HISTOLOGIC®
Technical Bulletin for Histotechnology

SAKURA

Vol. XLI, No. 2 December 2008

Managing Editor, Nancy Klemme
Scientific Editor, Vinnie Della Speranza, MS, HTL(ASCP) HT, MT

The Importance of Water Quality in the Histology Laboratory

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Abstract

Water is ubiquitous in histology laboratories. Not only is it the main component in many of the reagents prepared in the laboratory (buffers, stains, rinsing solutions), but it is also used in tissue flotation baths, tissue processors, water baths, etc. However, it is often taken for granted, and its potential impact on experimental outcomes overlooked. While it is well known that purified water should be used in most cases, various procedures refer to the use of "deionized," "distilled," and "double-distilled" water, making it confusing as to which type of water should be used. In addition, bacterial contamination of the water should be prevented, which may be difficult, even when using excellent laboratory practices. In the present study, water produced by a water purification system and combined with reverse osmosis, ion exchange resins, electrodeionization, and a germicidal ultraviolet (UV) lamp was used. The resulting purified water was used to prepare reagents, as well as in water baths and/or rinsing solutions for hematoxylin and eosin (H&E) staining and silver staining. The results obtained were all satisfactory, including the silver staining, which is known for being very sensitive to water quality. In conclusion, water purified with a combination of reverse osmosis, ion exchange, and electrodeionization is suitable for a wide array of histology experiments.

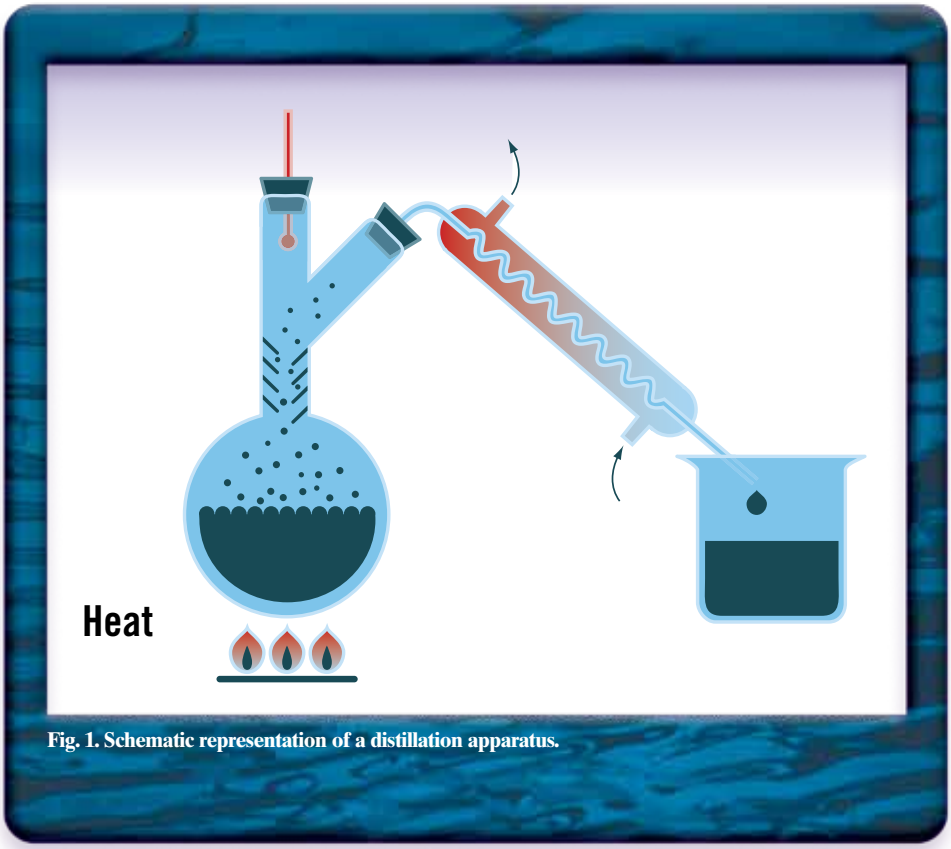

Fig. 1. Schematic representation of a distillation apparatus.

IN THIS ISSUE

Introduction

Histology laboratories use water in various ways. Histological procedures refer to the use of tap, deionized, or distilled water, making it confusing as to which type of water is preferred. In the present study, water produced by a water purification system combining reverse osmosis, electrodeionization, and a germicidal UV lamp was used.

The purified water obtained was used to prepare reagents and rinsing solutions for the common H&E stain, as well as for Grocott's methenamine silver (GMS) stain.

Water Contaminants

Tap water may contain many compounds, in addition to water molecules, including:

- Salts, inorganics: Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, Na^+, Ca^{2+}, Pb^+, Fe^{2+}, Mn^{2+}, SiO_4, etc.
- Organic molecules: humic acids, phenols, tannins, herbicides, pesticides
- Particles, colloids
- Microorganisms
- Dissolved gases

Water is the main component in many of the reagents prepared in the histology laboratory (buffers, stains, rinsing solutions), but the compounds listed above may interfere with the quality of the final staining. Silver stain quality, for example, is known for its sensitivity to water contaminants.

The water used in tissue flotation baths, tissue processors, and water baths may also interfere with the final results. For example, organic molecules present in water may be used as food by bacteria, allowing the bacteria to proliferate rapidly in water baths. Depending on the season and other factors, tap water composition may vary from one day to another. Because it is necessary to obtain reproducible and reliable results in the laboratory, it is therefore important to use purified water of a reliably consistent quality.

Water Purification

Most histology textbooks and procedures refer to the use of either deionized or distilled water.

Deionization

Many facilities have an in-house supply of deionized water. The deionization process removes ions from water via ion-exchange resins (Fig. 2). Once saturated (no more exchange capacity), the resin bed may be regenerated to strip away accumulated ions through physical displacement.

The potential difficulties are:

- Only ions are removed (neutral organic molecules remain in the water)
- Resins, once exhausted, can leach high levels of contaminants all at once
- Repeated resin regeneration cycles lead to broken resin beds and may release particles and organic molecules into the water
- Resin beds are conducive to bacterial growth
- Users of central systems need to rely on others to maintain the system and change the resins on time

Distillation

Distillation removes a wide range of contaminants. The water contained in a boiler is heated up. The vapor is then condensed and the purified water is collected in a receiving flask (Fig. 1). This may be repeated (double-distillation). During this process, it is expected that contaminants initially present (ions, organics, particles, and bacteria) will not distill and will remain in the boiler.

