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Expedited Bone Throughput Using Microwave Decalcification

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Abstract

Histology laboratories supporting toxicology testing are routinely tasked with production of very large numbers of histologic specimens from standard laboratory animal species. Because of this, minimizing slide preparation time is an ongoing challenge.

One step that is especially time consuming is the traditional decalcification of bones by manual immersion in decalcifying agents at room temperature. This is particularly true for large laboratory animals such as dogs and primates. Among other factors, the rate of bone decalcification is dependent upon size of the specimen, age of the animal, type of decalcifying agent, and methodology employed.

One potential method for decreasing the overall processing time for bone specimens involves the use of microwave decalcification. The goal of this study was to determine the shortest microwave times that would provide adequate decalcification without compromising tissue quality. Bones of various types (sternum, rib, femur, femorotibial joint, and nasal turbinate) from eight animal species were decalcified in a microwave processor for variable time periods. Adequacy of bone decalcification was evaluated on an hourly basis for small animals (eg, mice and rats), and every 2 hours for larger animals (eg, dogs and monkeys), until decalcification was considered to be complete. Depending on the size and type of specimen, the time for complete decalcification of small animal bones was reduced from a period of 1-6 days

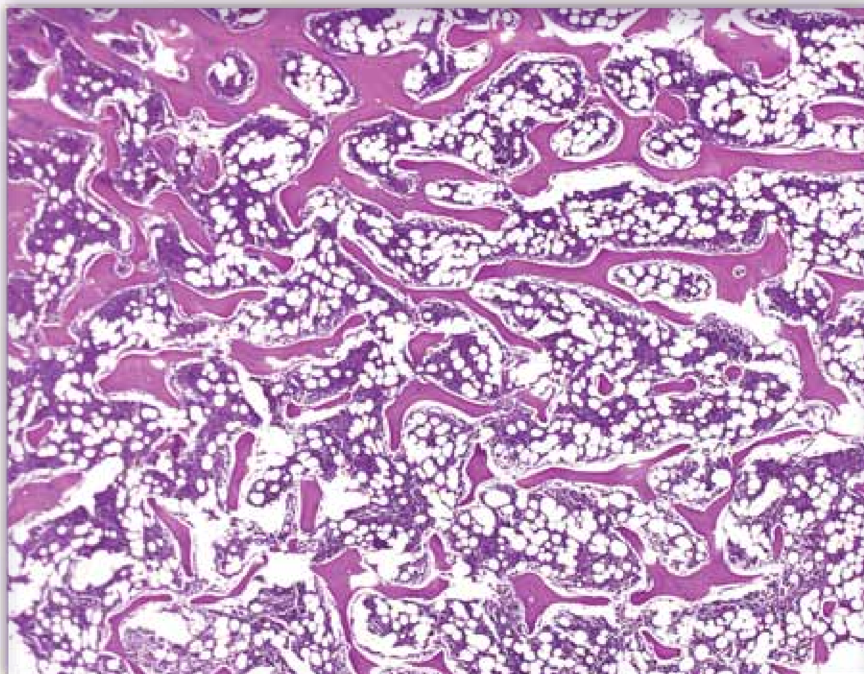

Fig. 1. Microwave decalcified dog femur. Stained with hematoxylin and eosin (H&E); 100X

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required with standard immersion decalcification to 1-16 hours when using microwave decalcification. For larger animal bones, the amount of time for decalcification was reduced from 3-12 days to 8-26 hours.

Microwave processing significantly reduced the time for bone decalcification and, as a result, we suggest using this method as the standard for larger animal bones, and on a case-by-case basis for small animal species. By using this methodology, slide submission by the histology laboratory to the pathologist can be expedited significantly.

Introduction

In our laboratory, there was an increased need to expedite preparation of slides for pathology review, so methods for optimizing the efficiency of various steps for microscopic slide preparation were considered. Since the traditional manual method of decalcifying bones is one of the most time-consuming steps in slide preparation, an evaluation of microwave decalcification was conducted. The goal of this study was to determine if microwave conditions would significantly shorten the time required to produce adequate bone decalcification without compromising tissue quality. Traditionally, decalcification of bones from research animals, most notably large animals, can take from several days to a week or longer. In this study, various bones from several animal species were decalcified using both a conventional procedure and the newer microwave methodology. The same decalcification solution was utilized for both methods. The microwave technique was compared to traditional decalcification by evaluating both the time required to achieve adequate decalcification as well as tissue quality.

Materials and Methods

Various types of bone, including sternum, rib, femur, femorotibial joint, and nasal turbinate were collected from the following eight animal species: mouse, rat, rabbit, guinea pig, ferret, dog, mini-pig, and monkey. Larger animal bones were pretrimmed to an approximate thickness of 3-4 mm; small animal bones (femur, joints, and nasal turbinate) were left whole initially and then trimmed after partial decalcification, as needed. Any bone that was too large initially to fit into a standard tissue cassette (Premiere®, CNA Scientific, Manassas, VA) was wrapped, along with its cassette, in gauze during decalcification.

The TissueWave™ 2 microwave processor (Thermo Scientific®, Kalamazoo, MI) was used for microwave processing (Fig. 2). Bones were loaded into the processing basket and placed in the processor chamber. The chamber was filled to the required level with Formical 2000™ decal solution (Decal Chemical Corp., Tallman, NY), which includes formic acid as its main ingredient. Although the capacity of the processing basket was listed as 74 cassettes, it was determined that the placement of cassettes in every other slot (for a maximum of 37 cassettes) produced superior results (Fig. 3). The processing temperature of the decalcification solution was set at 40ºC, the air agitator was set to "on", and the power control was set at 100%. The temperature of the decal solution during microwave processing was 38ºC-40ºC.

Fig. 2. Loading standard cassettes containing bones into the microwave processor.

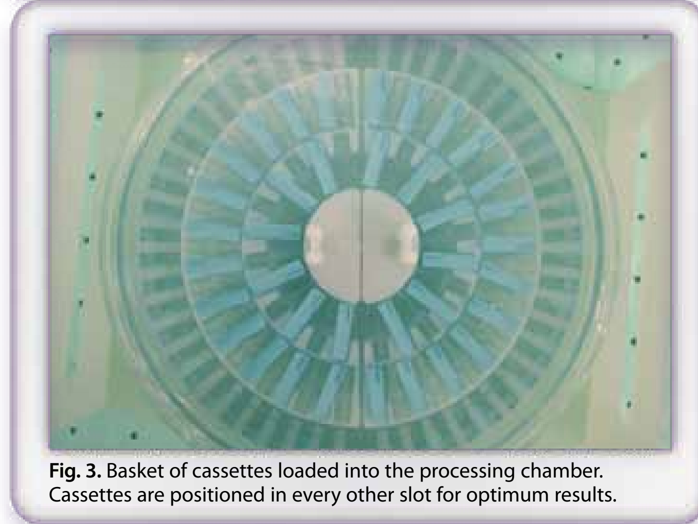

Fig. 3. Basket of cassettes loaded into the processing chamber. Cassettes are positioned in every other slot for optimum results.

Small animal bones were examined at 1-hour intervals and large animal bones were examined at 2-hour intervals during working hours until decalcification was complete. Bones were removed from the processor, rinsed in tap water, and loaded on a Tissue-Tek® VIP® 5 tissue processor (Sakura Finetek USA, Inc., Torrance, CA) for overnight processing. The bones were embedded in Paraplast® (Leica Micro Systems, St. Louis, MO) paraffin-embedding medium, and sectioned at 4 µm thickness. After the slides were stained with hematoxylin 2 and eosin-Y (Thermo Scientific®, Richard Allan Scientific®, Kalamazoo, MI) and coverslipped, they were submitted to three board-certified toxicologic pathologists (EPL, Sterling, VA) for evaluation.

Results

Compared with a traditional bone decalcification method, microwave decalcification significantly reduced bone decalcification time (Table 1). Time was reduced in all species and each type of bone evaluated, but was most significant for large animal bones. During subsequent studies in which super-sized slides and cassettes were required for very large bone samples, microwave processing was also found to reduce decalcification times significantly.

