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Understanding the Iron Stain

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Introduction

One of the simplest procedures performed in the Histopathology Laboratory is the Iron Stain, or Prussian Blue Reaction. The procedure was first described by Perls in 1867, and has been a constant in the demonstration of hemosiderin in tissue and bone sections since that time. Numerous staining protocols have evolved over the years that employ various concentrations of solutions to achieve the same result: the demonstration of hemosiderin.

Staining Mechanism

The Prussian Blue Reaction involves the treatment of tissue sections with acid solutions of ferrocyanides. Any ferric ion (+3) present in the tissue combines with the ferrocyanide and results in the formation of a bright blue pigment called Prussian blue, or ferric ferrocyanide. This is one of the most sensitive histochemical tests and will demonstrate even single granules of iron in blood cells. It is a stable pigment and sections stained with the technique may be stained with numerous other stains without loss of the Prussian Blue Reaction.

$$3\ K_4Fe(CN)_6 + 4\ Fe^{+3} \xrightarrow{HCl} Fe_4[Fe(CN)_6]_3 + 12\ K^+$$

Numerous procedures have been developed over the years for the demonstration of hemosiderin.[1-5] Each of the procedures uses varying concentrations of solutions of potassium ferrocyanide and hydrochloric acid to produce the Prussian Blue Reaction. In an attempt to determine the 'best' procedure, our laboratory stained serial sections of spleen with each of the various dilutions and found staining to be identical in all cases. In fact, dilutions as low as 0.01% still demonstrated hemosiderin deposits in the tissue sections. All reactions were carried out at room temperature.

Reference	Potassium Ferrocyanide	HCl
Sheehan and Hrapchak	1%	2%
Bancroft and Stevens	2%	2%
Carson	2%	2%
Preece	10%	20%
McManus and Mowry	10%	10%
Lillie	10%	0.5%

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No reader should utilize or undertake procedures in *Histo-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. The procedures discussed in these articles represent the opinions and experiences of the individual authors. Sakura Finetek U.S.A., Inc. assumes no responsibility or liability in connection with the use of any procedure discussed herein.

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Diagnostic Applications of the Iron Stain

Iron is stored in the bone marrow and spleen in the form of hemosiderin (Fig 1). Hemosiderin may also be demonstrated in macrophages of the efferent lymphatics and alveolar spaces of the lung. Hemosiderin occurs as yellow to brown intercellular granules and is a crystalline aggregate of ferritin.

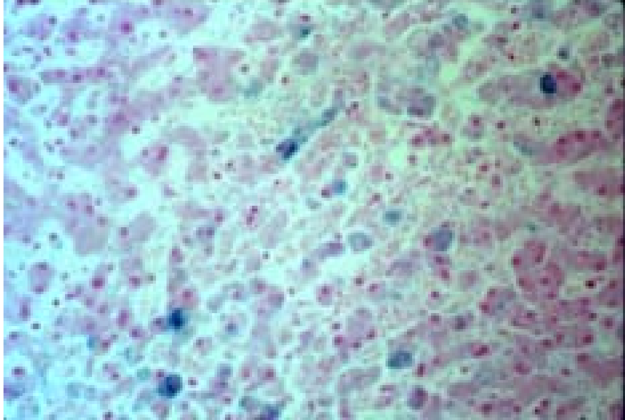

Fig 1: Liver section (Modified Iron Stain , 320×)

Excessive amounts are present in the disease states of hemochromatosis and hemosiderosis. In hemochromatosis, the iron deposits will be found in the liver and pancreas, causing significant damage. In cases of hemosiderosis, excess iron is found in the liver, spleen, and lymph nodes. These macrophages are associated with heart failure. The iron stain may also be used to demonstrate asbestos fibers in cases of asbestosis. The asbestos fibers are inhaled and become trapped in the lung tissue. The fibers soon become coated with protein, which contains hemosiderin, and can be demonstrated with the Prussian Blue Reaction. The asbestos fibers with their protein coat are called "asbestos bodies" (Fig 2).

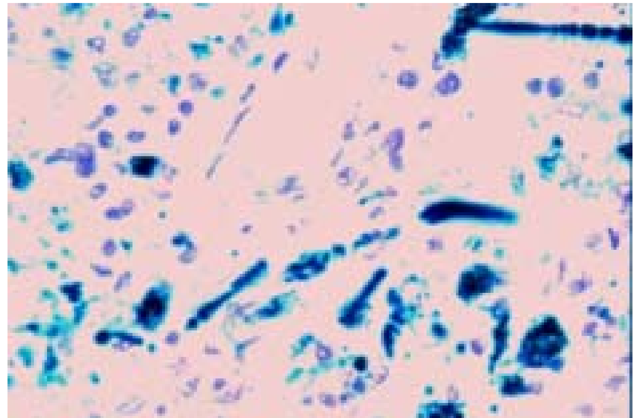

Fig 2: Asbestos bodies (Modified Iron Stain, 300×)

Fixation

Any well-fixed tissue. Avoid use of acid decalcification on bone sections.