The potential difficulties are:

- Some specific ions and a number of organic molecules may be carried along with the water vapor, or may co-distill with water
- The recipient vessel may contribute to recontaminate the distilled water
- Maintaining a distillation apparatus may be cumbersome, especially in hard water areas

Advanced Water Purification Technologies

Distillation has long been considered the gold standard of water purification, but today newer purification technologies have proven their efficacy and robustness. Using a combination of purification technologies (Fig. 3) has advantages over distillation or resin deionization: the various technologies are complementary and remove a wider spectrum of water contaminants than either deionization or distillation.

- *Reverse osmosis* is a membrane-based technology that removes a wide spectrum of contaminants
- *Electrodeionization* is a self-regenerating process and is composed of ion-exchange resins, a semipermeable membrane, and an electrical current
- *Ultraviolet (UV) light* at a wavelength of 254 nm inactivates bacteria

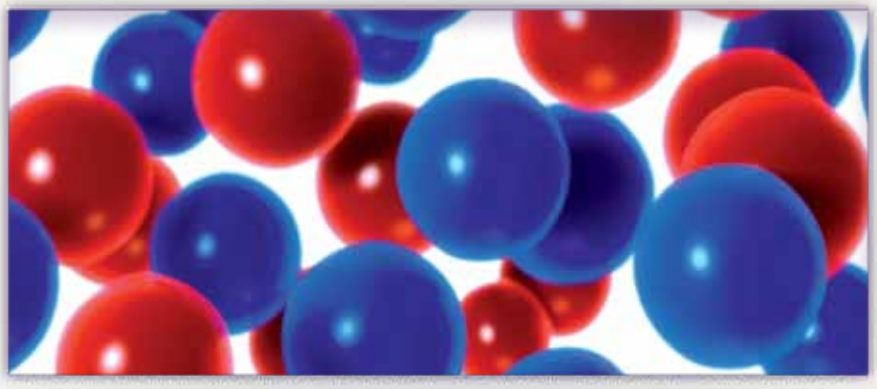

Fig. 2. Schematic representation of ion-exchange resin beads.

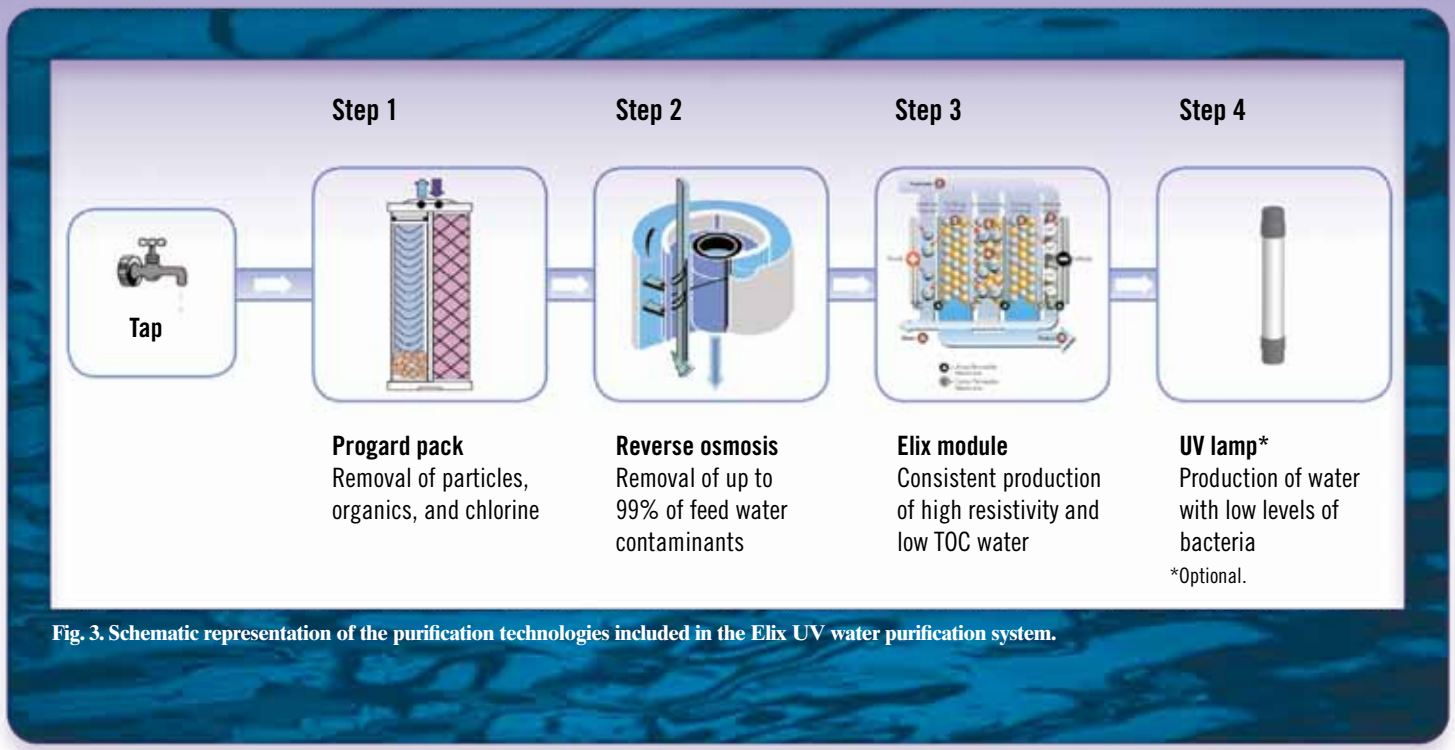

Fig. 3. Schematic representation of the purification technologies included in the Elix UV water purification system.

Materials and Methods

In this study, water was purified using an Elix® 5 UV Water Purification System (Millipore, Billerica, MA). This system typically delivers water with low levels of organic contaminants (total organic carbon <30 ppb in-line), high resistivity (>10 MΩ.cm), and low levels of bacteria.

While it is well known that purified water should be used in most cases, various procedures refer to the use of "deionized," "distilled," and "double-distilled" water, making it confusing as to which type of water should be used.

The purpose of this study was twofold:

- Assess the suitability of water purified with the Elix system when performing commonly used staining procedures such as H&E staining and Grocott's methenamine silver (GMS) staining
- Investigate which common water contaminants might have an effect on those procedures

H&E staining was performed using a robotic slide stainer. For the bluing step, either Scott's Tap Water Substitute Concentrate or Blue Buffer 8™ Solution (both from Surgipath Medical Inc., Richmond, IL) was used.