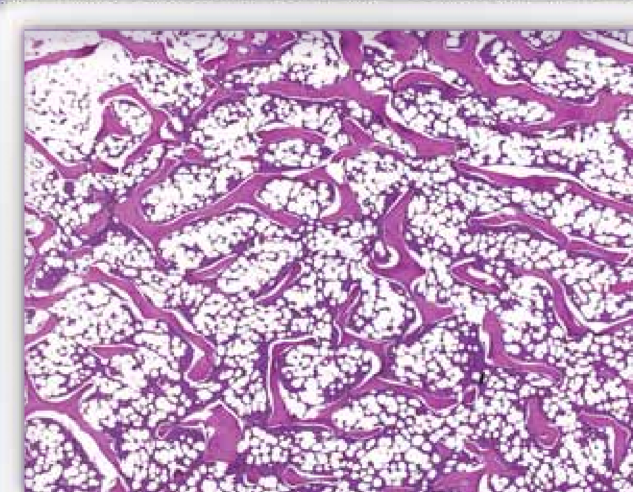

Fig. 4. Traditionally decalcified dog femur. H&E, 100X

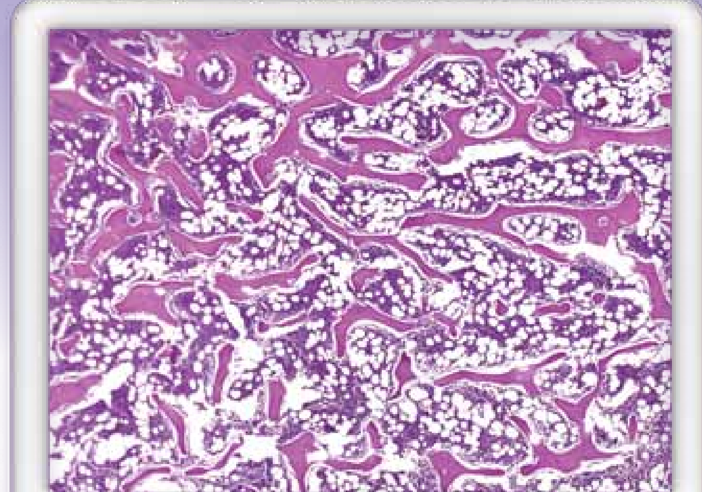

Fig. 5. Microwave decalcified dog femur. H&E, 100X

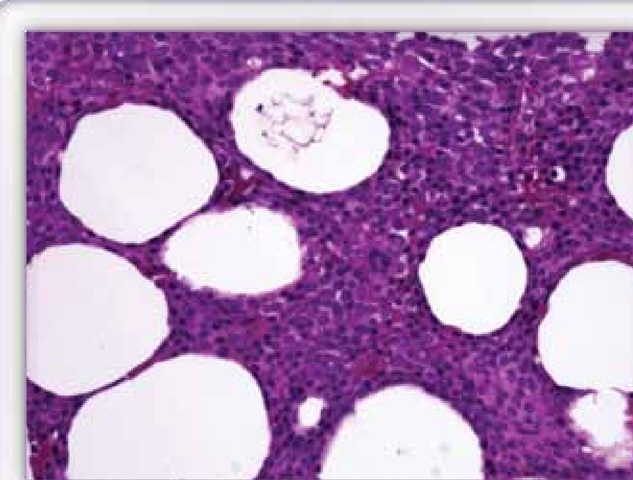

Fig. 6. Traditionally decalcified dog femur. H&E, 400X

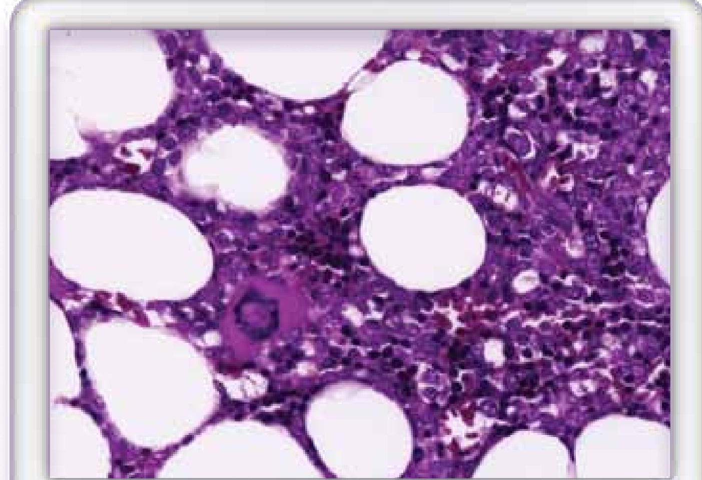

Fig. 7. Microwave decalcified dog femur. H&E, 400X

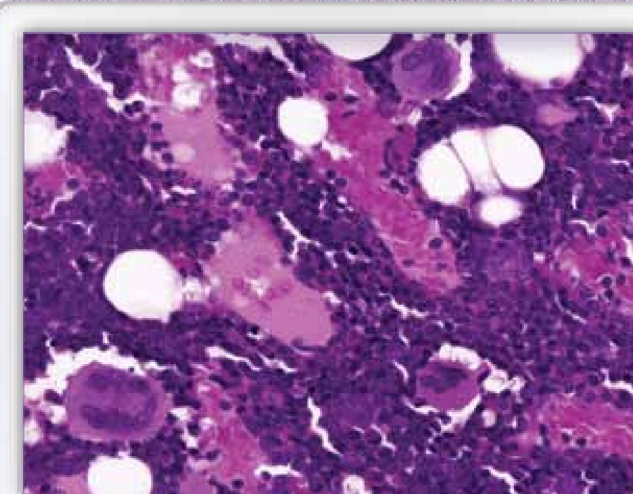

Fig. 8. Traditionally decalcified rat sternum. H&E, 400X

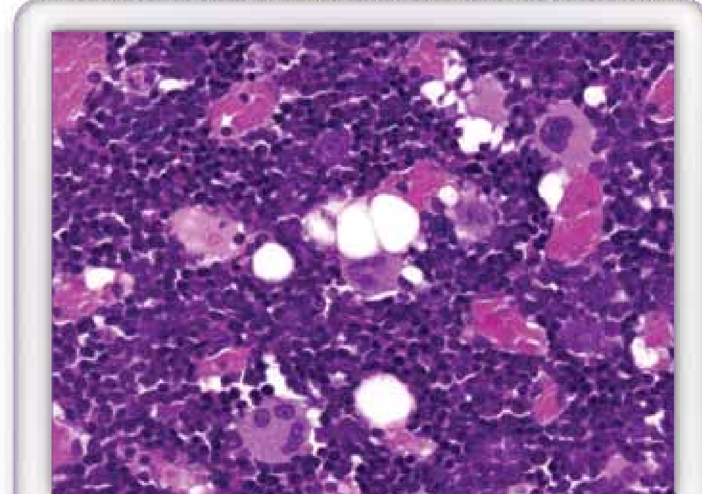

Fig. 9. Microwave decalcified rat sternum. H&E, 400X

Table 1. Traditional vs Microwave* Decalcification Times

BONE TYPE	TRADITIONAL DECAL TIME (DAYS)	MICROWAVE DECAL TIME (HOURS)	TRADITIONAL DECAL TIME (DAYS)	MICROWAVE DECAL TIME (HOURS)	TRADITIONAL DECAL TIME (DAYS)	MICROWAVE DECAL TIME (HOURS)	TRADITIONAL DECAL TIME (DAYS)	MICROWAVE DECAL TIME (HOURS)	TRADITIONAL DECAL TIME (DAYS)	MICROWAVE DECAL TIME (HOURS)	TRADITIONAL DECAL TIME (DAYS)	MICROWAVE DECAL TIME (HOURS)
	MOUSE		RAT		RABBIT/GUINEA PIG		FERRET		DOG/MINI-PIG		MONKEY	
STERNUM	1 d (overnight)	1-4 hr	2-3 d	4-8 hr	3-4 d	7-12 hr	4-5 d	8-10 hr	4-5 d	10-12 hr	4-5 d	12-14 hr
RIB	1 d (overnight)	1-4 hr	2-3 d	4-8 hr	3-4 d	7-12 hr	4-5 d	8-10 hr	4-5 d	10-12 hr	4-5 d	12-14 hr
FEMUR	1 d (overnight)	1-5 hr	3-4 d (trim after 2 d)	4-12 hr (trim after 8-10 hr)	4-6 d[†] (pretrimmed)	9-16 hr[‡] (pretrimmed)	3-5 d (trim after 2-3 d)	14-20 hr (trim after 9-14 hr)	6-7 d (pretrimmed)	18-22 hr (pretrimmed)	7-10 d (pretrimmed)	20-24 hr (pretrimmed)
FEMUR/ JOINT	1-2 d	2-5 hr	3-5 d (trim after 2 d)	4-12 hr (trim after 8-10 hr)	4-6 d[†] (pretrimmed)	10-16 hr[‡] (pretrimmed)	5-7 d (trim after 2-3 d)	14-22 hr (trim after 9-14 hr)	7-10 d (pretrimmed)	20-26 hr (pretrimmed)	10-12 d (pretrimmed)	20-26 hr (pretrimmed)
TURBINATE	1-2 d (trim after 1 d)	2-7 hr (trim after 2-5 hr)	3-6 d (trim after 2 d)	7-14 hr (trim after 8 hr)	4-6 d (pretrimmed)	12-16 hr	4-7 d (pretrimmed)	12-18 hr (pretrimmed)	7-10 d (pretrimmed)	16-22 hr (pretrimmed)	10-12 d (pretrimmed)	18-24 hr (pretrimmed)

* Bones may remain immersed in decalcification solution for several hours following completion of microwave decalcification.
† Guinea pig, trim after 2-3 days with traditional decalcification.
‡ Guinea pig, trim after 9-14 hours with microwave decalcification.

The quality of bone sections following microwave decalcification was considered to be very good to excellent by the reviewing pathologists. No adverse effects on structural preservation were noted, and nuclear detail was demonstrated to be sharp (Figs. 4-9).

Conclusions

Significantly reduced processing times were achieved when bone samples (Fig. 10), especially from large animals, were decalcified in a microwave processor. Tissue quality following microwave decalcification was determined to be comparable or superior to the results obtained using traditional decalcification methodology. Microwave processing proved to be an efficient and reliable procedure for the decalcification of bones from laboratory animal species.

Acknowledgments

The authors would like to thank Bernie Wolfe (technical support), Dr. Jeffrey C. Wolf (photographs and slide evaluation), Dr. Kathleen Funk (slide evaluation), Nelson Wilson (manuscript review), and Cyndi Bono (poster design) from Experimental Pathology Laboratories Inc., and Dr. Sarah Hale (slide review) and Theresa Sharp (supplied some of the bones for method development) from Covance Laboratories, Inc.

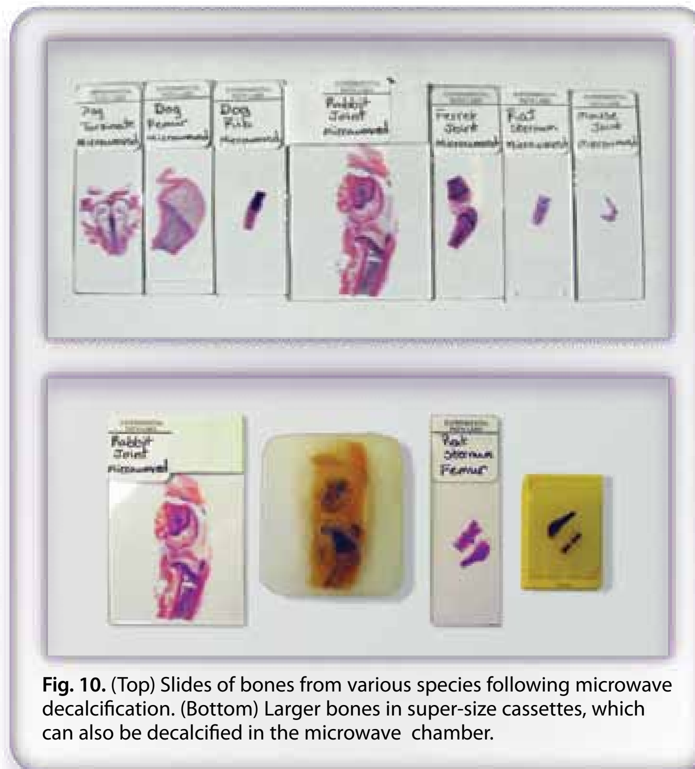

Fig. 10. (Top) Slides of bones from various species following microwave decalcification. (Bottom) Larger bones in super-size cassettes, which can also be decalcified in the microwave chamber.

Cryopreservation Method Optimization for Mouse Brain Tissues

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Abstract

Cryopreservation of whole mouse brains can result in morphology artifacts that affect the evaluation of this tissue. In researching diseased mouse models, these artifacts interfere with the interpretation of disease pathology.[1] In order to improve preservation of tissue architecture with minimal artifacts, several fixation and cryopreservation techniques were evaluated. In this study, fixation of central nervous system (CNS) tissue consisted of perfusion with 4% paraformaldehyde (PFA), postfixation in PFA, followed by immersion in 30% sucrose. This method was compared to either unfixed samples or immersion fixation in 10% neutral buffered formalin (NBF), with or without transferring to 30% sucrose. Prior to freezing, all brains were transferred to cryomolds filled with OCT™ compound (Sakura Finetek USA, Inc., Torrance, CA). During freezing, the cryomolds were partially immersed one by one in a container of cooled 2-methylbutane by holding them with long forceps until solidified. This method was compared to either immersing the cryomolds in liquid nitrogen or placing all cryomolds together in a shallow container filled with cooled 2-methylbutane. All brains were sectioned at 20 µm on a cryostat and stained with hematoxylin and eosin (H&E) and Nissl stains, which were used to determine the best fixation and cryopreservation methods. Based on the observation of stained sections, results indicate that the method using PFA perfusion and postfixation, cryoprotection with sucrose, and freezing multiple cryomolds in a shallow container with 2-methylbutane eliminated traditional freezing artifacts and was more efficient than traditional methods of fixation and cryopreservation for mouse brain tissues.