Solutions

0.2% aqueous Hydrochloric Acid

Hydrochloric Acid, concentrated. . . . . 0.2 mL
Distilled water . . . . 100 mL

0.1% aqueous Potassium Ferrocyanide

Potassium ferrocyanide crystals . . . . 1.0 g
Distilled water . . . . 1000 mL

Nuclear Fast Red (Kernechtrot) counterstain

Nuclear Fast Red . . . . 0.1 g
5% aqueous Aluminum Sulfate . . . . 100 mL

Use a Pyrex beaker to prepare this solution. With the aid of heat, dissolve the Nuclear Fast Red powder in the 5% Aluminum Sulfate. Cool, filter, and add a grain of thymol as a preservative.

PROBLEM	CAUSE	SOLUTION
Staining solution turns bright blue when prepared	Potassium ferricyanide used instead of potassium ferrocyanide	Remake stock solutions using potassium ferrocyanide.
Diffuse background staining present in tissue sections	Coplin jar contaminated with iron (eg, iron hematoxylin or ferric chloride)	Repeat procedure using chemically clean glassware.
Fine, granular blue deposit on sections	Iron solution heated during staining procedure	Repeat procedure, staining at room temperature.
Sections cloudy after dehydration	Sections not washed after Nuclear Fast Red counterstain	Return sections to running water and wash well prior to dehydration.

Method

1. Decerate and hydrate sections to water.
2. Mix equal parts of Hydrochloric Acid solution and Potassium Ferrocyanide solution immediately before staining. Immerse sections in the solution for 20 minutes at room temperature.
3. Wash thoroughly in distilled water.
4. Counterstain with Nuclear Fast Red for 5 minutes.
5. Rinse sections in distilled water.
6. Dehydrate, clear, and mount in synthetic medium.

Results

Iron (ferric form) . . . . .bright blue
Nuclei . . . . red
Cytoplasm . . . . pink

Troubleshooting

It is difficult to not be able to perform the iron reaction adequately. Problems encountered with the procedure are usually the result of contamination of the solutions or the staining containers, or the result of the wrong chemicals being used in the procedure.

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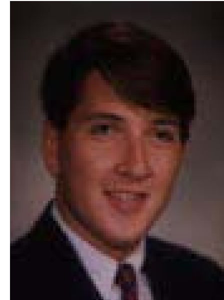
NSH Region IX Symposium Scheduled for April 1998 in Toronto

The National Society for Histotechnology, Region IX, is presenting a symposium entitled "Histopathology: Partners in Diagnosis" to be conducted at the Primrose Hotel in Toronto on April 3 to 4, 1998. Members employed in hospitals and research laboratories would find the symposium particularly useful. Topics to be included for discussion are: breast cancer, heart and lung transplantation, conflict management, oncology, in situ, autoimmune diseases, grossing, pediatric forensics, apoptosis, immunology, and automated special staining. For information about registration, please contact Lynn Yawney at (416) 596-3101, ext. 3112.

Meet Rob Hutchinson: New Sakura Sales Representative Named for North Carolina, South Carolina, and Tennessee



It must be a great job to get Rob off the slopes of Colorado and into a new career with Sakura Finetek USA, Inc. Currently a resident of the "Queen City" of Charlotte, North Carolina, Rob is a 26-year-old native of Roanoke, Virginia, and a graduate of Western Carolina University with a B.S. degree in Environmental Science and a dual emphasis in Public Health and Industrial Safety. He's also certified in CDC Foodborne Disease Control, Epidemiology, and Occupational Safety.

About the skiing. Rob became a ski and race instructor at Eldora Mountain resort in Colorado. He also worked as a Certified Inspector for the State of Virginia. A current member of The North Carolina Society of Histopathology and the Clinical Laboratory Management Association, Rob says: "I am excited about my new career with Sakura Finetek and the wonderful team of individuals I'll be working with. I'd also like to send a special hello to everyone in the Carolinas and Tennessee. See you soon!"

In his spare time, Rob's hobbies include snow skiing (no kidding!), golf, mountain biking, coaching youth soccer, and participating in the Kappa Alpha Order fraternity Alumni Association.

Welcome aboard, Rob!

Decaled or Not Decaled That Is the Question!

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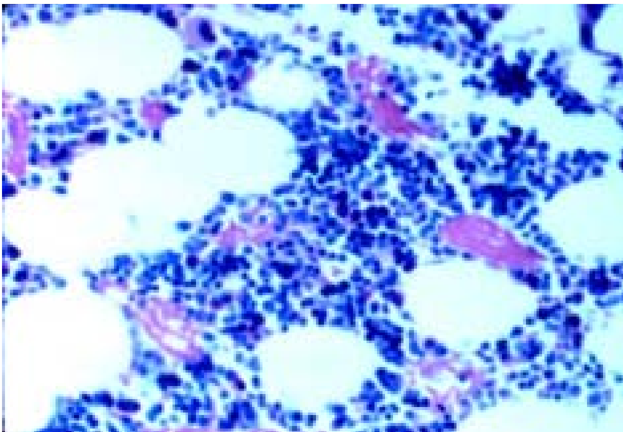
It all began on January 10, 1994. The wind howled outside and the mercury in the thermometer stopped short on eight degrees. Christmas vacation was a thing of the past; it was back to school for everyone. I approached with caution. Having never been in this situation before, I did not know what to expect. I took a deep breath, said a quick prayer and began my task.