For the GMS staining, tissue sections of *Pneumocystis carinii* were mounted on Superfrost® slides (Erie Scientific Co., Portsmouth, NH), and a commercial staining kit was used (Poly Scientific R&D Corp., Bay Shore, NY). The methenamine silver nitrate solution was prepared with each type of water. The remainder of the procedure was the same for all slides. All slides were done in duplicate and were evaluated using identical lots of all applicable reagents for each set of slides. Four types of water were tested:

- Elix purified water
- Distilled water
- Service deionized water
- Tap water

Solutions of methenamine silver nitrate were prepared with Elix purified water and one of the following contaminants in order to assess their impact on staining*†:

- Metals: potassium chromium sulfate (1 ppm), cupric sulfate (1 ppm), nickel sulfate (1 ppm), ferrous sulfate (1 ppm), DKW/Juglans basal salt mixture (contains a mixture of minerals)
- Chlorinated disinfectant: sodium hypochlorite (4 ppm)
- Detergent residues: sodium triphosphate (100 ppm)
- Silica: sodium silicate (10 ppm)
- Bacteria byproducts: endotoxin (1000 EU/mL)
- Common organic contaminant: humic acid (1 ppm)

*Humic acid was supplied by Biocontrol Network (Brentwood, TN). All other contaminants were supplied by Sigma-Aldrich Co. (St. Louis, MO).

†All contaminants above are measured in ppm, however, the equivalent units are mg/L.

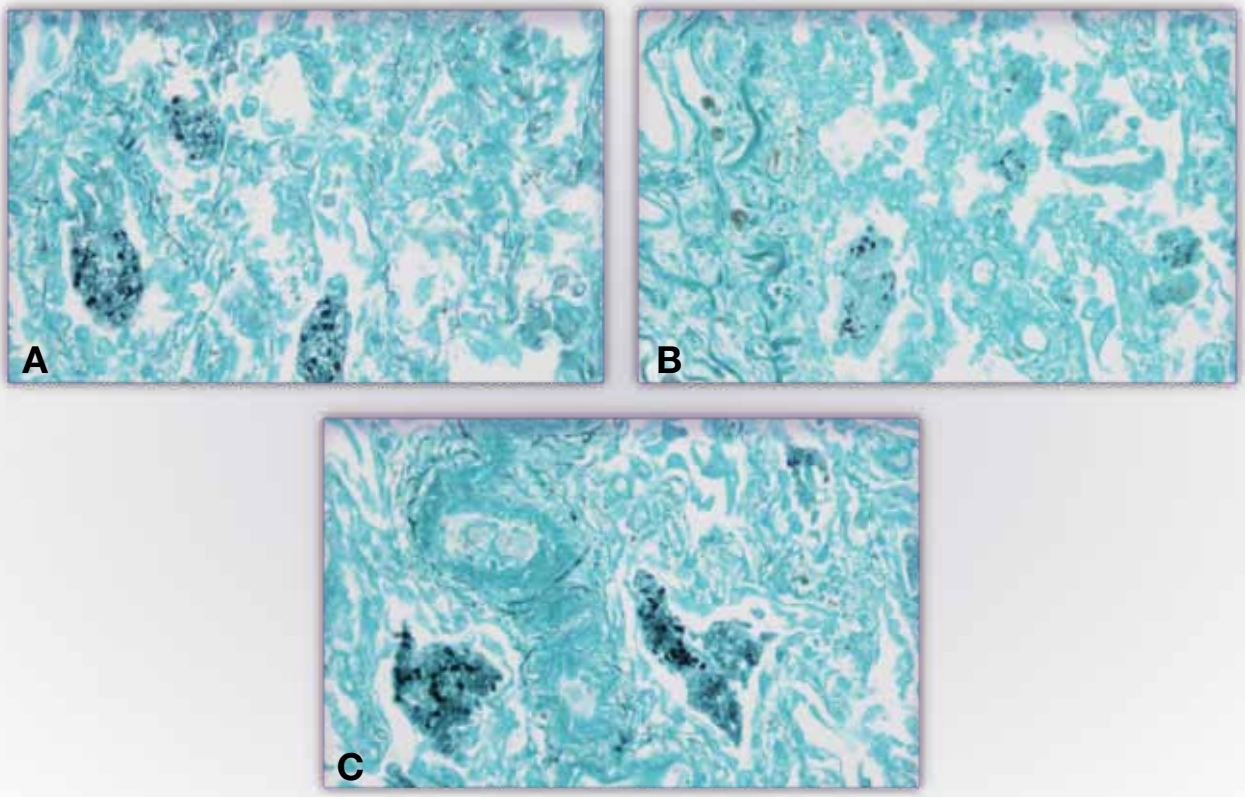

Fig. 4. Photomicrographs of lung tissue with *Pneumocystis carinii*, stained with GMS, 20X. GMS staining was done with solutions of methenamine silver nitrate prepared with (A) Elix purified water, (B) distilled water, or (C) deionized water.

Table 1. Contaminants in Methenamine Silver Nitrate Solution

Contaminants	Observations
Potassium chromium sulfate (1 ppm)	Negative for *P carinii*, no background staining
Cupric sulfate (1 ppm)	*P carinii* staining pale, elastic fibers darker
Nickel sulfate (1 ppm)	Only trace staining of *P carinii*, negative background
Ferrous sulfate (1 ppm)	*P carinii* moderately stained, elastic fibers dark, light staining of reticulum
DKW/Juglans basal salt mixture	Negative staining of *P carinii*, negative background
Sodium hypochlorite (4 ppm)	*P carinii* moderately stained, elastic fibers dark
Sodium triphosphate (100 ppm)	No *P carinii* identifiable, excessive background staining of elastic and reticulum
Sodium silicate (10 ppm)	No *P carinii* identifiable, excessive background staining of elastic and reticulum
Endotoxin (1000 EU/mL)	No *P carinii* identifiable, excessive background staining of elastic and reticulum
Humic acid (1 ppm)	*P carinii* barely identifiable through background, excessive background staining of elastic and reticulum, and collagen

