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Histo-Logic

Technical Bulletin for Histotechnology • Managing Editor, Gilles Lefebvre • Scientific Editor, Terri Staples, HT (ASCP), BS, HTL
Vol. XXVI, No. 1, March 1996

Adhesiveless Histologic Staining Techniques

Reynaldo T. Ruedas, AB, RMT, RT, HT
Riyadh National Hospital
Histology and Cytology Laboratory
Kingdom of Saudi Arabia

INTRODUCTION

Since the introduction of histologic techniques, adhesives of various kinds have been universally used for attaching sectioned tissues on the slides to prevent them from dislodging during the staining process. The types of adhesives commonly used are albumin, gelatin, starch, cellulose, sodium silicate, and resin. Methods for applying the adhesives involved either smearing the adhesive on the slides or dissolving the adhesive in the water used for the tissue flotation bath.

The problem with most adhesives is that they tend to take up stains during the staining process and thus cause a background artifact. This artifact could cause inaccurate interpretations, which in turn could cause inconvenience to both the pathologist and the patient. Further, excessive adhesive cannot be thoroughly cleaned from the slide, especially in the areas tangent to the sectioned tissues, without the risk of smudging or wiping off portions of the tissue section. To be able to get a clean and clear microscopic view of a sectioned tissue and component cells, adhesives must be totally eliminated from the slides.

METHODS AND MATERIALS

1. Sections are floated in a flotation bath containing distilled water. The desired section for staining is picked up on a pre-cleaned slide containing no adhesive.
2. Drain the slide with the sectioned tissue in a vertical slide carrier and place the carrier on a 45°C hot plate for 30 minutes or until dry. The tissue section, while wet, will appear opaque due to the presence of water between the slide and the sectioned tissue. This opacity will gradually turn translucent as the sectioned tissue blends with the paraffin.
3. When the entire section is translucent, lay the slide flat on the hot plate for 10 minutes to ensure prope attachment of the sectioned tissue on the slide.
4. Place the slide in a 58°C oven until the ribbon o paraffin melts. Deparaffinize and proceed t staining.

RESULTS

Serial sections of biopsy material were obtaine using the method described above and using traditional adhesive method. Slides were the stained with a routine H&E stain. The sectio without adhesive demonstrates a clean and clea background (Fig 1), while the slide with adhesiv shows blotches of bluish-pink artifact between tissu fragments (Fig 2).

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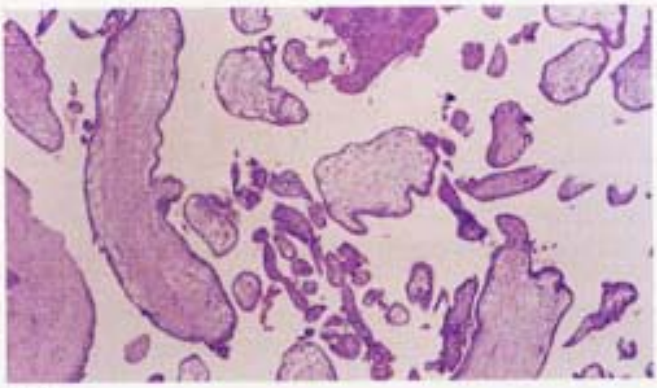

Fig 1

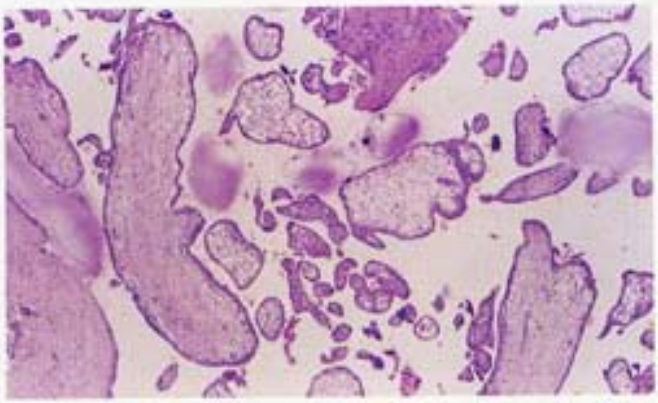

Fig 2

DISCUSSION

From repeated practice it has been noted that histologic sectioned tissue has its own adhesive property. This is perhaps due to its protein portion. Proper positioning of the slide in the vertical position right after mounting the paraffin ribbon guarantees astonishing results. Vertically, the water has more draining space and the surface tension of the water can break off easily from the edges of the tissue. In the horizontal position, more water remains trapped between the slide and the sectioned tissue for a longer time, especially if the tissue section occupies the whole width of the slide.