The six pieces of femoral head floating in the container of 10% formalin looked harmless enough. But, then again, you never can tell with femoral heads. They have been known to strike out on unsuspecting prey! I donned a pair of rubber gloves and placed five of the six pieces into a new container with 5% formic acid. The sixth piece remained in the 10% formalin as a control. With this first step completed, all there was left to do was wait.



Hemopoietic bone, Giemsa Stain (100×)

January 11, 1994: I entered the lab at 9:00 AM. As I was taking off my coat, I heard a very faint voice calling "Help! I'm melting, I'm meeeeelting…" I rushed over to the decal area, and there were the heads, one of which was screaming. Having the soft heart that I do, I removed this particular piece (whom I have so lovingly named Harriet) and placed her in a container of 10% formalin. The rest were only whimpering, so I left them in the acid.

January 12, 1994: A nasty northeast wind blew me in this particular morning, and I was greeted by another little voice pleading for help. This particular piece was so cute that I couldn't bear to hear his cries any longer. Butch was removed from the acid and placed in his very own container of formalin.

January 13, 1994: I didn't make it into the lab until 9:15 AM (never buy an electric alarm clock and have the power go off!). Again, the soft mews from decal called me over. It had been seventy-two hours, and another piece of bone couldn't take the heat. I saved Muriel, giving her a container full of formalin for her very own. Being in a foul mood, I decided to make the remaining pieces suffer. I carefully dumped out the formic acid, so as not to accidently lose one of the pieces down the drain, and refilled the container with fresh acid. Oh, the agony! What pain! I muffled their screams by shutting the decal room door.

January 14, 1994: Men! You can't live with them and you can't shoot them! My argument with "the boyfriend" the night before had me really ticked off, so all cries for help were ignored this morning. Luckily for the heads I had a change of heart that afternoon and removed them from the acid for the weekend. They enjoyed an all-expense paid trip to Formalin Land for three nights and two days.

January 17, 1994: I was in a great mood today. I bought a battery powered alarm clock, the boyfriend redeemed himself, and I loafed around all weekend long. Alas, the pieces of head were not to benefit from my mood. They were returned to a container of fresh formic acid and left to suffer….

January 18-22, 1994: During these four days, I periodically checked on the remaining two pieces. I poked them with a pin (to see how far it would go in, thus telling me how much further decalcification they needed) and tried to cut them with a scalpel blade. They weren't too thrilled with this idea, I assure you. On Friday, Moe and Larry were removed from the acid and placed in formalin for the weekend.

January 24, 1994: I attempted to perform a chemical test for decalcification on Moe and Larry today. I made up a 10% solution of ammonium oxalate and combined it with hydrochloric acid (5cc of each). The pieces were placed in this mixture for ten minutes. If the solution turned cloudy, Moe and Larry would have to be subjected to further formic acid. If at the end of the ten minutes the solution was clear, they could go on to bigger and better things. Like X-ray! Well, as it turns out, Moe and Larry failed their test. Their punishment was…the Formic Acid Chamber!

January 27, 1994: I repeated the chemical test again today and .... lo and behold, they passed with flying colors! Oh, hurrah! Tomorrow we go up to X-ray with not only Moe and Larry, but with Muriel, too (I wanted to see how much calcium was left in her).

January 28, 1994: The X-rays are back and the diagnosis is promising. Muriel has traces of calcium still left in her, but Moe and Larry are in the clear, (The rest of the pieces were chock full of calcium!) All three are placed in cassettes and are on their way into the tissue processor. Tomorrow, they will be embedded and cut. Hey, let's have some fun, shall we? I'll even stain them with Von Kassa's silver stain!

January 29, 1994: Moe, Larry, and Muriel are cut and ready to be stained. Moe and Larry are getting a regular H&E, but Muriel (the lucky girl!) gets the Von Kossa. I began Muriel's makeover by first hydrating her (xylenes and alcohols through water). I had 5% silver nitrate waiting for her in a coplin jar. She sat in the silver nitrate solution under a fluorescent light for fifteen minutes. The calcium salts turned a black-brown. Next, I washed the slide holding Muriel with several changes of distilled water. The slide then went into a coplin jar filled with 5% sodium thiosulfate (used to remove unreacted silver) for 3-5 minutes. After the allotted time was up, the slide was again rinsed in several changes of distilled water. I counterstained in Nuclear Fast Red for 3-5 minutes, rinsed in distilled water, dehydrated, cleared, and coverslipped with synthetic mounting media. The results were fascinating! The decalcified bone was pink and the calcifications were brownish-black. Amazing!