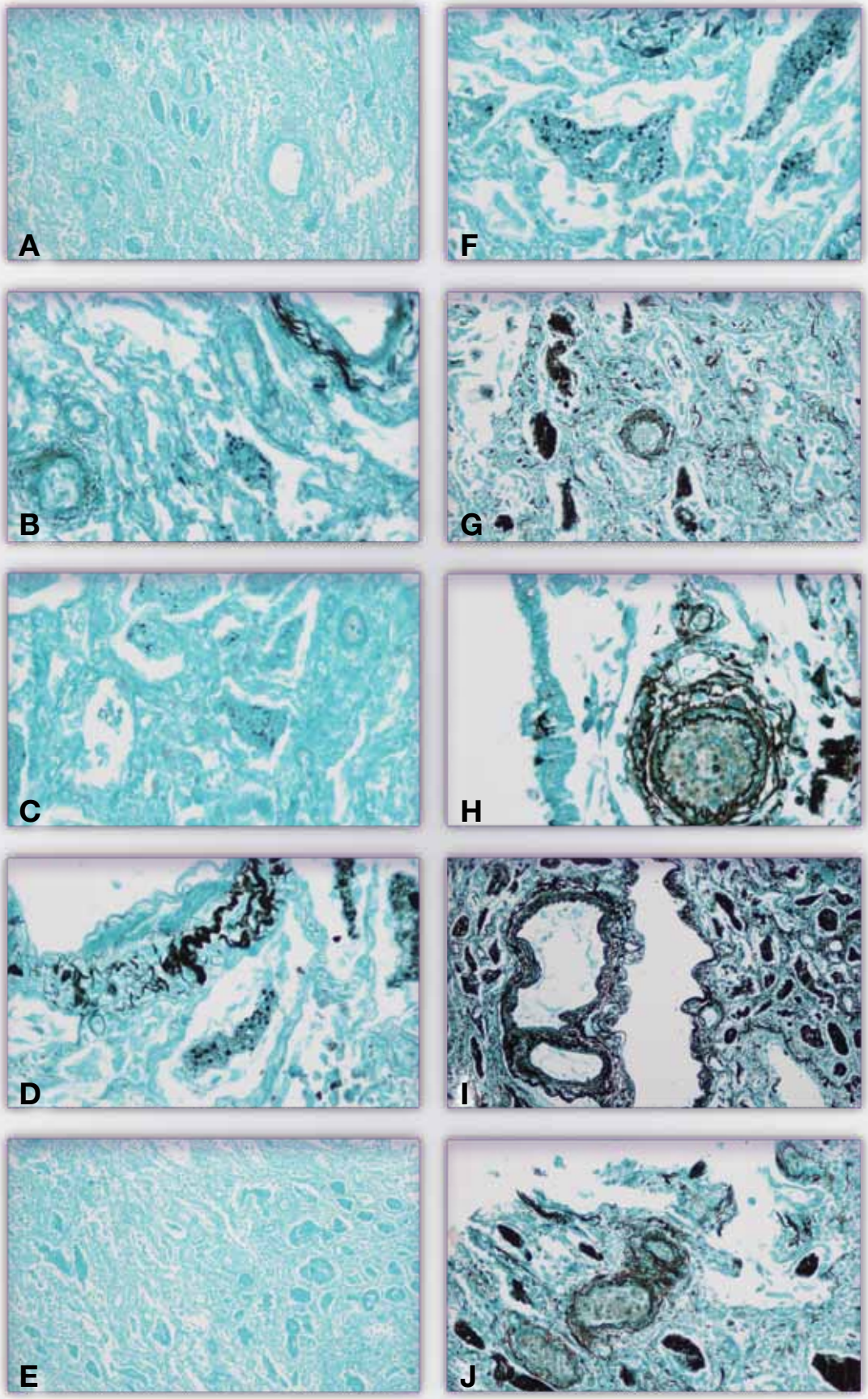

Fig. 5. Photomicrographs of lung tissue with *Pneumocystis carinii*, stained with GMS, 20X. GMS staining was performed using solutions of methenamine silver nitrate prepared with Elix purified water and one of the following contaminants: (A) potassium chromium sulfate, (B) cupric sulfate, (C) nickel sulfate, (D) ferrous sulfate, (E) DKW/Juglans basal salt mixture, (F) sodium hypochlorite, (G) sodium triphosphate, (H) sodium silicate, (I) endotoxin, (J) humic acid.

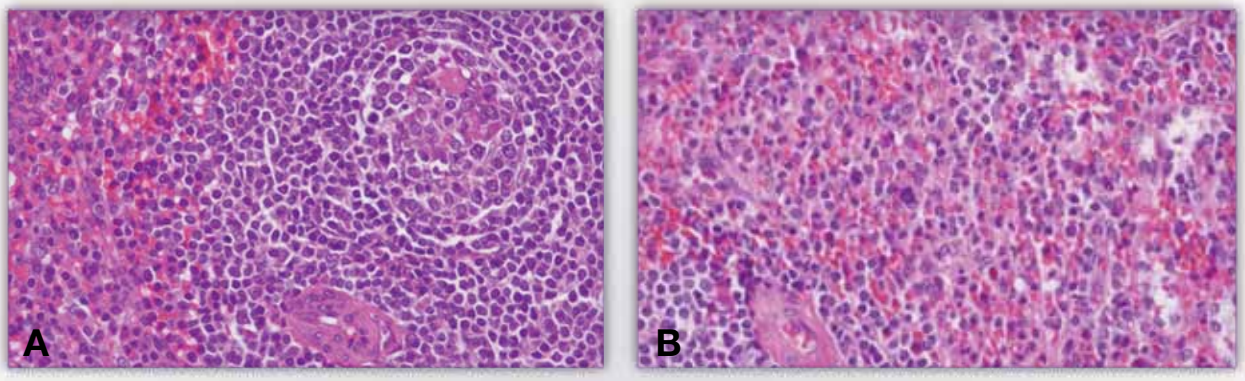

Fig. 6. Photomicrographs of tonsil tissue stained with H&E. 10X
Rinsing steps and Scott's tap water substitute prepared with (A) tap water or (B) Elix purified water.

Results

H&E staining proved to be very robust and was able to tolerate the various water types and water contaminants tested, with minimal impact to staining specificity and color.

When considering bluing reagents, the manufacturer recommends diluting Blue Buffer 8 Solution with *distilled water* while Scott's Tap Water Substitute is to be made with *tap water*. In this study, both Scott's Tap Water Substitute and Blue Buffer 8 performed well, regardless of the water used (Elix purified water, distilled water, service deionized water, or tap water) (Fig. 6).

Grocott's methenamine silver staining is used to stain fungi and often *P carinii*. It stains the cell walls of these organisms black-brown to black. Silver stains tend to have artifacts from background staining (often due to residues found in glassware or attributed to contaminants found in water).