Allowing the slide to dry prior to melting the paraffin is another factor in this method. When slides are placed in the oven while there is still water between the section and the slide, there is a tendency for the water to push the sectioned tissue away from the slide as the water expands during the heating process. This causes the tissue to warp or wrinkle. Consequently, when warping occurs, the tissue could become dislodged during the staining process. To prevent this from happening, sectioned tissue should be "waterless" before slides are placed in the drying oven.

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Alcian Blue-H&E-Saffron Stain for Gastrointestinal Biopsies

Terri C. Staples, HT(ASCP), HTL
Baptist Health System
Birmingham, AL

INTRODUCTION

Endoscopic biopsy procedures have become the preferred technique for diagnosis of the majority of gastrointestinal diseases. Histologic procedures that will accommodate the small, often fragile, biopsy specimens have been developed to help the histotechnologist produce a quality product to aid in patient diagnosis. Our laboratory has adapted a procedure brought to us by our pathologist from Vanderbilt University for use on gastrointestinal biopsies that allows us to conserve both time and techniques. This procedure is a combination stain using Alcian Blue, Hematoxylin & Eosin, and Saffron as a counterstain. Tartrazine may be substituted for Saffron for purposes of economy. When used as a routine stain on GI material, the AB-H&E-Saffron stain results in earlier diagnosis as it eliminates the need to do separate special stains for mucin and collagen. The Alcian Blue stains the acidic mucins in the specimen and the Saffron or Tartrazine stains the collagen. Other details in the specimen are demonstrated with the routine H&E stain.

FIXATION

Any good fixative is acceptable; however, we have experienced excellent results with Hollande's Fixative. Cut paraffin sections at 4 to 6 µm.

SOLUTIONS

3% Acetic Acid water

Glacial Acetic Acid	3 mL
Distilled water	97 mL

1% Alcian Blue

Alcian Blue 8 GN	1.0 g
3% Acetic Acid water	100 mL

Mix until dissolved and add a crystal of thymol as a preservative. Alcian Blue has a normal shelf life of 3 months.

Alcoholic Saffron Counterstain

Saffron	1.5 g
Absolute alcohol	100 mL

Mix well and filter. Add 1.0 mL of Glacial Acetic Acid as a preservative.

NOTE: Tartrazine Solution (Sigma #HT30-2) may be substituted for the Alcoholic Saffron.

0.5% Lithium Carbonate

Lithium Carbonate	0.5 g
Distilled water	100 mL

Routine H&E stain

PROCEDURE

1. Decerate and hydrate slides to water.
2. Place slides in 3% Acetic Acid for 3 minutes.
3. Place slides directly into 1% Alcian Blue solution for 10 minutes.
4. Wash well in water. Check microscopically for staining intensity. If background staining appears, rinse slides in 3% Acetic Acid to remove.
5. Wash slides well in water.
6. Place slides in 0.5% Lithium Carbonate for 1 minute. Wash well in water.
7. Stain slides in Hematoxylin for 6 minutes. Wash well in water, differentiate, and blue as with normal H&E stain. Check slides microscopically for nuclear detail.
8. Counterstain slides in Eosin for 2 minutes. Wash in water.
9. Dip slides in 95% alcohol and place in absolute alcohol, two changes, 1 minute each.
10. Stain slides in Alcoholic Saffron for 30 seconds or in the Tartrazine Solution for 1 to 5 seconds. Care must be taken not to overstain. If sections are overstained, decolorize slides in ammonia water, wash well, and restain, starting with Eosin.
11. Dehydrate, clear, and mount using a synthetic resin.

RESULTS

Nuclei	blue
Cytoplasm	pink to red
Mucin	aqua blue
Collagen	yellow
Smooth Muscle	salmon pink
Other Elements	shades of pink and blue

REFERENCE

Vanderbilt University Department of Pathology Special Staining Manual.

Combination Control Blocks

Cel Rutledge, HTL(ASCP)
Histopathology Department
San Francisco General Hospital
San Francisco, CA

Because we are a teaching hospital, we probably do more special stains and procedures than we would were we part of the private sector. While working with residents over the years, one consistent problem seemed to follow each resident rotation: that of trying to decide which would be the proper control to use for the stains they had requested.