January 30, 1994: My mission is now complete. I successfully decalcified two of the six pieces and have X-rays to prove it. O.K. now, all you pieces that didn't make it (including those of you that did). Let's pose for a picture. Ready? Say cheese!

The Paradigm Shift in Health Care

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I know you have all heard the phrase—*paradigm shift.* But, what exactly does it mean? A paradigm shift is a radical change in the way one views a process. The cellular phone created a paradigm shift in how we "reach out and touch someone," as did FAX machines and digital beepers. Immunocytochemistry created a type of paradigm shift in how we diagnose disease processes. Paradigm shifts are usually the result of discovery or technology.

Information technology is facing critical shifts: the shift from personal to network-based computing

Paradigm Shift: The New Promise of Information Technology discusses the new business environment that is shaping almost every facet of our lives today. This shift is being driven by technology, particularly information technology, and is characterized by several trends. These trends include a streamlining of all processes within a business, a focus on performance and effectiveness, and an emphasis on quality in all aspects of operations. Information technology is facing critical shifts: the shift from personal to network-based computing, the shift from stand-alone system islands to open integrated systems, and the shift from internal to inter-enterprise computing.[1] It is not difficult to relate the theories presented in ***Paradigm Shift*** to the health care industry as health care reform forces organizations to reinvent the health care delivery system.

Health care organizations today face a strong challenge from proponents of health care reform to become more productive and cost efficient. Hospitals are forming health enterprises with clinics, health maintenance organizations (HMOs), physician provider organizations (PPOs), insurance companies, and other related organizations to produce an integrated business focused on high quality, low cost health care. This restructuring of the health industry will have an enormous impact on information systems (IS) and will present new management challenges as network technology is used to follow this new paradigm in health care delivery. The ability to connect to the "information superhighway" will be critical to the success of any

health care enterprise, and the Chief Information Officer (CIO) will be the guiding force.

Health care has evolved into numerous independent and detached organizations that represent the various aspects of managing disease. These include acute and tertiary care centers, community hospitals, medical specialists, "Doc in a Box" facilities, and other health maintenance organizations. However, a shift is occurring that requires these independent facilities to form a cohesive system to provide cost-effective, quality care.[2] This shift is driven by health care reform mandates from Washington. In addition, health care providers have to shift from providing a disease-based delivery system to creating a community-based health care model, a proactive, disease preventative strategy. This model is referred to as an Integrated Delivery System (IDS), and is expected to be responsible for health care delivery to a predefined population. The IDS will have to deliver the health services at a fixed rate, known as capitation, creating a challenge for the IDS to "manage the care process to achieve the best clinical and economic outcomes."[2] The CIO of the IDS will need to coordinate the various pieces necessary to build the network, and to direct development of an electronic medium that will gather and assimilate the information needed by the members of the enterprise to provide quality, cost-effective care.

A shift is occurring that requires these independent facilities to form a cohesive system to provide cost-effective, quality care.

As health care providers shift from treating diseases to proactive health care delivery, there will be an increased need for information systems that can maintain, correlate, and manage the "lifetime patient medical record." The costs of health care will decrease as the most expensive medical resources, found in acute and tertiary care centers, are replaced by preventative care facilities spread across the entire community. Local Area Networks (LANs) and Wide Area Networks (WANs) will provide the technology necessary to allow the information maintained in the medical record to be available at any location within the health care network. Networks form the basis of the "information superhighway" that will enable all of the providers in the community to access, capture, synthesize, share, and evaluate all aspects of the medical information for all members of the community. This decentralization of information will result in changes in the management hierarchy, resulting in a more level organizational structure with fewer "chiefs" and more "Indians." The CIO will be instrumental in directing the workgroups formed, in maintaining high security procedures as information is made more available and vulnerable due to the proliferation of networks, and in keeping abreast of the new technology and its applications to the existing infrastructure.

Government intervention into health care reform has mandated that health organizations reduce the costs for providing health care. Although automation has been implemented that is designed to reduce personnel and improve the quality of patient care, the automation typically has developed around the existing health care systems and, therefore, has done nothing to reduce the costs of patient care. Helppie states that the entire health care operation must be reengineered to focus on the objectives rather than on the process.[3] The CIO will be responsible for defining the objectives of the new technology. Automation will lead to better dissemination of information, which will lead to better data collection, resulting in the ability to predict outcomes based on the best method of treatment. The entire process of medicine will be improved. "Process automation is key to reengineering care delivery through the use of strategically established protocols designed to optimize both clinical and economic outcomes while maximizing quality." It has been estimated that $36 million could be reduced from the annual health care budget if four new health care computing applications were implemented.[4] These applications include the electronic transport of patient information, electronic submission of health care claims, inventory management of materials and supplies, and video conferencing for training and remote consultation.