Elix water gave satisfactory results: good staining of *P carinii*, no background staining or staining of the elastic fibers (Fig. 4A). Distilled water lightened the staining of the organisms, though it did not contribute to background staining (Fig. 4B). Service deionized water appeared to intensify background staining as well as staining of *P carinii* (Fig. 4C).

Solutions of methenamine silver nitrate were prepared with Elix purified water, and common water contaminants were added. Staining quality was compared to the results obtained with Elix purified water (Fig. 5).

Conclusion

This study, though limited, indicates that the water used for silver stains should be free of silica, endotoxins (bacteria byproducts), and metals. In addition, detergents should be avoided (sodium triphosphate). H&E staining, on the other hand, proved to be very robust. Water purified with a combination of reverse osmosis and electrodeionization is suitable for a wide array of histology experiments, from commonly used and robust procedures (such as H&E staining) to more delicate ones (such as silver staining). It can be used in protocols that require distilled, deionized, or tap water, yielding good results. Using a water purification system provides peace of mind by delivering constant water quality and requiring only minimal maintenance.

All photos are courtesy of Ethel Macrea and Wendy Lange.

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Lectin Histochemistry: An Alternative to Immunohistochemistry for Identifying Specific Structures in Rat Renal Papillary Necrosis

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Abstract

Lectins are sugar-binding proteins that were first discovered more than 100 years ago in plants and are known to be ubiquitously expressed throughout nature. The purpose of this work was to use the carbohydrate binding specificity of lectins as a method to identify structures within the renal papilla that are affected in a model of renal papillary necrosis induced by 2-bromoethylamine (BEA). Histochemical staining with *Dolichos biflorus* agglutinin (DBA) and *Lotus tetragonolobus* lectin (LTL) identified the collecting ducts and confirmed that the collecting ducts were hyperplastic at 48 hours. Staining with peanut agglutinin (PNA) and *Amaranthus caudatus* lectin (ACL) identified the loops of Henle and confirmed that many of the loops of Henle were absent, which is consistent with necrosis. In conclusion, the ability of lectins to bind specific carbohydrates proved to be a useful alternative to immunohistochemistry for identifying explicit structures within the rat renal papilla.

Introduction

Lectins are a class of proteins that binds to carbohydrates, thus they have been defined as sugar-binding proteins of nonimmune origin. The study of the binding properties of these molecules has offered new information regarding the presence and compartmentalization of sugar residues in tissues. Lectins, as a rule, have at least 2 carbohydrate binding sites that allow cross-linking between cells or between sugar-containing residues.[1]

Because of the specificity that each lectin has toward a particular carbohydrate structure, it is possible to use lectins to evaluate changes in this unique class of molecules and their binding properties as an alternative to using immunohistochemistry techniques. With lectins, it is possible to visualize specific sugar residues and to obtain information on the location and potential changes in expression of the carbohydrate within tissue due to treatment. Qualitative and quantitative changes in lectin binding have been observed in a variety of cells and tissue in the course of cell division, maturation, and differentiation, as well as for other cellular events. Some applications for lectin use include, but are not limited to, detection, isolation, and structural studies of glycoproteins; investigation of carbohydrates on cells and subcellular organelles; studies of protein glycosylation; mapping of neuronal pathways; cell identification and separation; mitogenic stimulation of lymphocytes; diagnosis and targeting and selection of lectin-resistant mutants.[2]

Histochemistry is based on the same principle as immunohistochemistry, where there is a specific binding interaction between 2 molecules. Lectins are naturally occurring molecules derived from plants, animals, and bacteria and have a high affinity for binding specific carbohydrates (Table 1), whereas, in immunohistochemistry, antibodies are used to detect proteins. Antibodies are produced by means of monoclonal hybridoma technology or polyclonal antibody generation that targets a specific protein enabling the identification and localization of a wide range of molecules expressed either on the surface, in the cytoplasm, or in the nucleus of a specific cell type.

In this study, we explored lectin expression following administration of BEA, an agent known to induce papillary necrosis of the kidney. Upon examination of routine hematoxylin and eosin (H&E) slides of the renal papilla, which were harvested from rats exposed to a single dose of BEA, it was evident that the BEA produced a rapid and profound nephrotoxicity characterized by papillary necrosis and acute renal failure.[3]

BEA is a haloalkylamine found in soil fumigants and dry cleaning solution, and is used as a lead scavenger in leaded gasoline, and as a flame retardant in polyurethane foams. This compound has been widely used to study mechanisms of renal papillary necrosis (RPN), a potentially significant side effect caused by nonsteroidal anti-inflammatory drugs (NSAIDs). In rodent models, the development of NSAID-induced RPN requires several months of dosing. In contrast, BEA-induced RPN is an acute model and is thus useful for investigating mechanisms of drug-induced RPN.[4]

As illustrated in Table 1, the renal papilla is rich in a variety of carbohydrates, making this region an ideal one to apply lectin histochemistry in the overall evaluation of toxicity. In addition, the detection of various carbohydrates with lectins allows for the visual distinction of renal tubular epithelium, collecting ducts, and loops of Henle.

Materials and Methods

Animals and Treatments
Male Sprague-Dawley rats (Crl: CD [SD] IGS Br, Charles River Laboratories), 6 to 7 weeks old, weighing

Table 1. Lectins and Their Binding Target

Lectin or Agglutinin	Binding Target	Tissue Target
Amaranthus caudatus lectin (ACL)	Galactose	Ascending thin and thick limbs of Henle, distal convoluted tubules, cortical collecting duct, and outer medullary collecting duct
Dolichos biflorus agglutinin (DBA)	*N*-acetylgalactosamine	Collecting ducts
Lotus tetragonolobus lectin (LTL)	Fucose	Proximal tubules, collecting ducts
Peanut agglutinin (PNA)	Galactose	Mature distal tubules, thin limbs of Henle

approximately 250 g at initiation of the experiment were used. Rats were given either 150 mg/kg of BEA or vehicle (saline) via intraperitoneal injection. Rats were euthanized with CO_2 overdose at 4, 8, 24, and 48 hours after BEA treatment.

Kidney Histology

At each of the time points a portion of the kidney was prepared for histological evaluation. The left kidney was trimmed in a mid-hilar transverse plane to ensure collection and preservation of the papilla during sectioning. Samples were fixed by immersion in 10% neutral buffered formalin (NBF) for approximately 72 hours prior to processing and embedding, followed by routine H&E staining.