One of the questions asked on our special stain and procedures form is the "purpose of the stain" requested. This question was added to the form to ensure the correct control was used each time. However, this question often goes unanswered and, over the years, we have spent a lot of time waiting until the resident came to work before beginning the special stains for the day. It seemed there should be a better way of handling this problem. This is where the "combo" block was inspired.

We have been using combo blocks for approximately 10 years, and they have been a great help to us, both in saving time and reducing aggravation. Blocks can be made with almost any kind of tissue because there are no rules. The blocks can be devised any way necessary to suit your laboratory's needs.

Whether you cut your controls ahead of time or cut them each time you stain, this method will work. It is simply a matter of taking your already collected control blocks, melting them down, and then re-embedding them into a single block. Blocks can also be designed at the time you collect fresh or fixed tissues. I have found it helpful to label the cassette with the case numbers of the tissues you choose for the control to help identify future control cases. In addition, there is always someone who has an interest in finding out about one of the cases you are using as a control.

We occasionally use a block designed especially for fungus. It contains the six most common fungi diagnosed at our institution. This special block serves two purposes: we only have to keep one control block cut and stored, not six blocks as before, and the resident has a chance to look at all the organisms and how they stain.

Many of the tissues we use for controls contain more than one of the structures that we are looking for in our special procedures. The PAS is a good example. The kidney sample we use for demonstration of basement membrane also contains three kinds of fungi. This eliminates the need to have a separate control for fungus. Figures 1 to 4 demonstrate a few of the combo blocks we use in our laboratory.

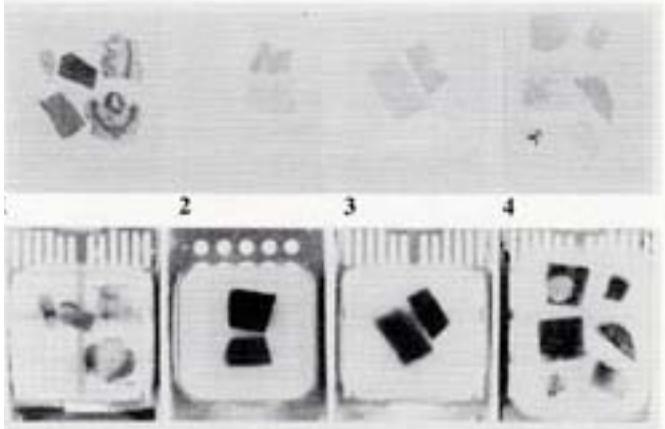

Fig 1. — Trichrome control of fallopian tube, liver, small bowel, appendix, and uterus.

Fig 2. — Fungus control with lung containing *Pneumocystis carinii* and tissue with *Aspergillus*.

Fig 3. — PAS control containing fungus-infiltrated kidney and a piece of liver.

Fig 4. — Fungus control containing tissues with *Aspergillus, Candida, Coccidioides, Cryptococcus, Histoplasmosis,* and *Pneumocystis carinii*.

ACKNOWLEDGMENT

wish to thank Carolyn Breaux for the photography.

A New Use for Mineral Oil

The Histotechnologists at Ottawa Civic Hospital
Ottawa, Ontario

In our laboratory we have traditionally rubbed used toluene onto our tissue processors, embedding centers, microtomes, and bench surfaces to remove the excess paraffin wax that seemed to accumulate everywhere. It appeared to be the most effective agent for this purpose, as conventional cleaning solutions did not work. With the growing concern to reduce fumes in the workplace, and the detrimental effect of toluene on our hands, we searched for an alternative. We have found an inexpensive and readily available substitute that has no unpleasant fumes and is actually beneficial to the skin. We use mineral oil obtained from the hospital pharmacy. We transfer the oil to squeeze bottles for easier handling. It takes much less scrubbing to remove accumulated wax than toluene and actually prevents wax from sticking to a surface where the oil has been applied. All of our equipment looks clean and shiny, it is easier to breathe, and we have to clean up less!