It is not surprising that all four of these applications can be realized with the use of LANs and WANs. Patient information can be maintained in an electronic medical record that can be accessed by everyone connected to the network. Billing information, generated automatically from the medical record, can be transmitted electronically by modem or network to the third-party provider, reducing costs by eliminating paperwork and reducing turnaround for accounts receivable (A/R).

The Baptist Health System of Alabama already uses an electronically submitted patient bill for several providers, including Medicaid and Medicare, and have reduced their average A/R by fifty days. Electronic ordering and inventory maintenance will allow health care organizations to reduce supply costs by monitoring what and how much is needed, as well as costs and outcomes. Information gathered from case management and outcomes studies will provide data to allow comparisons between products used and outcomes realized, enabling the health care provider to purchase the most cost-effective sutures and supplies. Rural areas will have access to specialists for patient consultations and professional training, reducing the costs normally associated with traveling to another hospital or training facility. It is easy to see that networked information systems will have a great impact on reducing health care costs.

Haigh reports that "...the overwhelming driving force for network development is physician/clinical information support."[4] The network will be instrumental in the final development of the electronic medical record that will give this support. "Health care decision makers can begin reengineering by giving physicians 'instantaneous access to hospital information'. This helps them to determine the patient's location, analyze test results, suggest diagnoses, enter orders, research medical libraries - even direct treatment protocols - so every patient in the institution receives the same high-quality care in a cost-effective, care-efficient manner."[3] Computer and communications technologies have promised to increase the efficiency and effectiveness of medical care and administration for several decades. However, information technology will not have an impact upon health care until cost and quality are measurably improved. Systems will have to be implemented that will give health care workers the tools to become more efficient. The open-systems approach of computer networks will improve the efficiency and effectiveness of the health care system. Networks allow systems to be downsized from the huge, centralized systems of the 1980's, and downsizing allows systems to be more cost-effective.[2]

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Seminar Date Set for Continuing Series in Histopathologic Technique

The only thing that's constant is change. To get a better idea of how the world of histopathology is changing, be sure to attend the next "Current Seminars in Histopathologic Technique" — scheduled for the Chicago area on September 20, 1997.

Sakura Finetek USA, Inc., in co-sponsorship with Richard-Allan Scientific, is proud to be involved in the ongoing seminar series. In an effort to continually provide educational support for the industry, we have once again contracted with Lab Management Consultants to present this comprehensive seminar in the various disciplines of histochemistry, quality control in H&E and special stain technique, carcinogenicity in dyes and reagents, and ergonomics and risk factors in the lab.

The day-long conference will feature these highly regarded speakers:

Mr. Lamar Jones, noted lecturer and winner of the prestigious 1995 McCormick Award, will be the featured speaker at the Chicago Seminar on September 20, 1997.

- Lamar Jones (Vice-President, National Society for Histotechnology)
- Jerry Fredenburgh
- Michael LaFriniere
- Jan Minshew

The Conference/Seminar is scheduled to be held in the luxurious Doubletree Guest Suites in Chicago's North Shore. All participants will get a chance to see technical presentations on routine and advanced instrumentation, focusing on how laboratory equipment is being designed to help improve lab applications and techniques.

The next Conference/Seminar is scheduled for December 6, 1997, in Fort Lauderdale, FL.

This session has been approved for Continuing Education credit hours. For more information about this and other scheduled Conference/ Seminars in this ongoing series — and to register — call: 1-800-725-8723, ext. 233. Or call Lab Management Consultants directly at 314-535-0578.

Date: Saturday, September 20, 1997
Time: 8:15 AM - 5:00 PM
Cocktail reception to follow.

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Technical Notes

Oil Red O Staining Artifacts

The following technical note was written by Lee G. Luna to be included in a past issue of Histo-Logic. It has recently been found and included here for the valuable information it contains.

Fig 1: Illustrates deposits of Oil Red O throughout the section. A fine precipitate can be seen if one observes this section at high power (400☐).

The most popular method for staining fat in tissue sections is the Oil Red O method with propylene glycol as the solvent. Frequently a fine precipitate forms on the tissue section and slide even though the solution was made properly and filtered prior to its use. This precipitate forms shortly after the staining phase of the procedure is completed. The precipitate is seen as a sheen on the surface of the solution and as a fine red crystalline material on the slides.

Although propylene glycol is fairly viscous, evaporation occurs from the Oil Red O solution. This results in a super-saturated solution. The resulting effect is the development of a precipitate and/or crystallization of the Oil Red O while in the solution container or on the microscopic slide (Fig 1). Crystal Oil Red O artifacts can be prevented by the addition of 25 mL of absolute propylene glycol to each 100 mL of the Oil Red O solution after it has been filtered.

Crystalline type artifacts may also be produced when slides are cleared of excess Oil Red O by dipping in 80% propylene glycol. It should be remembered that propylene glycol will not readily dissolve in Oil Red O at this percentage. This problem can be eliminated if one drains the slide for 1 minute after staining followed by 2 dips in absolute propylene glycol before immersing slides in the 80% solution.