Histochemistry

After fixation, kidneys were sectioned at 5 µm onto Gold Plus slides (Erie Scientific, Portsmouth, NH). The following lectins (Vector Labs, Burlingame, CA) were used to identify specific structures within the papilla of the rat kidney: *Amaranthus caudatus* lectin diluted 1:200, *Dolichos biflorus* agglutinin diluted 1:400, *Lotus tetragonolobus* lectin diluted 1:200, and peanut agglutinin diluted 1:350. Lectins were directly conjugated to biotin, and all sections were pretreated with the Avidin/Biotin Blocking Kit to block endogenous binding to biotin (Vector Labs), followed by 3% hydrogen peroxide treatment for 10 minutes to remove endogenous peroxidase, prior to lectin application. Lectins were diluted in Tris buffered saline (TBS) and incubated for an hour. Lectin carbohydrate binding was detected with a Ready to Use (R.T.U.) ABC-Elite HRP reagent (Vector Labs). An adequate amount of solution was applied to ensure complete coverage of the tissue section. Slides were then incubated for 30 minutes. This complex was made visible with 3´-diaminobenzidine tetrahydrochloride (DAB) (Dako North America, Carpenteria, CA) (Fig. 1). Optimal time of color development was determined by the end user and should be used consistently for the development of all samples. Sections were then counterstained with hematoxylin, Gills formula (Vector Labs), dehydrated, cleared with xylene, and permanently mounted under cover glass. All steps were carried out at room temperature. Slides were washed for 4 minutes with 2 changes of 1X TBS, pH 7.0, between each incubation step.

Results

BEA treatment caused a marked polyuria within 2 hours of dosing, but histopathologic changes were subtle at 4 and 8 hours after dosing and consisted of minimal to mild papillary karyorrhexis and pyknosis, with minimal papillary tubular dilation at 8 hours. By 24 hours, papillary lesions were more pronounced and varied from minimal to moderate. Lesions included individual cell death, increased interstitial spaces in the papillary tips, necrotic loops

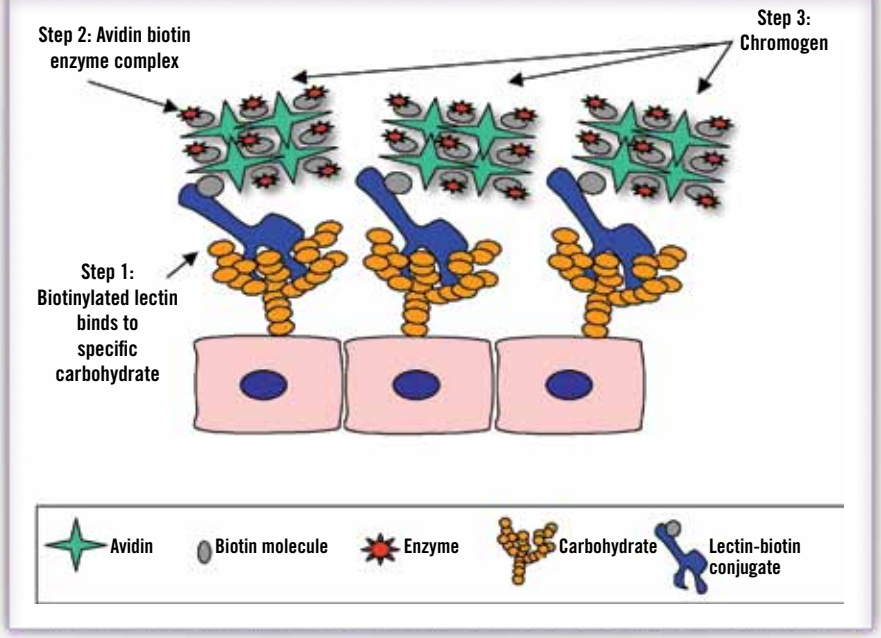

Fig. 1. Schematic of histochemistry protocol.

of Henle (2 of 9 rats), tubular dilation, intratubular proteinaceous fluid, and, in 5 of 9 rats, minimal to mild hypertrophic and hyperplastic regenerative changes (images not shown).

At 48 hours, there was evidence of both cell death and regeneration. In 8 of 9 rats, there were necrotic loops of Henle. At the same time, there was considerably more regeneration than at 24 hours. All 9 rats had hyperplasia and hypertrophy of the papillary collecting duct epithelium and urothelium (Fig. 2). To better define the affected tubules as loops of Henle and/or collecting ducts, lectin and agglutinin staining was performed on the samples collected at 48 hours.

Lectin histochemistry demonstrated moderate DBA staining of the collecting ducts with a localized pattern of expression along the luminal surface. A very similar pattern was noted with LTL, except for the absence of luminal staining due to the difference in lectin-carbohydrate binding specificity. Both identified the collecting ducts but a very distinct difference was observed with the BEA treatment, noted by obvious hypertrophy of this structure (Fig. 3).

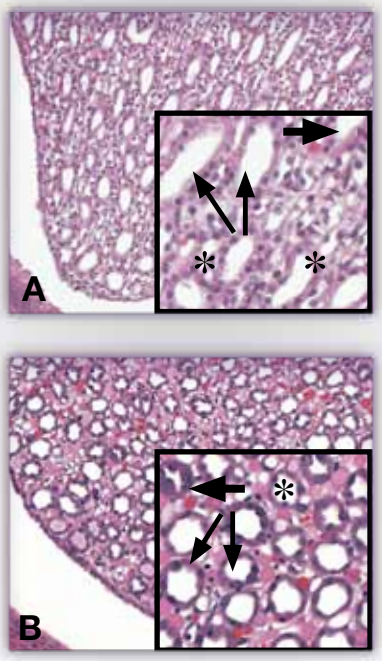

Fig. 2. Routine H&E stain of rat papilla (A) control animal and (B) BEA-treated animal (150 mg/kg) at 48 hours post exposure. Note hypertrophy of the collecting ducts (arrows) and atrophy of the thick (thick arrow) and thin (*) limbs of Henle. 200X

PNA and ACL showed expected overlapping patterns of expression because they bind to the same glycoconjugate, galactose. Both identified the thick and thin limbs of Henle, with the addition of PNA binding to the luminal surface of the collecting duct epithelium in the BEA-treated animal (Fig. 4). The affinity between a lectin and its receptor may vary a great deal due to small changes in the carbohydrate structure of the receptor. The additional staining observed with PNA in the treated sample is a case in point. It was clear by the staining result that the thick and thin limbs of Henle were necrotic and severely compromised by the treatment.

Discussion

For this particular study, lectins proved to be an excellent alternative to immunohistochemistry for identifying specific structures in necrosis for several reasons. First, lectin histochemistry is especially useful when specific antibodies are not commercially available for the detection of antigens under investigation or when the cost of purchase is not a viable option due to laboratory budget constraints. Second, lectin histochemistry proved to be a powerful tool for the identification of explicit structures affected by BEA treatment within the rat renal papilla.