Introduction

It is the brain's unique and multiplex structure that necessitates a meticulous procedure for the preservation of whole brains. Many histologists have struggled to find an optimal technique to fix and freeze brain tissue that avoids any artifacts or damage to brain tissue architecture. In this study, a variety of approaches were used to validate the best and most cost-effective method to fix and freeze brain tissue.

Materials and Methods

Tissue Collection and Preparation

Brains were harvested from 7-month-old YAC128 mice that were obtained from Charles River Laboratories International, Inc. (Wilmington, MA).

Fixation

Perfused brains were infused with 4% PFA in phosphate buffered saline (PBS) (Electron Microscopy Sciences, Ft. Washington, PA) using a Masterflex Console Drive perfusion pump (Cole-Parmer Instruments, Vernon Hills, IL) at 18 mL/minute, followed by immersion in PFA for 48 hours at 4ºC.

The brains that were studied using only full immersion fixation were first trimmed sagittally along the midline prior to placing them into 10% NBF for 48 hours at room temperature.

Sucrose infusion

Cryopreserved brains were incubated in 30% sucrose (Sigma-Aldrich, St. Louis, MO) in PBS at 4ºC until the tissue sank to the bottom of the tube.[2]

Freezing Methods

Samples were removed from their final solution prior to freezing, blotted on a paper towel to remove excess solution, then placed into a peel-away mold (22 x 22 x 20 mm) and covered in OCT. Samples were allowed to sit in OCT for 15 minutes prior to freezing in order to allow all bubbles to float to the top and the OCT to bind to the tissue.[3] For all 2-methylbutane (J.T. Baker, Phillipsburg, NJ) methods, a container suitable for freezing samples (small metal bowl as in Figs. 1 and 2, or a tripour beaker) was surrounded by dry ice.

Full immersion

Enough 2-methylbutane was carefully poured into the freezing bowl so that the cryomolds could be fully submerged. A few dry ice pellets were added to chill the 2-methylbutane. When the bubbling stopped, the cryomolds containing the brain/OCT samples were submerged completely as in Fig. 1.

Shallow immersion

A smaller volume of 2-methylbutane than what is used for full immersion was added to the bowl, so that the cryomolds could sit on the bottom of the freezing bowl without being fully submerged (Fig. 2). A few dry ice pellets were added to chill the 2-methylbutane prior to freezing any samples to ensure proper freezing temperature.

Forceps immersion

Brain/OCT samples were held by the outer edge of the cryomold with long forceps and immersed into precooled 2-methylbutane only up to, but not above, the top of the cryomolds. They were held in the 2-methylbutane by the histologist until completely opaque and solidified (Fig. 3).

Liquid nitrogen

Unfixed brains were bisected sagittally, placed into cryomolds, and covered in OCT prior to freezing. This method utilizes liquid nitrogen as a freezing agent analogous to the shallow immersion method described above.

Sectioning

The tissue blocks were mounted on chucks and sectioned with low-profile blades in a cryostat. The 20 µm sections were collected on charged Superfrost Plus glass slides (Erie Scientific LLC, Portsmouth, NH) and dried overnight at room temperature before storing in a -20ºC freezer.

Staining

All slides were postfixed in NBF for 5 minutes prior to staining with H&E. Nissl staining, also performed according to standard protocol, supported the results seen in the H&E slides (data not shown).

Photography

Microscopic photographs were taken with a 10X objective on a Nikon Eclipse 80i microscope equipped with a Nikon DXM1200F digital camera.

Results

Freezing

The full immersion method (Fig. 1), although faster at freezing specimens than both the shallow immersion and forceps methods, used the largest volume of 2-methylbutane. In this method, the tissues began to freeze from all sides at once, resulting in some cracked blocks (Fig. 4), which exposed the tissues directly to the cooled 2-methylbutane.

The shallow immersion method (Fig. 2) allowed multiple blocks to be frozen at the same time in a more ergonomic and efficient manner. Multiple samples could be placed into the bowl without the histologist having to hold each sample while it was being frozen. This process required only half the volume of 2-methylbutane than did the full immersion and forceps methods. But most importantly, because the top of the sample was the last to freeze, it allowed the OCT to expand upward as the block froze. This method consistently produced blocks without cracks (Fig. 5). The forceps method (Fig. 3) resulted in no cracked blocks (block not shown) but used a larger volume of reagent. This technique required a deeper level of 2-methylbutane and did not prove to be as time efficient as the shallow immersion method. It was necessary for the histologist to hold the block steady until it was hardened, allowing only one or two blocks to be frozen at a time.

Freezing the block in liquid nitrogen was the fastest method, but often resulted in cracked blocks (Fig. 6) due to the rapid drop in temperature.

Fixation

The evaluation of fixation results was based on three criteria: (1) tissue architecture, (2) differential staining, and (3) the presence of artifacts.

Unfixed tissue frozen in liquid nitrogen (Fig. 7) resulted in extensive tissue architecture damage. The differential staining was fair but the staining intensity appeared low and muddy. This method resulted in the most cracking and freezing artifacts of all methods used (not shown). In comparison, unfixed tissue frozen in 2-methylbutane by the shallow immersion method (Fig. 10) did not show as many cracks as with the liquid nitrogen method, but there was a reduction in staining intensity.

Tissue architecture was less preserved under the same freezing and fixation conditions without cryopreservation (Figs. 8 and 9) compared to those that were cryopreserved with sucrose (Figs. 11 and 12). The lack of sucrose resulted in poor differential staining throughout the section and splitting between granular and molecular layers throughout folia.

Although there was good tissue architecture and cellular detail in the shallow immersion/NBF/sucrose method (Fig. 11), the perfusion/PFA/sucrose method (Fig. 12) had far less splitting between granular and molecular layers. In addition, cellular details were more distinct and neuronal parenchyma was well preserved in the perfusion/PFA/sucrose method.

Fig. 1. Mouse brain tissue blocks being frozen by the full immersion method. The blocks are fully immersed in prechilled 2-methylbutane. The dry ice surrounding the bowl helps maintain proper freezing temperature.

Fig. 2. Mouse brain tissue blocks being frozen by the shallow immersion method. The blocks are only partially immersed in prechilled 2-methylbutane. Only half the volume of 2-methylbutane is required as that used in the full immersion and forceps immersion methods, and multiple blocks can be frozen at the same time.

Fig. 3. Mouse brain tissue block being frozen by the forceps immersion method. In this method, only one or two blocks can be frozen in cold 2-methylbutane at a time, requiring specific hands-on time by the technologist. It also requires a large volume of cold 2-methylbutane.

Fig. 4. Mouse brain tissue block frozen by the full immersion method, which shows noticeable cracks in the tissue and OCT as a direct result of tissue that was directly exposed to the cold 2-methylbutane.

Fig. 5. Mouse brain tissue block frozen by the shallow immersion method, which demonstrates no cracks as a result of freezing.

Fig. 6. Mouse brain tissue block frozen by the shallow immersion method using liquid nitrogen rather than 2-methylbutane. This block sustained significant cracks, even though this was the fastest freezing method of all the ones tested.

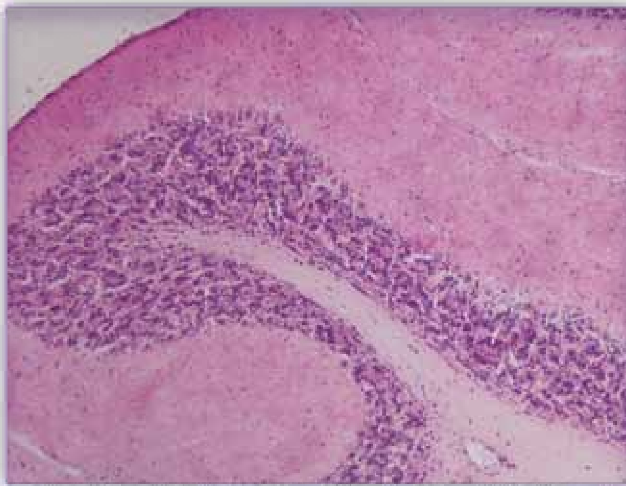

Fig. 7. Unfixed mouse brain (only cerebellum shown) frozen in liquid nitrogen, with no cryoprotection. H&E, 100X

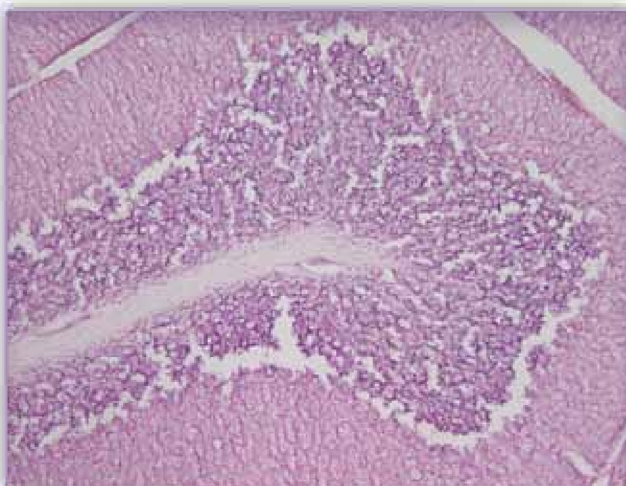

Fig. 8. Mouse brain (only cerebellum shown) frozen in 2-methylbutane by the shallow immersion method, postfixed with NBF, stained with H&E, with no cryoprotection. H&E, 100X

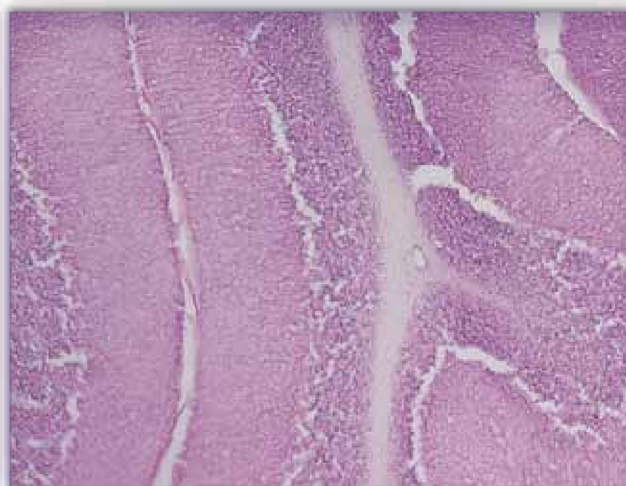

Fig. 9. Mouse brain (only cerebellum shown) perfused with PFA followed by freezing in 2-methylbutane by the shallow immersion method, with no cryoprotection. H&E, 100X