Some Oil Red O dye lots may produce crystals in sections several hours after staining and mounting of the coverglass. I suggest that some samples of Oil Red O and propylene glycol mixtures have different solubility levels in the fat cell substance itself. Staining of fat is largely a physical process resulting from the fact than Oil Red O dye is soluble in fat cells, much more so than in the propylene glycol that is used as the solvent. This inability of the Oil Red O to completely dissolve in the fat substances results in the availability of excess stain molecules. A preventive measure is to avoid washing sections too long after the nuclear stain as excessive washing at this step removes propylene glycol, which in turn allows for saturation of the fat dye forming dye crystals. Mounting the coverglass with glycerin-jelly from 1% aqueous glycol also prevents this problem. This change to the mounting procedure seems to enhance the intensity of staining.

Fixation: 10% buffered neutral formalin

Technique: Frozen sections

Solutions:

0.5% Oil Red O Solution

Oil Red O. . . . . 0.5 g
Propylene glycol, 100%. . . . . 100.0 mL

Add a small amount of propylene glycol to the Oil Red O and mix well. Large pieces of the dye should be crushed in the mixture. Gradually add the remainder of the propylene glycol, stirring periodically. Heat gently until the solution reaches 95°C. Do not allow temperature to go over 100°C. Stir while heating. Filter through coarse filter paper while the solution is still warm. Allow to stand overnight at room temperature. Filter through Seitz filter with the aid of a vacuum. If solution becomes turbid, refilter.

85% Propylene Glycol Solution

Propylene glycol. . . . . 85 mL
Distilled water . . . . 15 mL

Mayer's Hematoxylin Solution

5% Acid Water Solution
Hydrochloric acid . . . . 5 mL
Distilled water . . . . 95 mL

Glycerin Jelly
Gelatin . . . . 10 gm
Distilled water . . . . 100 mL

Heat until gelatin is dissolved. Add 70 mL glycerin and 1.0 mL liquid phenol.

Staining Procedure

1. Cut frozen sections and collect in distilled water.
2. Immerse sections in absolute propylene glycol for 2 minutes.
3. Immerse section in Oil Red O solution for 1 hour. Sections can be allowed to stand overnight.
4. Differentiate in 85% propylene glycol solution for 1 minute.
5. Rinse in distilled water, 2 changes.
6. Counterstain in hematoxylin solution for 1 minute.
7. Rinse in distilled water, 2 changes.
8. Differentiate, if needed, in acid water solution.
9. Wash sections in running water.
10. Neutralize sections in weak ammonia water.
11. Wash sections in running water for 2 minutes.
12. Mount in glycerin jelly.

Results
Fat . . . . red
Nuclei . . . . blue

Reminder

NSH
October 18-22, 1997
Columbus, Ohio

1998 Meeting
September 12-16
Salt Lake City, Utah

Welcome to Sakura US on the Net

The Internet is rapidly gaining acceptance and use as a responsive, always-available source of information for business and personal use. And the Sakura Finetek, U.S.A., Inc. website is quickly getting up to speed to meet your needs — now and at 2 AM if you need it.

Our address is **www.sakuraus.com** and in the near future here's what you'll find when you visit:

- **An overview of Sakura Finetek, U.S.A.**: a brief capsule of the 125-year history of the leading histotechnology company in the world — our products, our focus, and our dedication to the present and future needs of histotechnology, society, and world culture.
- **A brief product catalog**: a showcase of the reliable products available from Sakura Finetek, U.S.A. — a ready-reference guide to the state-of-the-art products manufactured and distributed by Sakura Finetek, U.S.A.
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right now.

The Art, Science, and History of Disposable Microtome Blades

Why All Blades Are NOT Created Equal

Histotechnologists are trained over a period of years to develop the special skills that are needed to obtain quality tissue samples consistently. It's tough, exacting work. The "tools of the trade" become extremely important — and few are more important than the microtome and its blades.

Materials: Only high-grade materials, from the finest stainless steel manufacturing sources, are used in Accu-Edge products.

Everything a precision microtome does is judged ultimately by the quality, precision, and accuracy of the sections it produces. The Tissue-Tek® Accu-Edge® Disposable Microtome Blades manufactured for Sakura Finetek by Feather combine state-of-the-art manufacturing with ongoing process improvement for unsurpassed consistency and sharpness.

Blade Punching: Quality-inspected materials are punched into final blade shape.

Feather began blade manufacturing in 1932 and today produces the world's largest volume of disposable blades for use in medical, surgical, and pathology applications. In 1978, the company introduced its disposable microtome blades and quickly displaced conventional, reusable blades in its markets. They also produce precision blade holders for many different types of microtomes.

Heat Treatment Processing: Using a special high-heat treatment with "flash cooling" tempering assures the proper combination of hardening and flexibility in every Accu-Edge blade.