A Technical Note: Storage and Handling of Uranyl Nitrate

Maureen Rose, MLT, ART
Markham Stouffville Hospital
Markham, Ontario

We had an interesting discussion with our Radiology people here regarding uranyl nitrate handling and storage. When testing the dry chemical for radiation emission, it was found that the chemical gives off a good deal of radiation unless it is kept in the lead canister in which it is shipped. In addition, we found our 1% solution on the staining shelf gave off radiation up to 3 feet away. Therefore, it is wise to keep any solution of this chemical in a lead container. Gloves should be worn at all times when using this chemical and extra precaution should be used when weighing and preparing reagents using uranyl nitrate.

Preparing this solution in a coplin jar saves large amounts of preparation and use of this chemical. All glassware used in the preparation and storage of this chemical should be washed by hand separate from other glassware. Gloves should, of course, be worn during washing.

We use our uranyl nitrate in the microwave oven, and the inside of our oven tested normal for radiation levels. The staining sink where we wash off the chemical from the slides tested normal, also. The real hazard with uranyl nitrate is in having the working solution stored too close to people (which we have done for years!) or in not having the dry chemical in a lead container. We did, at least, have our dry chemical in its lead container!

JUST A REMINDER

NSH MEETING

October 19-23

Albuquerque, New Mexico

Chemical Dropouts... and the Solution

Nancy Klemme, HT (ASCP)
Customer/Product Support, Department Manager
Sakura Finetek U.S.A., Inc.

Histology has new technologies with specialized techniques. Fewer people must do more in less time. New products and reagent substitutions are available. "The more things change, the more they stay the same." That old saying applies as we still acquire specimens, fix them, record gross descriptions, process by replacing tissue fluids with paraffin, embed the specimens, etc. Those unchanged things still form the foundation to understand new technologies and utilize new products.

We have become increasingly aware of the solid chemical component(s) of our reagent solutions — fixatives, normally — which precipitate out of solution when the chemical bond between them and the fluid or solvent is broken. "Salts, precipitates, buffer salts, formalin salts,..." whatever we label them, we have become more familiar with these materials as their accumulation has caused undesirable conditions — in our tissue processors and in our tissues. The presence of these solid components in solutions stabilizes pH, controls osmolarity, coagulates tissue protein, and acts as a mordant.

As visual beings, we may be unaware of the effect our processing fluids have on each other if a reaction is hidden from our view. Make the reaction visible by combining successively used reagents.

1. Into a clear graduated cylinder, measure approximately 100 mL of reagent.
2. Add 20 to 50 mL of the preceding reagent.
3. Note your observations. Tiny gas bubbles will rise to the surface.
4. Cover and allow the container to remain undisturbed for an hour to allow potential precipitates to drift to the bottom.

Note these observations as well.

When performing this exercise with the first reagent on the processor, the preceding reagent will be whatever reagent is already *in* the specimens (and sponges).

Any material that became visible during this exercise certainly has the capability of collecting in a comparative volume within your processor to become a barrier to fluid transfer. Tissue processor maintenance usually includes direction to remove this material before it becomes this barrier. Generally, warm tap water may be used in this line maintenance procedure. Check the pH of your tap water. You may already have this as part of your regular quality control checklist (especially if it is used in your automatic staining process). If your water is especially laden with minerals, distilled water might be a better choice. If the accumulated chemicals came from an acetic solution, an acidulated water can be used to aid in the precipitate removal. Likewise, water with a more neutral pH should be used to remove precipitates from a neutral pH solution.

Perform a line maintenance procedure on your Tissue-Tek® VIP™ as directed in your operating manual. Remove the drain filter to allow any dislodged material to pass freely into the retort. Once fluid has pumped in, you may stop instrument action to see if any material has been brought in. Record your observation. Initially, you may do this at each step of this maintenance procedure to note which position (if any) demonstrates the most precipitate material. Usually, the reagent immediately following fixation causes the fixative solid chemicals to fall out of solution. Include that step in your line maintenance. The step immediately following the fixative may not allow for complete replacement of all the fixative. This could be due to immersion time, ratio of reagent to tissue, reagent dilution, and the like. You may find a need to perform the "water wash" maintenance in that step as well. Based on the observations of how the reagents of your choosing react with each other, this "water wash" maintenance is responsibly done to include the steps where these chemical solids may accumulate.

We are directed by professional responsibility of specimen handling and personal safety responsibility to understand the products we use. The product insert or instructions which accompany the product are our first source of information. If that printed information is not readily available in your lab, call the supplier or manufacturer to have it sent to you. Verify with the manufacturer of your processor and the supplier of your reagents to confirm that your use of their products is the intended use for the specimen and for the product they supply.