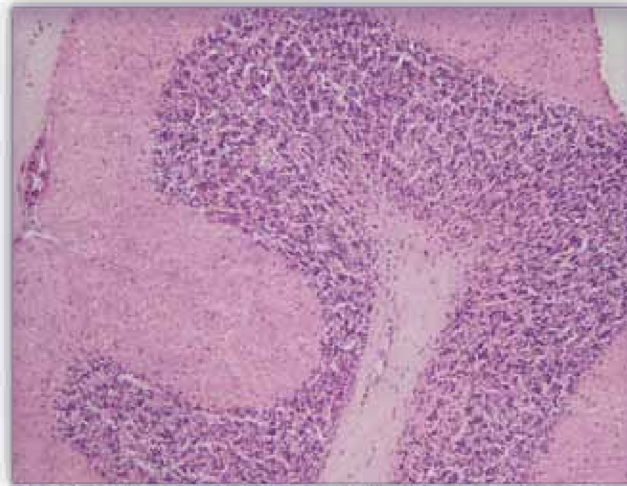

Fig. 10. Unfixed mouse brain (only cerebellum shown) frozen in 2-methylbutane by the shallow immersion method. H&E, 100X

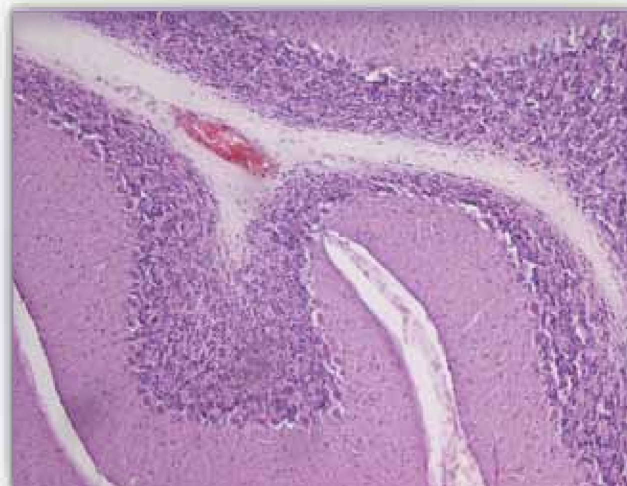

Fig. 11. Mouse brain (only cerebellum shown) fixed with NBF, followed by shallow immersion freezing in 2-methylbutane and cryoprotection with 30% sucrose. H&E, 100X

Fig. 12. Mouse brain (only cerebellum shown) perfused with PFA, postfixed with PFA, followed by shallow immersion freezing in 2-methylbutane and cryoprotection with 30% sucrose. H&E, 100X

Conclusion

The shallow immersion freezing method with chilled 2-methylbutane provided optimal microscopic results. This method allowed multiple blocks to be frozen at the same time. Samples could be left for a period of time without requiring a histologist to hold the sample while it was being frozen. This method saved not only time, but also valuable reagents. It required only half the volume of 2-methylbutane used in either the full immersion or forceps method. Because this method allows blocks to freeze from the bottom to the top, cracking of samples was extremely rare. As the tissue/OCT froze, the liquid layer (not yet frozen) on the uppermost surface allowed the sample to expand without creating a break. Due to these factors, this method consistently produced high-quality blocks without any cracks.

The addition of sucrose as a cryopreservation agent was critical. Both the PFA/perfusion and the NBF/immersion methods yielded samples that showed better architectural preservation and improved overall staining intensity compared to the samples that did not use sucrose in cryopreservation.

Although both fixatives showed good cellular detail, PFA appeared to retain the fine detail better than NBF. Perfusion fixation further improved the preservation, reducing artifacts as it brought fixative to deeper areas within the brain that may be affected by the slow penetration of an immersion fixation.

The combination of shallow immersion freezing, perfusion and postfixation with PFA followed by cryopreservation with sucrose has proven to be the best method to preserve the morphology of mouse brain tissue.

References

1. Pontén U, Ratcheson RA, Siesjö BK. Metabolic changes in the brains of mice frozen in liquid nitrogen. *J Neurochem*. 1973;21(5):1211-1216.

2. Rosene DL, Roy NJ, Barry JD. A cryoprotection method that facilitates cutting frozen sections of whole monkey brains for histological and histochemical processing without freezing artifact. *J Histochem Cytochem*. 1986;34(10):1301-1315.

3. Steu S, Baucamp M, von Dach GV, et al. A procedure for tissue freezing and processing applicable to both intra-operative frozen section diagnosis and tissue banking in surgical pathology. *Virchows Arch*. 2008;452(3):305-312.

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Is Rapid Tissue Processing the Right Choice for Your Lab?

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Abstract

Technologies that allow rapid tissue fixation and rapid tissue processing (RTP) have gotten increasing attention in clinical histology laboratories over the past 5 years as a result of the perceived benefits to patient care and lab operations. There has also been much reported about the problems that have been encountered with this technology. Some laboratories have chosen to abandon RTP, while others have used it as a catalyst for change that truly benefits patients and their treating physicians. Adopting RTP has even resulted in improved employee satisfaction as workplace constraints have been eased. The information in this article is a compilation of shared user experiences with the implementation of the Sakura Tissue-Tek® Xpress® (Sakura Finetek USA, Inc., Torrance, CA).

Why Adopt New Methodology?

Traditional workflow within anatomic pathology (AP) laboratories follows a linear progression that is familiar and comfortable for pathologists and technical staff. Patient specimens are batch processed and moved through a complex and labor-intensive series of technical steps that produce stained microscope slides used by a pathologist to make a diagnosis. By its very design, batch processing takes all patient specimens received during the workday and processes them late that same day; the slides from those specimens will be finished some time the next day. Because this has been the norm for so long, many fail to appreciate the significance of delayed diagnosis and delayed clinical treatment. Current case turnaround time (TAT) for specimen type (88305) in anatomic pathology may range from 24 hours to 60 hours, dependent upon the facility. Some AP laboratories have reported up to a 60% improvement in TAT following the adoption of RTP.[1]

In 2003, new technology—first-generation RTP—became available that would allow for continuous processing of patient specimens. Since that initial technologic launch, the concept of RTP presents laboratory leaders with the opportunity to restructure technical and clerical work steps to meet increasing demands for the most efficient work model that will be delivered in the most cost-effective manner. The Tissue-Tek® Xpress®, a rapid, continuous flow tissue processor, allows continuous loading of patient specimens for processing as soon as the specimens are fixed. Specimens no longer have to wait for the entire day's workload to be batch processed, but can be continuously moved through the sequential technical steps that produce the stained microscopic slide set for diagnosis. The question about *whether* to launch RTP has been replaced by *when* and *how* to launch it.

Today, in addition to the Tissue-Tek® Xpress®, there are several RTP instruments readily available from established vendors, including the Peloris (Leica Microsystems, Bannockburn, IL), the MARS System (Hacker Instruments & Industries, Winnsboro, SC), and the Pathos Delta (Milestone Medical Technologies, Kalamazoo, MI), among others now rapidly appearing on the horizon. Making the changes necessary to move from a batch processing mode to a continuous processing mode can be a journey loaded with anxiety. However, the ability to actively plan for and execute these changes can result in improved case TAT and more employee satisfaction. A systematic action plan can make the difference between planned, positive change and forced, negatively perceived change. Proactively performing a systematic appraisal of the entire existing workflow—including work process mapping; designing a structured, solid validation plan that includes all aspects of the testing sequence; actively involving stakeholders in the evaluations; and consistently using a broad communications plan to keep all staff informed—will give satisfactory results for all those impacted by the change to RTP.

Who Will Benefit, and How?

Pathologists have traditionally been identified as the end customer for all the activities leading up to and culminating in the stained slides. That view has broadened over the past several years, and now the focus is on the patient as the end customer. This philosophy change has impacted the laboratory, whose staff members are now forced to consider how they work, when they produce the work product, and for whom. There are benefits associated with RTP that align well with this change in direction. The added benefit that RTP offers means that its product can truly become a win-win for the new customer (patient) while still meeting the needs of the former primary customer (pathologist). RTP makes it possible for pathologists to provide diagnostic information to clinicians more quickly. But what does this really mean for the patient?

Faster diagnosis enables clinicians to commence or alter treatment plans that would affect inpatient length of stay (LOS). This is a quantifiable metric that is appealing to both hospital and clinic administrators, and can affect whether insurers choose to reimburse patients seeking care through a particular pathology practice. Hospital administrators can theoretically expedite turnover of surgical rooms or same-day surgery sites more quickly, resulting in additional revenue. This is a direct result of faster diagnosis from improved laboratory TAT. Hospital administrators may also be able to absorb more cases due to the potential for RTPs to move cases along using a 1-piece flow.

Clinicians could use same-day pathology diagnosis to begin same-day treatments for their patients before they leave same-day surgery clinics or are discharged from the hospital. Think about the opportunity for patient satisfaction in the following scenario:

A woman finds a lump in her breast one morning. She immediately calls for an appointment and is quickly moved through radiology/imaging where it is determined that the lump be evaluated by surgical biopsy. The woman is soon scheduled for outpatient surgery where the biopsy is obtained. The specimen is delivered to the lab and is quickly processed using RTP technology, followed by embedding, cutting, and staining. In a matter of a few hours, slides are delivered to the pathologist, who renders the diagnosis and calls the clinician. Based on that diagnosis, the clinician makes the decision to begin treatment immediately that day.

It might surprise you to know that RTP can influence staff recruitment and retention in the laboratory. It is no secret that our workforce continues to age, with a great majority of technically skilled workers moving ever closer to retirement. Adding to this problem is the fact that histotechnology schools continue to close, and those that stay open have decreased the number of students they graduate. Laboratory workers in histology and surgical pathology typically work odd hours, often beginning work in the middle of the night or late in the evening. This work schedule may not mesh with family responsibilities at a time when many laboratories seek to emphasize work-life balance as a recruitment strategy. Staff workplace satisfaction could be enhanced by reducing the kind of stress that comes along with the current batch processing work model. Labor-intensive work that moves along in a bolus would be distributed in a more orderly and continuous fashion (1-piece flow as in the Toyota Production System model and in accordance with Lean principles).

The ability to take a close look at which problems will be solved by adopting RTP versus the risk of staying with the current state of batch processing can prove to be a valuable exercise for laboratory leaders.