Only the highest grade of materials is used for Tissue-Tek Accu-Edge Disposable Blades. The special process — incorporating high-heat treatment, "flash cooling," and triple honing — ensures the proper amount of hardening and flexibility in every blade. Precision grinding, performed on grinding equipment developed by the company, is kept to micron accuracy for exact blade angle and shape. Platinum and chromium coatings are added to reinforce blade edge strength and long-life durability. Then the disposable

Intermediate Inspection: All disposable blades are inspected throughout the entire manufacturing process.

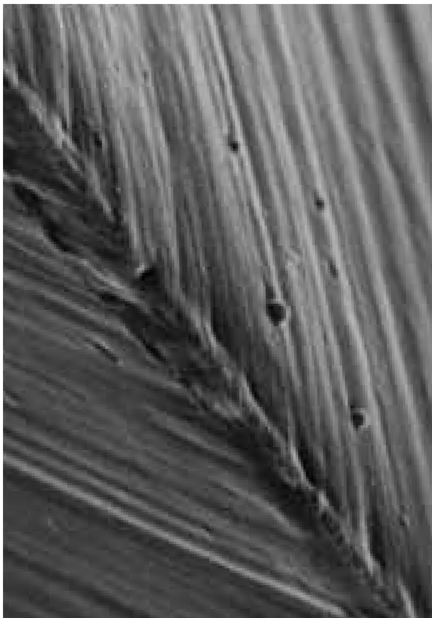
Edge Grinding: Precision grinding, within microns of tolerance, create exact blade angles and triple-honed shape — all performed in line on grinding machines manufactured by Feather.

blades are resin coated to produce a low-friction coefficient for smooth, precise, consistent sections.

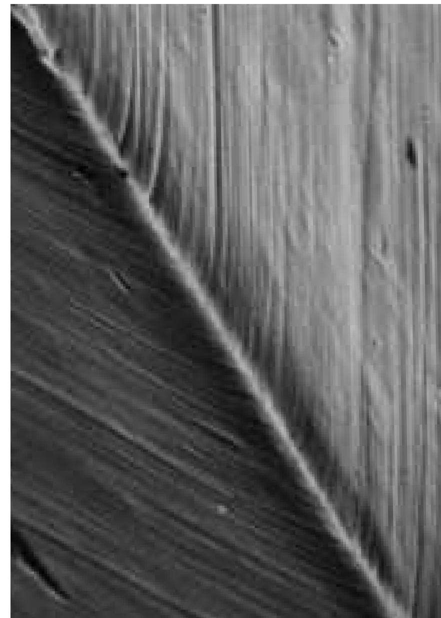
The result is microtome blades with a quality edge that can be seen. Compare the microphotographs at 10,000 X magnification that accompany this article. Note the surface unevenness of another brand's blade [left] due primarily to insufficient process preparation. The lack of luster on the cutting surface indicates a source of cutting friction which will result in inconsistent sections. The Tissue-Tek® Accu-Edge® cutting edge [right], however, is practically mirror smooth; the unique processing sequence used in creating Tissue-Tek Accu-Edge Disposable Blades virtually eliminates unevenness, resulting in low friction, reduced drag, and superior performance.

Quality control is important to ensure consistently superior performance. Maintaining a stringent inspection program is essential. It begins with analyzing raw materials, electron microscopy of cutting surfaces and edges, and includes continuous in-process and final QA inspection, and cutting-force and wear/abrasion testing — all with a single goal in mind: to ensure absolutely unsurpassed performance consistency within and across manufacturing lots.

The results are easy to see: Tissue-Tek Accu-Edge Disposable Microtome Blades deliver the kind of microtome performance you depend on for quality results.



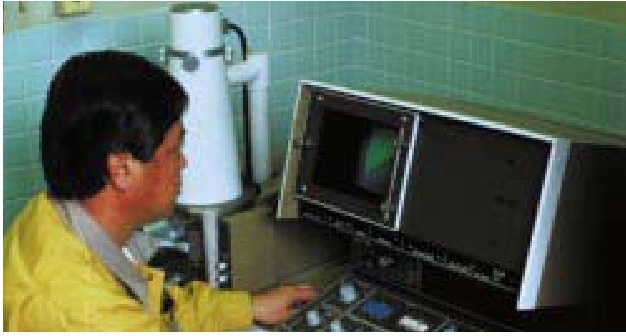
Competitive Blade
(electron microscope x 10,000)



Accu-Edge Blade
(electron microscope x 10,000)

Platinum Coating: Platinum and chrome coatings are added to reinforce blade edge strength and durability.

Resin Coating: An additional low-friction coefficient coating is applied to ensure smooth tissue sectioning without chattering.



QC Testing: All disposable blades are fully inspected to ensure consistent quality. The microtome sectioning test is performed lot by lot.

Delivery: All Tissue-Tek® Accu-Edge® Disposable Blades that have passed the rigorous quality control inspection are sent to distribution points worldwide.

A Modified Brain Processing Schedule

By Michael Pruitt HT (ASCP)
University of Louisville Hospital

To produce high quality tissue sections from brain taken at autopsy can present a tremendous challenge to the histotechnologist. All too often these tissues are fixed and processed along with the routine surgicals, and less than optimum results are obtained. This is due to the differences in the composition of brain tissue from the majority of routine surgical specimens.