Specific cells within tissue express a wide array of carbohydrates in the form of glycoproteins, glycoconjugates, and glycosaminoglycans. It is well documented that carbohydrates play an essential role in cell communication and cell signaling events, making lectins a superb tool for observing changes associated with cell behavior, development, and disease processes.[2] The kidney is an excellent example for lectin histochemistry staining and evaluation. Because there is an abundance of various carbohydrates expressed in the renal papilla, specifically within the epithelium of the collecting ducts and loops of Henle, lectin histochemistry was especially useful in identifying the defects associated with papillary necrosis and renal cortex damage.

This application, though powerful, may or may not prove to be advantageous in addressing all facets of biological processes. It is not without some shortcomings. Many lectins have an affinity for the same carbohydrate. Their presence or absence on a particular cell structure corresponds with apparent physiological changes, for instance, due to drug treatment. Therefore, lectins that bind to the same carbohydrate may well have a slightly different outcome, making interpretation of results difficult or confusing. It is imperative that the decision to utilize this technique is thoroughly explored to determine if it meets the needs in addressing specific questions that arise during the course of a study.

In summary, by utilizing lectin histochemistry, we were able to identify structures within the kidney papilla and make a definitive determination regarding the specific structures affected by the effects of BEA treatment. These data have clarified the functional irregularities caused by this lesion.

Lectin histochemistry enhanced the ability to define areas within the papilla that were not clear from the evaluation of routine H&E-stained slides. In this model, performing this technique proved to be an asset and should be considered as an alternative to immunohistochemistry when appropriate.

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Acknowledgments

I would like to extend a thank you to Donna Dambach, VMD, PhD, for sharing her experience and expertise with lectin histochemistry and Michele French for reviewing and editing this paper prior to publication.

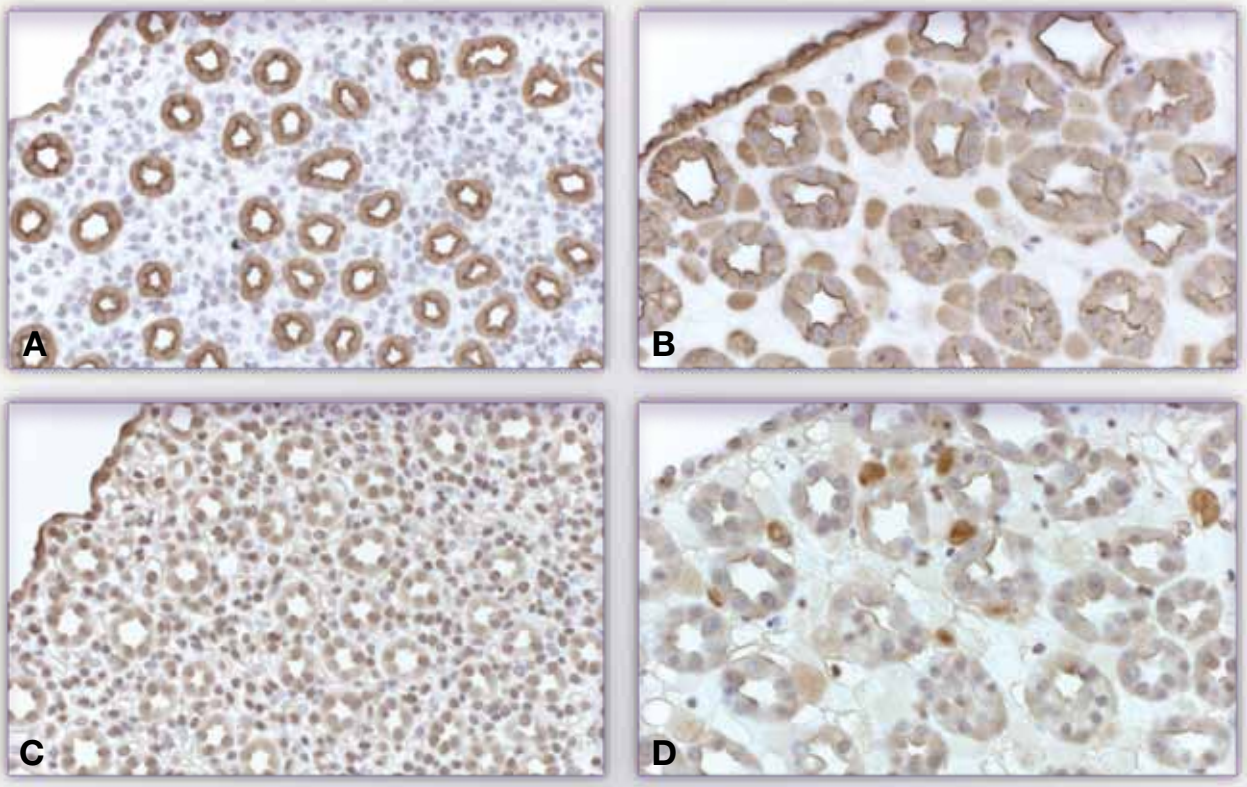

Fig. 3. Rat renal papilla histochemically labeled with *Dolichos biflorus* agglutinin (A, B) and *Lotus tetragonolobus* lectin (C, D). A and C show control animal, and B and D show BEA-treated animal (400X). A marked hypertrophy of the collecting ducts was noted with these lectins.