RTP: Problem-solving Capabilities

- The capability of meeting any institutional goal to provide a faster TAT for surgical pathology results
- The ability to position the institution to become a leader in the practice of surgical pathology
- The opportunity to get results in 4 to 5 hours, or less, versus a process that used to take 24 hours
- The opportunity to standardize work processes so they are no longer dependent on who is at work and available to do a certain bench task
- The ability to provide a consistent product, delivered on time, every time
- The opportunity to reassign the right work to the right person—defining tasks as either technical or nontechnical, and assigning those tasks requiring a higher level of problem solving to qualified staff—using this strategy as an opportunity to promote nontechnical staff to the next level of technical training and job opportunity
- The opportunity to reduce the cost per test
- The ability to eventually eliminate formalin and xylene from the workplace

RTP: Potential Problems to Be Addressed

- Clinician and pathologist practice behaviors may not be influenced to change
- The ability to deliver on the promise—if the laboratory infrastructure doesn't allow the lab to receive more specimens (outreach) for processing, or produce the right kind of report (informatics limitations), then success may be limited
- Specimen quality and integrity may need some technical work and assistance to tweak it for optimal results; validation plans, studies, and results documentation are critical
- Established work practices and workflow are difficult to change
- Keeping the product (specimen/cassette/slide) moving
- Ensuring that both ends of the workflow can accommodate the middle step (RTP), which can now be done more quickly. For example, having insufficient numbers of grossing personnel at the beginning and/or insufficient numbers of sign-out staff at the end can hinder the efficiency attained by the faster RTP step
- Anticipating data collection needs, designing a way to capture them, and delivering the data to the right person or place at the right time

Who Are the Stakeholders and What Are Their Roles?

Table 1. Stakeholders and Their Roles

Stakeholder	Involvement
Clinicians	Continuous communication between them and the pathologist to assess patient care needs
Pathologists	Work closely with director of surgical pathology to guide diagnostic services for patient
Resident pathologists and other medical trainees	Provide insight and suggest ways in which residents can achieve superior education and training, and to provide patient services
Laboratory staff: technical clerical support	Participate in various work groups that will address specific issues such as workflow, skills building, scheduling, and staffing levels, and the training and competency assessment necessary to implement RTP
Physicians using outreach laboratory services	There is marketing potential to these clients for same-day or 24-hour TAT

Planning for Change

The central issue is about changing the behavior of the people involved within the established batch processing workflow. Behavioral changes happen when an incident or an opportunity can truly speak to people's feelings and emotions. Most of us entered this field because we were inspired by emotions such as empathy in being able to help patients, or the ability to use technical skills to influence patient treatment, diagnosis, and prognosis. It feels good when we learn that our technical tasks and actions contributed to a good patient outcome. Understanding this basic need and how it is met in the workplace can offer managers and pathologists insight into planning adequately for the type of change needed to adopt RTP.

In successful transitions from traditional batch processing to RTP, change leaders must find ways to help the staff see the opportunities related to RTP in ways that influence their emotions, not just their technical knowledge, skills, or beliefs. Using imaginative and powerful demonstrations or stories can build the case for adopting RTP, and ultimately alter staff behavior sufficiently to overcome the operational and technical obstacles related to such large-scale change. Many people do not handle large-scale change well. They make predictable mistakes, which occur because they have little exposure to highly successful conversions from batch processing to RTP.

The goal of this article is to share change strategies from a number of successful conversions by RTP users and to provide them as a

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resource to laboratories currently investigating RTP. Change journeys are highly individual, but the commonality is that we all seek to "do it right" in a way that best matches the individual institutional needs but, above all, in a way that ultimately benefits our patients.

Building the Case for Change Starts With a Thorough Analysis of the Current State

You must understand exactly where you are right now (current state) before you can know where you want to go (future state). Using a standard work process map to identify each individual work activity along the batch processing route will form the foundation for 1) painting a physical picture of a linear process in which each single step is affected by the previous step; 2) demonstrating the complexity of this multistep process; and 3) identifying overlapping or redundant steps in which the same piece of work (tissue specimen) is handled, packaged (cassette and block), and touched (grossing, processing, embedding, cutting) by many hands along the way. Identifying opportunities to streamline the process with fewer steps, such as reducing or eliminating delays and stops along the way with fewer hands on each specimen, can contribute toward reducing or eliminating errors. The less often the tissue is handled or labeled reduces the chance that specimen identification errors will occur.

Case Study

A high-volume clinical laboratory with a large physical footprint is faced with 1) staffing shortages; 2) a requirement that the lab incorporate a 15% increase in case volume within the next year; and 3) clinician demand that case diagnosis be available sooner than the customary 24-hour TAT. The group is hesitant to change, fearing more work and stress on an already burdened system. Some staff members endure fatigue and ergonomic problems related to repetitive work being done in a short and intensive time period. They all share in the lifting and hoisting of drums containing the toxic chemicals used in the tissue processors and the xylene/alcohol recycling instrument. Constant interruptions from frequent phone calls and pathologist visits in the lab contribute to a "controlled chaos" atmosphere. RTP is suggested for consideration.

The project manager invites the group to a presentation. They arrive with skepticism. To their surprise, they enter the conference room to discover 2 separate stacks of items.

*Stack #1**

25 boxes of protective gloves in various sizes
7 5-gallon drums of 100% ethanol (ETOH)
4 5-gallon drums of 95% ETOH
16 4-gallon box cubes of 10% neutral buffered formalin (NBF)
3 containers of paraffin
Funnels, reagent hose feeders, and various other implements used for changing out tissue processors

**This represents the types of reagents, packaged in the current form, needed during 1 week of laboratory operations for the current average block volumes.*

Stack #2†

3 boxes of protective gloves
2 1-gallon containers of Processing Reagents 1, 2, 3, and 4

†This represents the types of reagents, packaged in the current form, needed during 1 week of tissue processing for the proposed changeover to the RTP, using the current block counts.

- *Which laboratory operation and task-related expectation is more appealing?*
- *Can such an exercise, using information and props from your own operation, make a visual impact on staff members?*

Communicating the Opportunity to Clinicians, Faculty, and Staff

Many efforts toward making change in both public and private sectors begin with an announcement that "Change" is coming. Sometimes there is little explanation, background, or justification given. People often come away from these announcements feeling upset, angry, and cynical. These announcements leave out important information. What should be included in the preliminary communications and activities? Present a persuasive kickoff speech that honors the past, or status quo, by recognizing past successes, acknowledging people's contributions, and highlighting attitudes that helped in past crises. By showing appreciation for the hard work people have done, you build the bridge to the future state by using their past actions as examples of what they are capable of doing in the future state of RTP.

In a similar manner, when talking about the future of RTP, discuss the target goals for your laboratory and how the characteristics and actions demonstrated by the staff in the past will help with the challenges faced by this large-scale change. Focus on future improvements in quality, patient care and service, cost reductions, and TAT. Efforts to teach new instrumentation and methodology through education and training, often delivered by the vendor, can demystify the process. Providing the opportunity for something new—RTP—to become familiar can make it achievable.

Recommendation 1: Write a detailed on-site demonstration plan that addresses aspects including recruiting for, and assigning, leadership roles and responsibilities, facilities planning, and the actual technical team (grossing, fixation, processing, embedding, staining, cutting, and delivery of stained slides). Recruit and designate "pilot pathologists" who are willing to be part of the assessment and understand the crucial need for detailed results documentation.

Recommendation 2: Request an on-site demonstration of the RTP; the pathologists and staff members involved can use tissues from their work areas. Obtain standard operating procedures from laboratories in which RTP has been successfully introduced. Assign staff members to read and critique with an eye toward anticipated obstacles or problems.

Recommendation 3: Design and use a start-up cost analysis worksheet with RTP.

Recommendation 4: Executive planning
- Design a step-by-step plan
- Create the process improvement project proposal
- Engage in team building
- Execute all related tasks
- Documentation, documentation, documentation

Recommendation 5: Communicate. Communicate some more.

Part 2 will appear in the next issue of HistoLogic *and will continue with the technical aspects of planning and executing the introduction of RTP into the laboratory.*

My Life-changing Opportunity to Train Histotechs in Africa

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Throw your dreams into space like a kite, and you do not know what it will bring back, a new life, a new friend, a new love, a new country.
—Anaïs Nin

Most lucky people who work in histopathology labs are just that—lucky. Every day, we are privileged to be an important part of the healthcare team, assisting others in diagnosing and caring for patients. We get to work with some of the best histotechs in the world, we work closely with some of the best pathologists in the world, and we have the best instrumentation and supplies in the world! We are encouraged and allowed to be active in continuing education and social activities with other histotechs—activities that open doors and allow travel to places far from home.

OK, maybe you don't agree with all of that, but it has always been my opinion. Early in my histology career, I realized that I could continue to learn and progress in the lab profession if I chose to do so. Over the many years of my histology career, I have been one of the lucky ones—and I have made very purposeful choices to be involved, to learn more, and to befriend and learn from other histology professionals. Because of my choices, my luck has returned to me many times over! Let me explain.

During the first 10 years or so after I found myself in histology—and I loved it almost immediately—I just worked in the lab. Of course, I was learning, but I didn't know there were options available for advancement, continuing education opportunities, and professional meetings to attend. I guess one might say that I was paying my dues at the bench, performing the daily work that is the histology lab. But eventually I found myself in a position that afforded me a trip to my first professional meeting. In 1984, I traveled from my home in Lubbock, Texas, to Kansas City, Missouri, for my first National Society of Histotechnology (NSH) Symposium. What a trip—a plane ride, a "fancy" hotel, meeting other people who actually know what a histotech does, exhibits of instrumentation one could only dream about, and even parties! I was hooked, and after that meeting, I have attended every annual NSH meeting except for two. I am now a histology educator, having left the daily work of the lab for teaching about 16 years ago.

From that Kansas City meeting until now, my life as a histotech has taken me many places. I've gotten involved in different activities and met many histotechs and other pathology-related professionals. In addition to my activities with NSH, I have served on committees at the American Society for Clinical Pathology (ASCP), and worked with the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS). And my life as a histotech recently became even more exciting and rewarding. Are you ready for a trip to Africa?

In December 2007, I received an email from the ASCP asking if I would be interested in traveling to Africa to assist with development and revisions of the laboratory training programs' histology curriculum. My first thoughts: Africa? Are they crazy? However, after learning more about the work to be done and the group I would travel with, and after receiving encouragement from my family and support from my employer, I agreed to seize the opportunity.

The President's Emergency Plan For AIDS Relief (PEPFAR) was a commitment of $15 billion over 5 years (2003–2008) from US President George W. Bush to fight the global HIV/AIDS pandemic. The program initially aimed to provide antiretroviral treatment (ART) to 2 million HIV-infected people in resource-limited settings, to prevent 7 million new infections, and to support care for 10 million people (the "2–7–10 goals") by 2010.[1] The program's funding was administered through the Centers for Disease Control and Prevention (CDC), granted to agencies for use in countries worldwide to treat and prevent HIV/AIDS. Early in the program, it was evident that in order to provide treatment to people, the disease first had to be diagnosed—and the need for improved laboratory services was revealed! The ASCP realized that they had members with knowledge that would be helpful, so the ASCP Institute for Global Outreach was formed for this purpose. They applied for, and received, some of the PEPFAR monies, and began work in many African countries as well as in other HIV/AIDS-ravaged countries worldwide. For several years, lots of hard work was done by many medical technologists (MTs) to improve the diagnostic abilities of laboratories in large hospitals as well as in isolated rural areas of the world. Great improvements were made, the programs were successful, and the antiretroviral drugs were made available to the people who needed them.