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Meet the Folks Who Answer to You

Nancy Klemme Rob Fujimura Sunny Hong

We'd like to introduce you to the real people behind the voices at Sakura's Customer/Product Support lines.

Rob Fujimura is a Pathologist Assistant. His histology-related work experiences span over a decade at two major southern California hospitals. He's also worked for both the Los Angeles County and Orange County Coroner's Offices. "I would be on histology rotation every other week. Coverslipping was among the duties in this rotation schedule and I was really glad when we got the Tissue-Tek Coverslipper." Rob's inventive nature appreciated the precision as well as the speed of the unit. "Actually, it was kind of entertaining."

Sunny Hong has her HT and HTL certification from ASCP. Her undergraduate degree is in biology and she has a graduate degree in hospital administration. Sunny has 10 years of histology experience specializing in neuropathology. She recalls using the Tissue-Tek VIP Tissue Processors. "I think we took their reliability for granted. We had good processing and they didn't really demand special attention." Sunny also has over 3 years of experience in product troubleshooting.

Nancy Klemme has been a histologist for over 25 years. Her previous laboratory employment was in Wisconsin. For nearly 17 years, Nancy has been in hundreds of laboratories for product demonstrations, consultations for troubleshooting, and instructional presentations. In the past, she has conducted state and regional workshops (including NSH accredited). Nancy is the manager of the Customer/Product Support Department and also teaches the VIP-E Series operator training classes.

The Customer/Product Support Department receives and documents calls relating to use of the Tissue-Tek products the customer has purchased from Sakura.

Instrument, component, or operational failures are rare, fortunately, and relatively easy to identify and correct. The Customer/Product Support Specialist will ask questions and sometimes have you go through some instrument interaction to help isolate the problem. When the qualifying data yield repair authorization, our department dispatches the service or directs the return process of smaller products if the repair is to be done at Sakura. An abundance of documentation is done to assure that anyone involved with your product receives the information they need.

It is when instruments have not actually "gone down" that a lot of data have to be collected to accurately identify the cause of the negative condition which prompted the call to Sakura. Sometimes, as you can imagine, emotions run high, but we've been in your shoes and know how it feels. We still need measurable data to move toward variable identification as we move toward helping you get back in control of those variables.

Examples of our telephone consultation calls include undesirable staining or processing results but no equipment failure. Each call is customized to your particular need. What works at one facility may not be subjectively appreciated at another. So with your particular set of facts, we will work toward problem resolution *together*.

HOURS OF OPERATION

The hours of operation are easy to remember. The Sakura hotline can be accessed 24 hours a day, 7 days a week.

For specimen-related problems and any other technical issues, Nancy, Rob, and Sunny are available from 7:00 AM to 5:30 PM Pacific Time. Just call 1-800-725-8723, and then choose option #2 from the automated attendant message at any time during these normal business hours, Monday through Friday.

Outside these hours, Emergency Technical Service is also available. After calling the toll-free number, choose option #4 from the automated attendant message center. Effective May 1, you'll be connected with a Sakura Service Representative, further improving our service to you.

Remember this about Sakura Customer Service: it's more than just instrument focused. We want to help. Call us.

Meet Your Local Sakura Representatives — Up Close and Personal

Sakura Sales Representatives are special people, we like to think. We also think it would be a good idea to let you know something about each of them so that you get a better idea about the quality we depend on to serve you better.

Antoine Arbuckle covers the territories of Texas and Louisiana for Sakura. He's a graduate of Rice University and has been involved in health care sales for the last 5 years. His hobbies include sports, games, and personal computers.

Judy Bordyn, Regional Sales Specialist for Kentucky and the southern parts of Illinois and Indiana, was a Technical Product Specialist with Lab-Tek® products for Miles Inc. and worked as a "troubleshooter" on the original Tissue-Tek® VIP™ Processor. She lists piano and skiing as her hobbies.

Charles Boxenbaum is the Sakura Regional Sales Specialist for Wisconsin, upper Michigan, and the northern parts of Illinois and Indiana. A Chicagoland native, Charles' career includes 12 years of medical sales experience and 8 years of pathology sales. In his spare time he enjoys gardening, camping, and family activities.