The main difference in brain tissue from others is the enormous amount of lipids, which make up over one half of the total dry weight of the human brain. Myelin is a complex lipid abundant in the white matter and acts as a protective insulating sheath around the nerve fibers. Myelin is made up of a protein, cholesterol, a phospholipid, and cerebrosides which are lost during processing, leaving behind a resistant proleolipid. Because these tissues are so dense, dehydration needs to be slow, and any abrupt change in the concentration of alcohol will produce shrinkage and distortion.

Another difference that affects the handling of brains is the tremendous amount of water contained in the tissue. Water comprises 70% to 80% of the total mass of the human brain. This large percentage of water causes the brain to be extremely soft so that it can't be examined or sectioned without first being well fixed and hardened.

Our laboratory purchased a new VIP Tissue Processor last year, allowing us to use our 12-year-old VIP for exclusively processing brains. This has enabled us to set up a long process and also segregates neural tissue from the rest of the surgical specimens. Under the new CAP guidelines each laboratory must have a procedure for dealing with Creutzfeldt-Jakob disease, and separating brain tissue from the rest of the specimens is essential.

In our laboratory brains are placed in 20% NBF for at least 1 week, then washed well in running water before grossing. After sections are taken, the cassettes are placed back in 15% NBF for approximately 5 days. When the sections are well fixed they are processed according to the following schedule with the pressure/vacuum turned off:

1. 50% alcohol for 12 hours
2. 70% alcohol for 12 hours
3. 80% alcohol for 2 hours
4. 90% alcohol for 2 hours
5. 95% alcohol for 2 hours
6. 100% alcohol for 1 hour
7. 100% alcohol for 1 hour
8. 50% Histosol/50% absolute alcohol for 1.5 hours
9. Histosol for 1.5 hours
10. Histosol for 1.5 hours
11. Paraffin for 1 hour
12. Paraffin for 1 hour
13. Paraffin for 1 hour

Fixing the tissue before it is placed in the processor allows for extra dehydration stations, which enables a slow and gentle dehydration. This prevents the tissue from becoming too brittle. The extra time in the dilute alcohol helps to remove the lipids allowing for penetration of the subsequent solutions. This procedure has produced consistent high quality results for our laboratory.

1. Lillie, RD. *Histopathologic Technic and Practical Histochemistry.* 3rd ed. New York, NY: McGraw-Hill; 1965:34,412
2. Disbrey BD, Rack JH. *Histological Laboratory Methods.* Baltimore, Md: Williams & Wilkens;1970:224-226.
3. Sheehan DC. *Theory and Practice of Histotechnology.* 2nd ed. Columbus, Ohio: Battelle Press; 1980:261.

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And it's still working very well, thank you.

The Tissue-Tek® VIP™ System remains the leading vacuum infiltration processor for a very simple, traditional reason: it just *keeps* working. Batch after batch. Day in, day out. Year after year.

That's ***proven*** reliability—the kind that the laboratory professionals at Straub Clinic & Hospital in Honolulu, Hawaii, have come to depend on. That's where Tissue-Tek VIP Serial Number 4660-81001 was installed in 1981…and is still working today. It was a good investment then—just like today's new Tissue-Tek VIP would be an even better investment for today and the future.

So when it's time to specify the most reliable tissue processing system for *your* lab, specify Tissue-Tek VIP. A wise decision for a very long time.

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Spock? Captain Kirk? Captain Nemo? The Rocketeer? Yoda? Darth Vader? Chewbacca?

Partytime at NSH — And You're Invited, But...

Party plans are now set for NSH. We've taken the meeting theme — ***Discover the Future of Histotechnology*** — and given it a bit of a spin to make the annual party a true adventure into the future.

Step into the future of histotechnology aboard the **Starship *Sakura*** and journey to the **Richard-Allan Galaxy** at the far reaches of the **Allegiance Universe**. For entertainment, there's a laser show, a Robot, Cyber Pac, Laser Storm tag, and an M-4 Motion Theater. And that's just for openers.

There's also food, refreshments, and a DJ to play your favorite music. Add to that the atmosphere of the Grand Ballroom of the Hyatt Regency, Columbus — and you've got all the right makings for a grand evening.

But...you've got to have the right look to get in!

You've got to appear in your future costume — your favorite sci-fi character. Jabba the Hut, The THING, Alien, Wonder Woman, Flash Gordon, Dale Arden, Spider Man, Batman, Robin, The Joker...you get the idea.

Be sure to be there, in costume...and we'll beam you up, Techie!

Where: Grand Ballroom
Hyatt Regency
Columbus, Ohio

When: Saturday Night
October 18, 1997
8:00 PM til....

Sponsored by Sakura Finetek, U.S.A., Inc., Allegiance, and Richard-Allan Scientific.

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Terri Staples, ***Histo-Logic*** Editor, 1000 16th Avenue South, Birmingham, AL 35205. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.

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