In 2007, the healthcare agencies in some of the countries where ASCP had been working realized that they needed to improve their laboratory education in order to have a well-trained workforce. Again, the ASCP Institute was willing to offer assistance by funding some of their members who are laboratory educators. The MTs began traveling to Africa to work with the laboratory training programs' faculty and administrators, sharing knowledge and expertise in course design and teaching methods. In the United States, histology and medical technology training are separate, but in most other countries of the world, the training is a combined curriculum. Because there were no histotechs involved with the ASCP group, that aspect of the curricular expertise was lacking in the groups of MTs who traveled to Africa. In late 2007, they realized the need for a histotechnology educator for the team of volunteers. This is how I came to receive the opportunity to travel to Africa.

Travel to African countries is not equivalent to a vacation trip to Europe! Many immunizations are required, including ones for yellow fever and other diseases that you never hear of in the US. I had a total of 11 shots plus the malaria-preventive pills required. I learned about which foods were safe to eat and which to avoid. The ASCP group provided conference calls on procedures, expectations, and travel arrangements. The 2008 trip included 1-week visits to both Rwanda and Tanzania in sub-Saharan (southeastern) Africa. I was joining a group of MTs and ASCP Institute staff members, none of whom I knew; I met them upon boarding the plane in Brussels, Belgium, heading to Kigali, Rwanda. The trip to Africa is long and tiring, with 9-hour flights from Chicago to Brussels and again from Brussels to Kigali, taking 2 full days to complete.

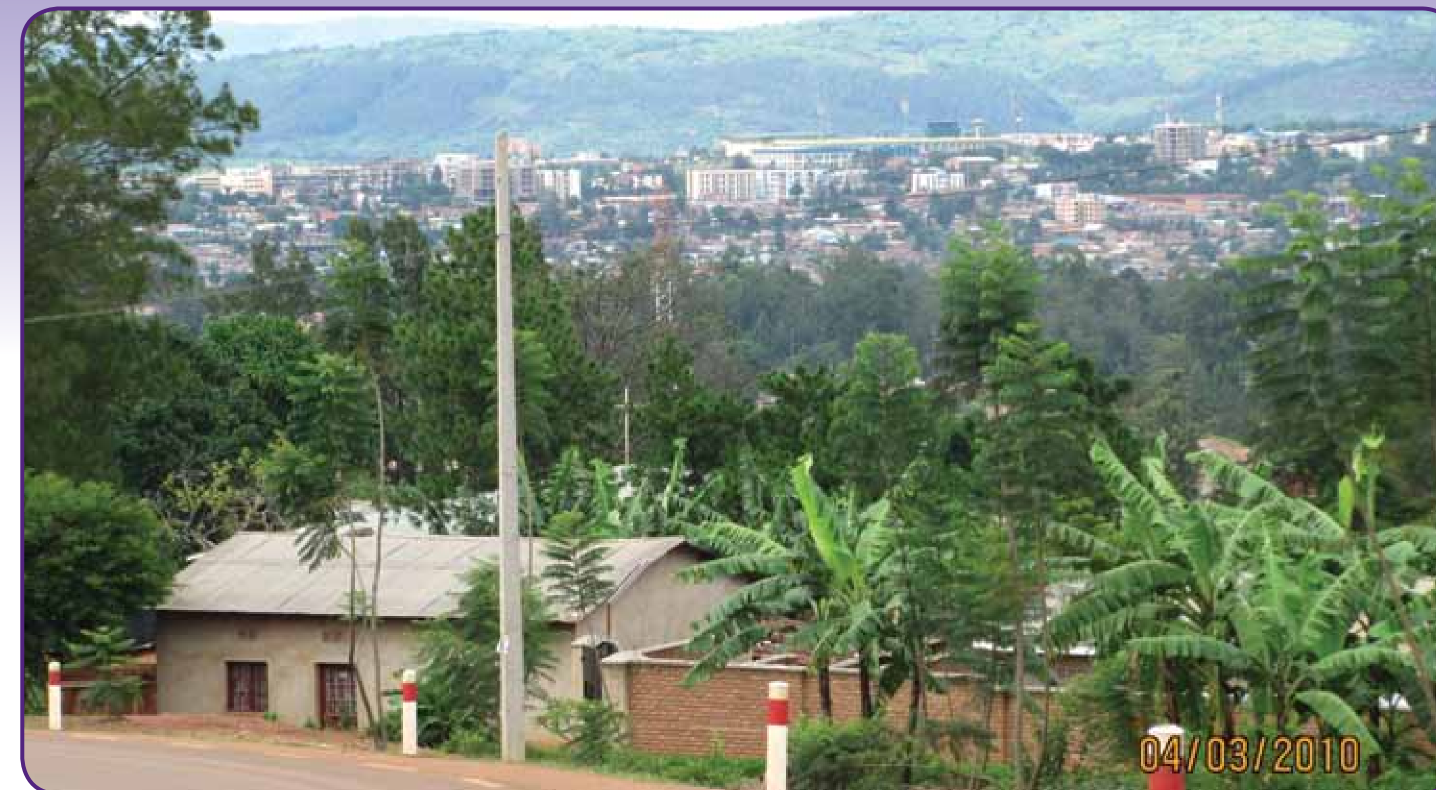

Fig. 1. The view driving into Kigali, Rwanda.

The Hotel de Mille Collines was our home for the week in Kigali, and it was the hotel that the movie *Hotel Rwanda* was about. Since the genocide in 1994, it has been updated, but it was still without air conditioning and had only one working elevator (the door was operated manually from the inside). Each room had windows, and mine did have a screen to prevent insects from coming in, so I got air circulation into the room. Some rooms, however, had no window screens and were much less comfortable. Temperatures during our stay ranged from 65°F-85°F accompanied by high humidity, which often made sleeping difficult.

Rwanda is a beautiful small country, about the size of Maryland. There are many hills, and it is known as the country of 1000 hills (de milles collines). Vegetation is lush; coffee and tea are major crops. Having been colonized by France and Belgium, many influences from those countries remain. In 2008, the official language was still French, although most urban Rwandaise speak English; Kenyarwandan is the first and local language of the people. We found that menus and signage are often printed in all three languages. Soon after our arrival, we were taken by hotel drivers to exchange our US dollars for Rwandan francs in neighborhood storefront establishments where they used handheld calculators to figure the exchange rate for our currency. We also went to the Genocide Memorial, where I learned more about the 1994 atrocities in the country. During a period of 100 days, approximately 1 million people were killed by neighbors and acquaintances. The city and countryside are dotted with sites of large massacres and mass graves. Due to the resilience of the people and government, Rwanda is now considered the safest and least corrupt country in Africa.

The people of Rwanda are friendly and welcoming. As a country, they have worked diligently to overcome the horrors of the genocide. The religions of Rwanda are primarily Christian, with a minority Muslim population. The average age of all Rwandans is 19, with the life expectancy in the 40s. One of the teachers I met was happy to tell me that his 60-year-old mother was still alive—a rarity in their world!

Our work in Kigali began in earnest at the Kigali Health Institute (KHI). This facility houses educational programs in healthcare careers including dentistry, laboratory sciences, physiotherapy, and nursing. The campus previously was a military base, and the buildings now housing administrative offices, dormitories, classrooms, and laboratories are the former barracks used by the soldiers. None of the buildings are air conditioned and there are no elevators to access the four floors. Many of the students are disabled from polio or injury, and they climbed the stairs frequently to get to and from classes.

Each day we met with the Laboratory Sciences faculty, in addition to other administrators and faculty of other programs within the Institute. They were reviewing their entire laboratory programs' curriculum at the institutional level, and much of the time was spent with explanations and justifications for changes. Our team of ASCP volunteers also provided expertise to their faculty in specific areas, such as clinical chemistry, hematology, microbiology, and histotechnology. We each met with our counterparts—the teachers of those specific

courses—and discussed what was in place and how it could be improved to better educate students. We offered help with teaching techniques, based on their conditions and constraints.

This individual time with our counterparts was the most meaningful for me. I met with Dr Venerand Bigirimana, the pathologist who taught the histology courses. Not surprisingly, he had been teaching the classes as beginning pathology courses, based on tissue identification and recognition of normal microanatomy and pathologic changes. Students were not taught what we consider histotechnology—the fundamentals of fixation, processing, embedding, cutting, and staining. Rather, they were being trained to be assistant pathologists.

There were only two anatomic pathology-trained pathologists in Rwanda at the time, and they both gave me invaluable input regarding the practice of histotechnology in the country. There was only one working histology lab in Rwanda, and it was in a hospital in the city of Butare, 2 hours to the south of Kigali. Dr Louis Ngendihayo was the pathologist in Butare, and his histotech was a young man named Leopold. Previously, Leopold had been sent to Brussels for 3 months of training in histotechnology, which made him the only trained histotech in Rwanda. Leopold traveled by bus to Kigali to meet with me, and we immediately formed a friendship that only fellow histotechs can appreciate. We spoke the same language—that of histology.

Dr Bigirimana came to the school and asked me and three of the MTs to accompany him to his laboratory at King Faisal Hospital in Kigali. I was especially excited to see the hospital facility, and to be away from the school for a few hours. The lab was fairly modern, having been updated significantly with PEPFAR funding and ASCP efforts in recent years. The laboratory facility, like the entire hospital, was not air conditioned; windows were open for circulation. One chemistry analyzer was housed inside an air-conditioned room to accommodate operating requirements; even the pathologist's office was not cooled. Dr Bigirimana was eager to show me the space that would eventually house the histology laboratory and the instrumentation that had been acquired toward that goal. However, stainers, cryostats, and microtomes were still in shipping crates since there were no people trained to operate them and no reagents to run the tests. When I asked what I could do to help, he quickly responded that I should stay and get the lab operational!

All of the Laboratory Sciences department's faculty were male; most were young and were recent graduates of the program. Because of the genocide and AIDS epidemic, there are limited numbers of educated professionals in the country. Some other African countries send professionals to work in the KHI for 1- to 2-year periods in an effort to fill the knowledge gaps. Obviously, the goal of the country is to become self-sufficient again, depending on their own professionals to train new generations of healthcare providers.

The ASCP group hosted our Rwandan colleagues with a reception at the hotel, complete with a DJ (who played mostly American and country-western music), dancing, party snacks, and drinks. It was such fun to see the same people who had been all business during the week have such a relaxed and enjoyable time during the social event!

Some of the precautions we were advised to take during our trip centered around food and drink. We were not to eat any food that was raw (salads, fruits that we had not peeled ourselves), milk (except the hot milk that was provided with coffee or tea), local water (we were provided with bottled water even for brushing our teeth), and ice (obviously made from local water).