Sheldon Brown is a Sakura Regional Sales Specialist covering Maryland, Delaware, Virginia, Washington, DC, and parts of Pennsylvania. His career in medical product management and sales spans nearly 25 years, most of it in histology and cytology product sales to clinical and research markets.

Sharon Claus is the Sakura Regional Sales Specialist who covers Georgia, Alabama, and Mississippi. A resident of Marietta, Ga, Sharon has been in sales over 5 years with experience in chemical sales to her credit. Her hobbies include skiing and running.

John Corrigan, Regional Sales Specialist for southern California, Nevada (south), and Hawaii, has been with Sakura for nearly 4 years following his graduation from the University of California, Santa Barbara. He was a pathology assistant at Hoag Hospital in Newport Beach, Calif. He loves to surf the net in his off time.

Raphael Cuevas is the Sakura Territory Manager for Florida, Puerto Rico, and the Caribbean. He has over 20 years of experience in medical, surgical, and laboratory equipment sales in addition to marketing and management positions. His hobbies include bike riding with his 2-year-old daughter.

Robert Edmonston is the Sakura Regional Sales Specialist for the greater New York City and New Jersey areas and parts of New York, Connecticut, and Pennsylvania. Bob began as a Lab-Tek products specialist. In 1986, he became a hospital representative with Miles. He has received the "Golden Forceps" and "Eagle" awards for sales excellence.

David Gainous, Regional Sales Specialist for Minnesota, North and South Dakota, Nebraska, and Iowa, holds a degree in biochemistry from the University of Colorado in Boulder. He has been selling laboratory equipment for 14 years. His hobbies include tennis, weightlifting, and computer applications.

Elise Green graduated from Bowling Green State University and now resides in Toledo, Ohio. Her territories are Ohio and lower Michigan. Prior to joining Sakura, she was a sales specialist in the medical industry, valuable experience in her multifaceted role now. She has a wide range of interests that include reading, skiing, and biking.

John Green is a Regional Sales Specialist assigned to Alaska, Washington State, Oregon (north), Idaho, and Montana. John graduated as a medical technologist and worked at UCLA Medical Center. His sales career began in 1971 with Scientific Products. His ambition is to become a life master at bridge.

Bill Irwin, Regional Sales Manager, Eastern United States, joined Sakura in 1988. A graduate of the University of Texas at Arlington, Bill has a long sales career beginning with Deknatel, Inc. medical sales, Miles Inc., Rubicon Corporation (laboratory computer systems), and Citation Computer Systems. His other interests include golf, tennis, and snow skiing.

Melissa Killen is the most recent addition to the Sakura sales team. She's a former medical technologist, originally from St. Louis, Mo. Melissa is assigned to northern California, Nevada (north), and Oregon (south). She enjoys outdoor athletics, reading, and arts and crafts.

Bill Lannon, Regional Sales Specialist for Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, and parts of Connecticut and New York, makes his home in Duxbury, Mass. Bill has been affiliated with the Tissue-Tek product line since 1982 and finds the time to golf, ski, and officiate college and high school football.

Scott McKelvey is the Regional Sales Specialist for Upstate New York, Western Pennsylvania, and West Virginia. He holds a BA degree in biology from Washington and Jefferson College and an MT (ASCP) degree from Latrobe Area Hospital School of Medical Technology. Scott served 4 years with the 82nd Airborne Division. His hobbies include golf, downhill skiing, and mountain biking.

Dianne Megrdichian is Regional Sales Specialist for Colorado, Utah, Wyoming, New Mexico, Arizona, and part of Texas. A Rhode Island native, she now resides in Denver. Her prior experience includes work with optometrists. She enjoys biking, running, volleyball, skiing, reading, and travel.

Brian Rolerkite is the Regional Sales Specialist for Kansas, Missouri, Arkansas, Oklahoma, and part of Illinois. Using his degree in medical technology, Brian began his career by going into cardiac histopathology research with the VA. He moved into sales with Lab-Tek and Miles Scientific. He joined Sakura in 1995.

Scott Schultz is Regional Sales Specialist in North and South Carolina and Tennessee. He's a graduate of North Carolina State University with a degree in biological sciences. His background is in pharmaceuticals. Currently residing in Raleigh, NC, Scott finds time to enjoy basketball, tennis, travel, water skiing, and coaching Little League baseball.

