyl green counter stain provided the best results for argyrophil impregnation because it did not interfere with the granules, and it did not affect the quality of black-and-white photography.

Staples' next article, which was titled, "Methods for Staining Campylobacter Pyloris," won her the Golden Forceps Award at the 1989 NSH Symposium/ Convention in Las Vegas. "I was on cloud nine," she said. "I was so excited and so honored. It's like getting a good-citizenship award for helping an elderly lady cross the street."

The Golden Forceps Award is sponsored by the Diagnostics Division of Miles Inc., and recognizes annually the writer of the best scientific article in *Histo-Logic*. Staples' article was published in the September/October 1988 issue.

"All of a sudden there was this big commotion about Campylobacter," Staples recalled. Her goal in writing the article was to explain what Campylobacter is, and why it is important to find it in tissue. She also wanted histotechnologists to be aware that there was a choice of methods for staining Campylobacter. Her article provided details on three methods: A modified Steiner technique, the Gimenez technique, and a modified one-hour Giemsa stain.

"I had never heard of *Campylobacter* before," she continued. "But I'm an inquisitive person. So when I started to get requests for *Campylobacter*, I did some research and found out what it was,"

Staples is a firm believer in the importance of gaining a full understanding of what histotechnologists do. "It isn't always enough to simply know how to perform a certain staining procedure," she said. "You have to understand what you're doing, and why you're doing it."

The article was originally written for her state society newsletter for which she writes many articles. When Lee G. Luna, editor of *Histo-Logic*, saw it, he asked Staples to expand the article and submit it to *Histo-Logic*. She was happy to oblige.

She has recently written another article that will soon be published in the *Journal of Histotechnology*. It discusses dilute ammoniacal silver nitrate solutions for reticulin and argentaffin. "Histotechs have only each other to teach each other," she said. "We have to share what we know. I write because people need to know,"

Staples has always been a proponent of education. When she moved to Birmingham, she completed her bachelor's degree by attending classes at night. She earned her degree from the University of Alabama in 1986, then passed the HTL registry in 1987. She is currently enrolled in graduate school.

Also in 1987, Staples returned to Montclair Baptist Medical Center to become supervisor of histology.

She devotes a lot of time and effort to her state society, as well as the NSH. She is currently president of the Alabama Society for Histotechnology. As Region 3 director for the NSH, Staples works hard to keep the lines of communication open within her territory, which includes North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Tennessee, and Puerto Rico. Last year, she helped the Mississippi state society arrange a continuing education meeting. She'll be going back to present a workshop for its 1990 meeting.

Staples is on three committees for the NSH, including the Education Committee, the Quality Control Committee, and the Continuing Education Subcommittee. She is scheduled to present two workshops at the 1990 NSH Symposium/Convention in San Antonio—one on preparing for the HT and HTL registries, and one about CAP work-load recording and quality-assurance monitoring in the histology lab.

Staples has big plans for the future, too. "There's so much I want to do," she said. "I want to see the status of my profession elevated. I want to see histotechnologists get to the level where medical technologists are now."

Incidentally, even with her busy schedule, Staples does manage to set aside some free time for herself and her family. She is the team mother for her son's baseball team. And on weekends, she sings in a rock-and-roll band.

Most people would like to have 48 hours in every day. But those who know Staples, know exactly what she would do with the extra time. She would get even more involved in her work. She would take on even more responsibilities. She would take advantage of still more educational opportunities. And she would wish for 72 hours in every day.

Cleaning Plasticware "Ruined" by Metallic Microwave Staining

Frank Razzaboni Charles River Laboratory 251 Ballardvale St. Wilmington, MA 01887

Metallic staining of tissue sections by microwave oven is being performed in a ever-increasing number of histology laboratories. Occasionally, overstaining happens, and a heavy black film coats the inner wall of the plastic coplin jars. In most cases, this can be cleaned with 50% bleach/water solution. However, if the plastic coplin is allowed to stand overnight with this film, conventional methods (including acid cleaning) may fail to remove it. I have found that 5% sodium thiosulfate currently used to dezenkerize slides will clean plastic coplins with this film. Pour the 5% sodium thiosulfate into the jars and let it stand overnight, then clean them as usual the next morning. The sodium thiosulfate used in cleaning was ready to be discarded, so the procedure proves to be very economical. Other concentrations used in various laboratory procedures would probably work equally well.

To receive your own copy of *Histo-Logic*⁵ or to have someone added to the mailing list, submit home address to: Miles Inc., Diagnostics Division, P.O. Box 70, Elkhart, IN 46515.

Method for Reducing Biopsy Sponge Artifacts in Biopsies and Soft Tissue

Daniel B. Kelley College of Medicine – University Hospital The Milton S. Hershey Medical Center

Biopsy sponges can create microscopic artifacts in small biopsies and soft-tissue samples. A useful method of avoiding this is to cut shallow depressions or linear slices in the sponges so that the specimen is contained, yet not compressed.

This is accomplished by bifolding the sponge and cutting with scissors along the folded margin, or laying the sponge flat and incising 2 or more parallel lines. The residual sponge is then removed by folding and excising between the lines to create a trough.

Other benefits of this method include orienting small biopsies on edges (e.g., G.I. skin shaves) and easily confining and locating small (soft) tissue samples (needle biopsies).

The editor wishes to solicit information, questions, and articles relating to historechnology. Submit these to: Lee G. Luna, *Huno-Logic* Editor, 7605-F, Airpark Rd., Gaitherdurg, MD 20879, Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.

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