Fig. 2. The author, Glenda, and Leopold, the only trained histotech (3 months of training in Brussels) in Rwanda.

I realized quickly that I missed iced drinks, and the locally grown vegetables and fruits that we couldn't have looked so very appetizing and tempting! Bottled Coca-Cola and Fanta products were available, and they were served chilled, opened at the table for our assurance of cleanliness. Sandwiches had to be ordered without lettuce and tomato. All restaurants are open-air, even in the hotels. Mosquito repellent was suggested anytime we were outside, in addition to the malaria preventive drugs we took daily. Thankfully, none of our group became ill during our trip.

After our week in Kigali, several members of the group then traveled to Tanzania. After plane connections in Nairobi, Kenya, we arrived at Mount Kilimanjaro airport in the evening. Navigating the airport was an adventure, and then we boarded a mini-bus for the trip into Arusha, where the meeting with laboratory educators was to be held. Unlike Rwanda, Tanzania was settled by the British. Consequently, their money is the Tanzanian pound and driving is on the left side of the road. The highway from the airport into Arusha is a two-lane road, and there were many pedestrians, bicyclists, and hand-pulled carts alongside the road. It was quite frightening to travel after dark—meeting headlights on the "wrong" side of the road, and knowing that we were passing so closely to so much foot traffic on the nonexistent shoulder of the roads. I was happy to finally arrive in the city!

The hotel in Arusha was much nicer than our Kigali accommodations. The Kibo Palace is a 5-star hotel, catering to American and European travelers. The facility was very modern and new; each room had its own air conditioner, the floors and stairways were made of native woods, and the grounds were manicured. Imagine my surprise in the morning when I looked out the window and realized we were in the equivalent of what would be considered an American slum, which turned out to be the majority of the city.

We were there on Easter Sunday, and several of us went on a day trip into the countryside, driven in the hotel van. The driver took us to a park with beautiful waterfalls near the foothills of Kilimanjaro. We hiked down damp and slippery trails to the bottom of the falls, where we found several groups of young people celebrating their holiday with music, dancing, and playing in the very cold natural pool. I couldn't resist taking off my shoes and wading for a few minutes, only to realize what a risk I had taken going barefoot there! On our trip we saw vegetation that is only grown in miniature in the US—banana trees with loads of fruit, avocado trees, coffee plants,

Fig. 3. Histology lab personnel at King Faisal Hospital, Kigali, Rwanda.

and flowering trees that I recognized as being shrubs in Texas! The people of Tanzania speak Swahili as well as English, and we were often greeted with "Jambo" (hello) as we drove past pedestrians on the rural roads. On the trip back into the city, our driver pulled to the side of the road and stopped. I have to admit that I was a bit alarmed until he pointed out a view of Mount Kilimanjaro. He said that it was rare to be able to see the top of the mountain because it is almost always shrouded in clouds, but there it was. What a special day!

Our meetings got underway on Tuesday. The group of educators was much larger than the one in Rwanda, as this group incorporated faculty from five of the laboratory education programs in different locations throughout Tanzania. They had met once previously with the ASCP MT group, and this was a follow-up visit to see what implementation had occurred since that earlier meeting. Faculty representatives from the various laboratory disciplines met with the MT educators, discussing revisions and teaching techniques. Presentations were made by several of the educators and our group gave positive and constructive feedback on their teaching methods. The only histology educator was a woman from the school on the island of Zanzibar, and what I learned from her was very disturbing. She told me that they had very few supplies, and that five students shared one microtome and one microtome blade during the 3-week training period. Other aspects of the training, such as embedding and staining, were similarly inadequately supplied. I met with the Ministry of Health's representative, and he was most eager to hear my ideas for improvement in the training of histology technicians. I was not able to visit any hospitals in Tanzania, so I am not aware of the actual practice of histotechnology in that country. After the week in Arusha, I flew back home to Texas via Dar es Salaam, Amsterdam, and Chicago.

This 2-week trip forever changed me. All my life I had been aware that I am blessed and I live a safe and secure life. Until this trip, however, I had no idea just how much we are all blessed. Even though each histology lab in the US is unique, we generally

Fig. 4. Backlog of tissue blocks in the Butare, Rwanda histology lab.

have the supplies, the instrumentation, and the personnel we need to do our jobs. We do not have to use one disposable blade for an entire day's cutting, nor do we have to skimp on reagents for staining. Similarly, training programs in this country generally have books, access to computerization and copying services, technology to support teaching, and personnel to deliver the curriculum to students. We do not have to lecture to students who have no access to books or printed lecture notes or have no method of learning the laboratory skills necessary to become histotechs. We all have access to a high standard of living compared to that of people in other parts of the world.

Aside from becoming more acutely aware of the opportunities that we take for granted, I also realized that I wanted to do more. When I came home, I started spreading the news about my trip. I gave a short talk at the Texas Society for Histotechnology meeting that spring, I wrote a short article for NSH In Action, and I talked to groups of people when I could. Donations started pouring in for supplies such as embedding wax, processing cassettes, microscope slides, coverslips, books, money, and gloves. These donations came from individual labs, students in training programs, histology product companies, and individual histotechs. One of my students also donated art supplies that are now being used for teaching. It has been amazing and overwhelming! The ASCP Institute pays for shipments of these supplies to Africa; they have also sent some of the supplies to other countries that are also receiving PEPFAR funding.

Although I enjoyed my time in Tanzania, I made a closer connection to the people and the training program in Rwanda. So, when I was given the opportunity to return to KHI in the spring of 2010, of course I was happy to go!

Read about my return trip to Rwanda in the next issue of HistoLogic.

Histological and Histochemical Staining of Sections Using an Inverted Vial Device

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Abstract

A simple technique was developed for staining ordinary paraffin-embedded or frozen sections mounted by conventional techniques. The method utilizes a device made of a small glass vial and a disk of filter paper, which controls the staining solution inside the vial. Staining takes place by inverting the vial upside down over the tissue section. The staining solution inside the vial can be retained for further use by returning the vial to an upright position. The filter paper disk affixed to the top of the vial also helps to keep the solution free of debris, which makes for cleaner staining.

Introduction

Histochemistry and immunohistochemistry (IHC) techniques have proven to be invaluable tools in clinical and medical research. However, one of the limitations is the extensive volume of reagents, some of them quite costly, that these methods require. Many containers used for staining paraffin-embedded or frozen sections can be found on the market from a variety of sources. These containers are of different types, materials, and capacity, and include the glass Coplin staining jar, high-density polyethylene and polypropylene staining jars, EasyDip™ slide staining system (Simport, Beloeil, QC, Canada), slide staining dish, Lab-Tek™ Chamber Slide System (Thermo Scientific/Nalge Nunc International, Rochester, NY), and chambered coverglasses, to name just a few. All of these containers require a relatively large volume of reagents, which may be used only once before being discarded. The development of microfluidic chambers allows for the use of small volumes of reagents, but they are not suitable for staining ordinary tissue slides. Although these devices are innovative and useful, they are limited to incubation and simple one-step staining reactions, such as those used for tissue culture. In this study, we implemented a device that can be used for staining single slides, and allows reagents to be used multiple times. This can result in substantial cost savings for the laboratory when it is necessary to stain single slides.

Materials and Methods

Description of the Device

The device is very simple to construct. A 5-mL brown glass vial with a wide mouth and flattened rim is used, which can hold as little as 1 mL or as much as 5 mL of staining solution, and accommodate tissue sections of up to 1.0 cm^2. A piece of filter paper is cut to a size slightly larger than the opening of the vial; it is affixed to the vial rim by applying an adhesive (glue stick) to the rim of the vial. Before staining, the filter paper disk is pierced by a pin several times to increase its porosity. After filling the vial with staining reagent, the slide containing the section is positioned onto the filter paper disk that is fixed to the mouth of the vial. For staining, the slide is fixed above the filter paper disk using the same glue, which can be easily removed after staining. After the staining is finished, the vial is rotated back to the upright position (Fig. 1). This maintains the reagent securely and saves it for further application to another slide. The filter paper disk prevents any debris from getting into the vial. Used reagent can be stored for a long duration in the same vial and can be used multiple times.

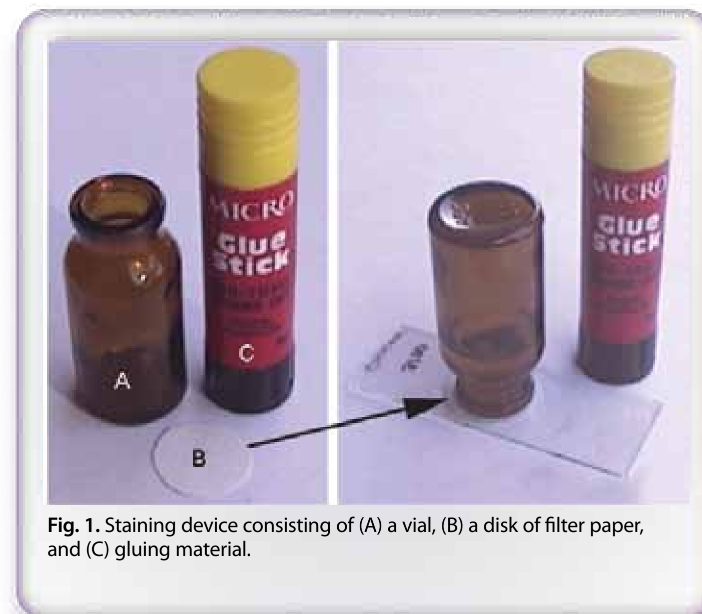

Fig. 1. Staining device consisting of (A) a vial, (B) a disk of filter paper, and (C) gluing material.

Results

Our utilization of an inverted reagent vial for IHC staining makes it possible to perform all sample preparation steps including washing, fixation, staining, and antibody dilution with a wide range of costly reagents in a manner that minimizes the volume of solution necessary for staining. Slides can be stained individually without significant loss of staining reagent. Specimens can also be tagged with multiple markers while maintaining reagent integrity by preventing cross-contamination. There is no need to use a moisture chamber as there is no loss of reagent through evaporation. Also, liquid reagents are held securely without splashing. No leakage of the reagent through the space between the glass slide and the paper disk was observed. Specimen drying, which can result in nonspecific antibody staining, is avoided as the section remains moist throughout the incubation period. Traditional horizontal IHC staining can sometimes result in the loss of tissue sections that inadequately adhere to the glass slide. We did not experience this problem when the inverted vial technique was utilized in our laboratory. This method is also ideal for IHC staining of tissues mounted on glass coverslips. The rim of the vial perfectly holds the slide or coverglass evenly in an inverted position.

Summary

The inverted vial device is made from very inexpensive supplies found in any histology laboratory and is very simple to make. The inverted vial technique for IHC staining of paraffin-embedded or frozen sections is most useful when the need arises to stain single slides. This method economizes on the use of staining reagents while maintaining clean staining and avoiding cross-contamination. It is also an economical solution because reagents can be stored in the vial and used multiple times.