Gary Toponce, Sakura Regional Sales Manager for the Western United States, has been in medical and laboratory product sales for over 22 years. Gary is one of Sakura's original sales representatives and is centered in the San Francisco Bay Area. His sales experience includes Lab-Tek products for Miles Inc. and A/O Cambridge Instruments. He enjoys snow skiing, rollerblading, fishing, and archery.

Robert Weingard is Sakura National Sales Manager. He has 7 years of experience with biological research laboratories and over 25 years of sales in bio/histology including Lab-Tek products. Bob was part of our original sales force.

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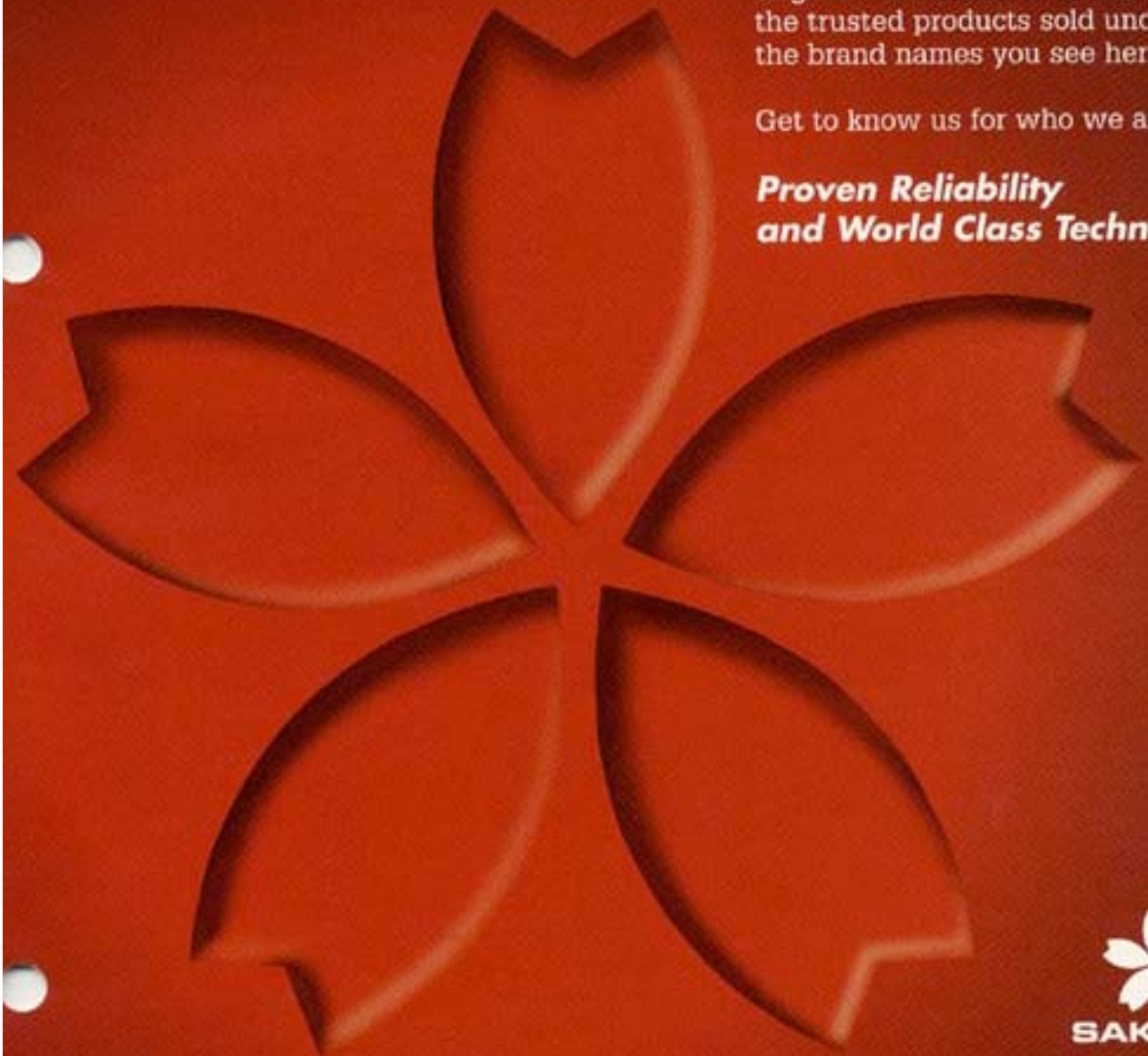

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