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Optimization of a Fast Schiff Reaction for Tissue Staining

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Abstract

The periodic acid-Schiff, or PAS, reaction is a staining technique for the detection of glycogen in tissue sections such as liver, heart, kidney, colon, and skeletal muscle, which can be performed in either paraffin-embedded or frozen tissue. The basis of this reaction is the oxidation of carbohydrates induced by periodic acid. This reaction produces aldehyde groups that react with Schiff reagent, forming a bright fuchsia stain in the tissue section. The standard procedure requires incubation with a 0.5% aqueous solution of periodic acid followed by Schiff reagent for 15 minutes. The incubation time with Schiff reagent can be shortened by using a microwave oven, but this method has several drawbacks. Among others, the final reaction depends on the energy absorption related to volume, size, composition, and starting solution temperatures. Most importantly, rising reagent costs result from the need to discard all the reagents after microwave-driven incubations.

The purpose of this study was to optimize a faster Schiff reaction by assessing the effects of temperature and incubation time on the final staining intensity without the use of a microwave oven. Tissue specimens from kidney, colon, and liver were sectioned at 4 µm and incubated with Schiff solutions preheated to three different temperatures: room temperature for 15 minutes (standard protocol), 60°C for 10 minutes, and 65°C for 5 minutes. The slides were reviewed by two pathologists, and they reported that no differences were observed among the four conditions. These results suggest that a shorter, faster Schiff reaction can be achieved without compromising the quality of the stain. This time-saving modification can be achieved without the added expense of a microwave oven.

Materials and Methods

Formalin-fixed paraffin-embedded tissues were sectioned from tissue control blocks used daily in our laboratory that were proven to have a high content of glycogen. These tissues were kidney, colon, and liver biopsy, sectioned at 2, 4, and 4 µm, respectively. Sections of each tissue were dried, deparaffinized, and hydrated to distilled water (dH_2O). Oxidation was carried out with 0.5% periodic acid solution, which results in the formation of aldehyde groups through carbon-to-carbon bond cleavage. Parallel slides from each tissue were rinsed in dH_2O and then placed in Schiff reagent (Sigma-Aldrich, St. Louis, MO) at one of three temperatures (room temperature for 5 and 15 minutes; 60°C for 10 minutes; and 65°C for 5 minutes). Following Schiff reagent incubation, the slides were washed in warm running tap water for 10 minutes after which they were counterstained in hematoxylin (Richard-Allan Scientific, Kalamazoo, MI) for 2 minutes, rinsed with tap water, and placed in the bluing solution for 1 minute. The slides were dehydrated, cleared, and mounted (Fig. 1).

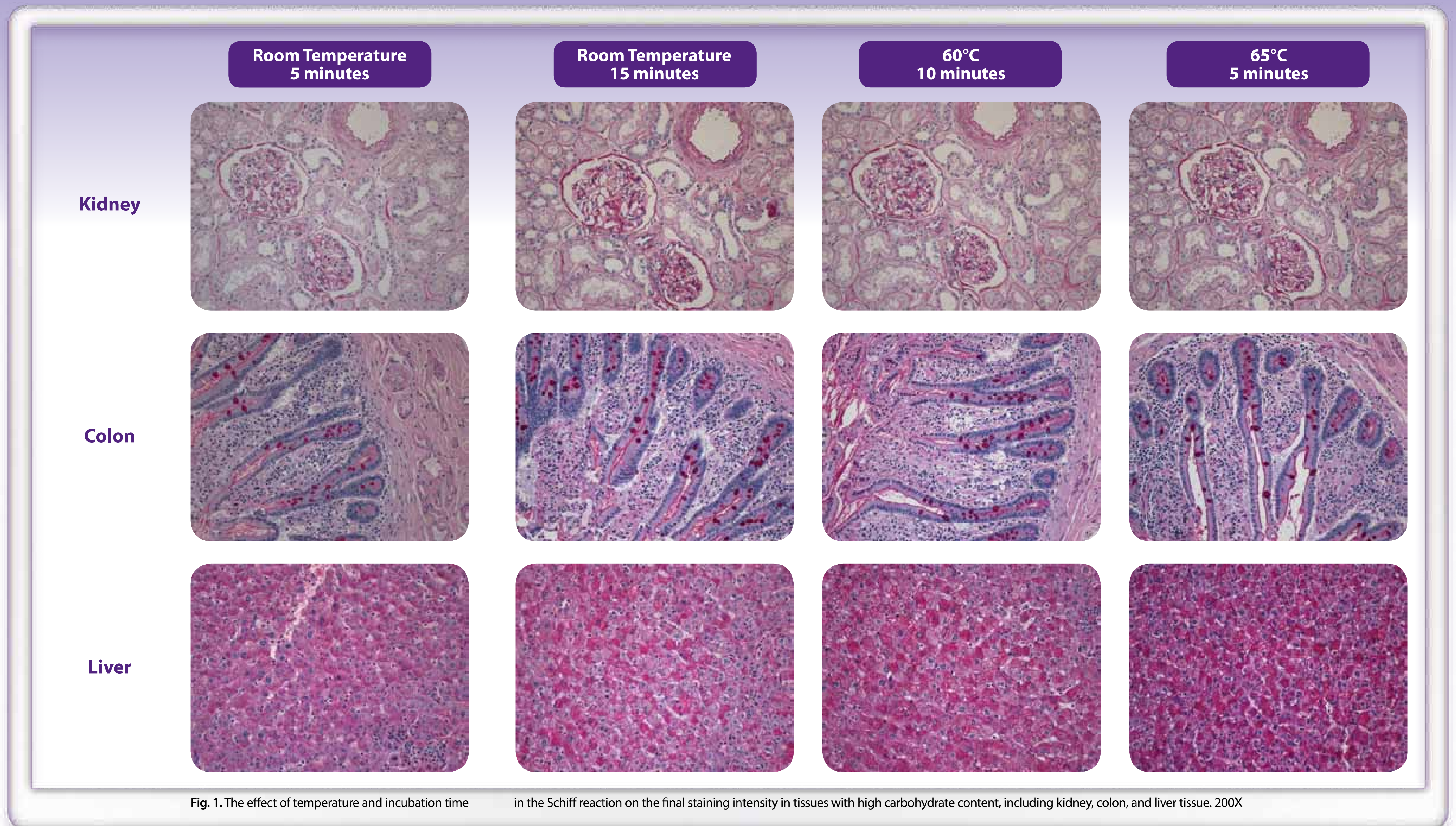

Fig. 1. The effect of temperature and incubation time in the Schiff reaction on the final staining intensity in tissues with high carbohydrate content, including kidney, colon, and liver tissue. 200X

Conclusion

Although PAS staining is not routinely performed on liver or colon tissue in our laboratory, we evaluated these tissue types along with kidney tissue to assess the effect of temperature in the Schiff reaction on tissues with high carbohydrate content. At elevated temperatures, we observed no significant difference in Schiff staining intensity, which appeared equivalent to room temperature staining for 15 minutes. However, the Schiff incubation for 5 minutes at room temperature resulted in a pale stain. This confirmed that elevated temperature is necessary in order to reduce staining time. The staining result using Schiff reagent at 65°C for 5 minutes was virtually identical to the standard 15-minute incubation at room temperature. Incubation time can be reduced by two-thirds by simply elevating the temperature of the Schiff reagent with an inexpensive water bath, which will improve lab turnaround time and efficiency without a large investment in a laboratory-grade microwave oven.

Bibliography

Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques*. 5th ed. New York, NY: Churchill-Livingstone; 2002:173-175.

Carson FL, Hladik C. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, IL: American Society for Clinical Pathology; 2009:137-141.

Kayser K, Bubenzer J. Microwave-assisted staining procedures in routine histopathology. *Histochem J*. 1990;22(6-7):365-370.

Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis, MO: CV Mosby Co; 1980:164-166.

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✔ Mark Your Calendar!
Educational Opportunities in 2011

JUNE

2-4 **Missouri Society for Histotechnology**
Site: Resort at Port Arrowhead
Lake of the Ozarks, MO
Contact: Sharon Walsh
Email: userwalsh@sbcglobal.net

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Issues in Processing Breast Specimens**
Speaker: I-Tien Yeh, MD, Professor, Dept of Pathology
University of Texas Health Sciences Center
San Antonio, TX

22 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Clone Wars: Muridae vs Leporidae (Are You Team Mouse or Team Rabbit?)**
Speaker: Jeff Gordon, BS
Cell Marque
Rocklin, CA
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

24-26 **Mississippi Society for Histotechnology**
Site: Lake Terrace Convention Center
Hattiesburg, MS
Contact: Kimberly Wright
Phone: (601) 288-1064
Email: KWright@forrestgeneral.com

JULY

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Understanding the Finances of Laboratory Management**
Speaker: Elizabeth Sheppard, MBA, HT(ASCP)
Ventana Medical Systems Inc.
Tucson, AZ

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **The Cell Cycle and Cancer**
Speaker: Traci DeGeer, BS, HTL(ASCP)QIHC
Ventana Medical Systems Inc.
Tucson, AZ
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

AUGUST

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **A Systems Approach to H&E Staining**
Speaker: H. Skip Brown, MDiv, HT(ASCP)
Leica BioSystems
St. Louis, MO

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Estrogen and Progesterone Testing Standardization for Breast Cancer**
Speaker: Elizabeth Sheppard, MBA, HT(ASCP)
Ventana Medical Systems Inc
Tucson, AZ
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

SEPTEMBER

16 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **IHC Tips and Troubleshooting from a Preclinical Research Perspective**
Speaker: Anne C. Lewin, BS, HT(ASCP)QIHC
Senior Research Scientist, Bristol-Myers Squibb
Princeton, NJ

16-21 **National Society for Histotechnology Symposium/Convention**
Site: Duke Energy Convention Center
Cincinnati, OH
Contact: Aubrey Wanner
Phone: (443) 535-4060 or register online at www.nsh.org
Fax: (443) 535-4055
Email: aubrey@nsh.org

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Lower G.I. Biopsies: Tissue Identification, Diseases and Stains**
Speaker: Mitual Amin, MD
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

OCTOBER

21 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **The Cryostat and Technique**
Speaker: Mari Ann Mailhiot, BS, HT(ASCP)
Leica Microsystems
Deerfield, IL

26 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Taking It to the Bio "Bank": The Journey of a BioSpecimen to a BioMarker**
Speaker: Barbara Pruetz, BS, HTL(ASCP)
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

NOVEMBER

16 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Violence in the Workplace: What Can We Do to Prepare?**
Speaker: David Tate, MS
Purdue University
West Lafayette, IN
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: ***In-situ* Hybridization for Beginners**
Speaker: Bonnie Whitaker, HT(ASCP)QIHC
Ohio State University Medical Center
Columbus, OH

DECEMBER

21 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Safe Storage of Laboratory Chemicals**
Speaker: Peggy A. Wenk, HTL(ASCP)SLS
Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

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