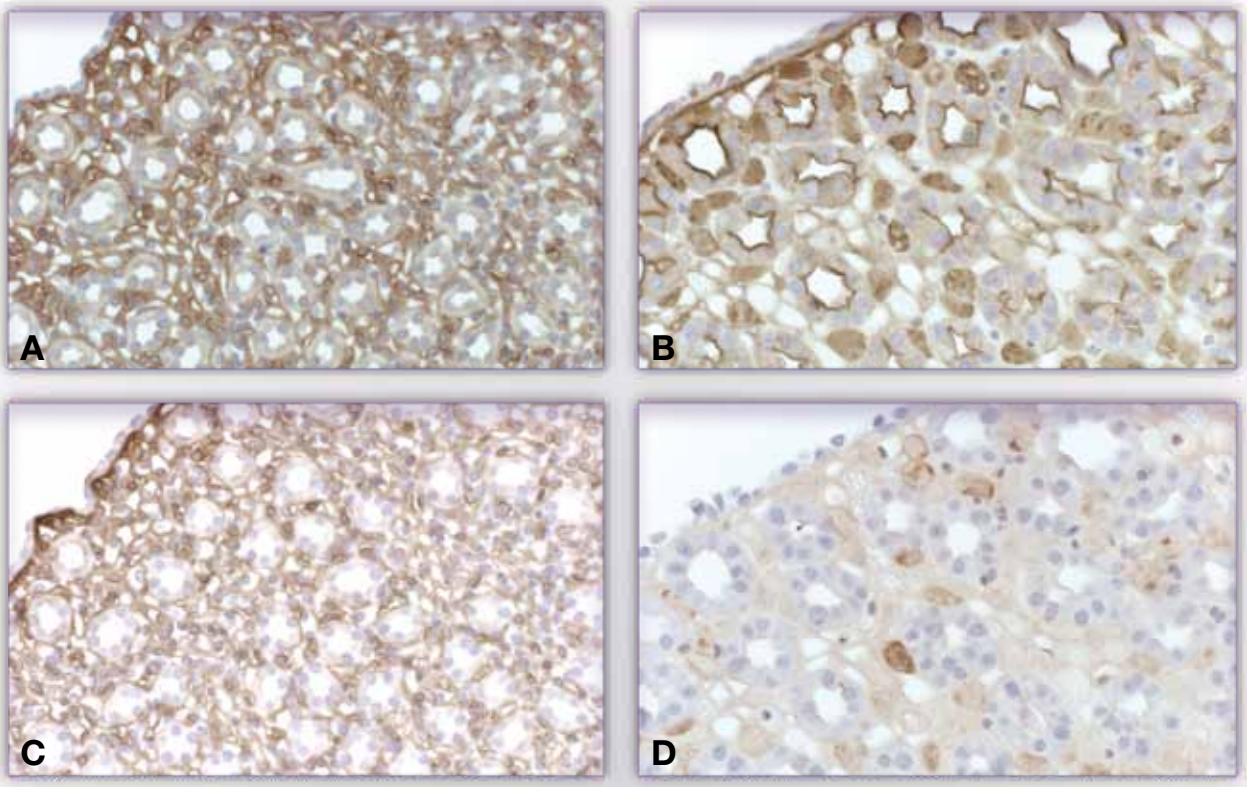

Fig. 4. Rat renal papilla histochemically labeled with peanut agglutinin (A, B) and *Amaranthus caudatus* lectin (C, D). A and C show control animal, and B and D show BEA-treated animal (400X). Loss of staining is consistent with necrosis of the thick and thin limbs of Henle.

Comparison of Decalcification Methods on Rodent Femurs

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Abstract

Decalcification is the process by which calcium is removed from tissue. This removal is necessary prior to processing, embedding, and sectioning in paraffin. Overdecalcification can result in a lack of nuclear staining and altered cytoplasmic and structural staining, so the endpoint must be closely monitored. There are 2 methods of decalcification: acid and chelation. Acids ionize and dissolve the calcium salts and work relatively quickly; however, they can affect the staining ability of tissue if they overdecalcify. Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), bind the calcium ions, but this is a relatively slow process. Chelating methods are usually recommended when subsequent immunohistochemical staining is to be performed because anecdotal antigenicity is more robust with this method. In our lab, we routinely use formic acid buffered with citrate to decalcify. There are many other specialized products available and we evaluated some of these products and compared them to our standard protocol:

- Buffered formic acid (BFA) (trisodium citrate)
- Formical-4™
- Immunocal™
- RDO™
- RDO *Gold*™
- EDTA

We compared variables such as length of time in solution, ease of cutting, and overall quality of staining to determine the best possible method depending on desired priority.

Introduction

No one decalcification method is ideal for every stain or laboratory setup. In this experiment, we set out to observe the effects of various decal solutions on staining results, while also considering ease of use and time requirements. We chose to evaluate hematoxylin and eosin (H&E), toluidine blue, CD68 (an immunohistochemical stain for macrophages and osteoclasts), and tartrate resistant acid phosphatase (TRAP) (an enzyme stain for osteoclasts). Through experimentation using mouse femurs, we have demonstrated several methods that yield valuable results. The length of time necessary to decalcify with any method is dependent on the size of the sample. The following is an overview of each method.

Buffered Formic Acid (BFA) (trisodium citrate) (Poly Scientific R&D Corp., Bay Shore, NY)

This combination of formic acid and citrate buffer is the most commonly used method of decalcification in our lab. The sample should be fixed first for a minimum of 24 hours then placed in the BFA. Completion of decalcification is determined by a chemical endpoint test.

Formical-4 (Decal Chemical Corporation, Tallman, NY)

This fixative decalcifier is a combination of formic acid, formaldehyde, and EDTA. Fresh tissue can be placed directly into Formical-4 to fix and decalcify simultaneously. This will cut down on the overall time necessary for fixation and decalcification. Completion of decalcification is determined by a chemical endpoint test.

Immunocal (Decal Chemical Corporation)

This is a combination of semiconductor-grade formic acid and highly purified reagent-grade water. According to the manufacturer, Immunocal "will not destroy antigen sites during decalcification, so it can safely be used to demonstrate antigens in decalcified bone."

RDO (Apex Engineering Products Corporation, Aurora, IL)

This is a proprietary formula that has hydrochloric acid as the active ingredient. RDO is a rapid decalcifier, meaning it decalcifies samples in as little as 3 hours.

Table 1. Decalcification Materials and Methods

Decalcifier	Length of Time in Decalcifier	Comments
BFA	5 days	Chemical endpoint
Formical-4	4 days	Chemical endpoint
Immunocal	5 days	Chemical endpoint
RDO (See inset photo in Fig. 1)	3 days	Deliberately overdecalcified for demonstration purposes
RDO	3 hours	Chemical endpoint
RDO *Gold*	3 hours	Chemical endpoint
EDTA	11 days	No chemical endpoint

RDO *Gold* (Apex Engineering Products Corporation)

This is a proprietary formula that also has hydrochloric acid as the active ingredient. Apex states that "RDO *Gold* is specifically designed for when biopsies contain minimal amounts of calcium and will provide less aggressive decalcification on both large and small tissue samples that the nucleic acids within the cell are not altered."

Decalcifying EDTA pH 7.2-7.6 (Poly Scientific R&D Corp.)

This is a chelating solution. There is no chemical endpoint to determine complete decalcification. Since this is a very slow process, samples may have to remain in this solution for months before they are completely decalcified.

Materials and Methods

Rat femurs were fixed in 10% neutral buffered formalin (NBF) (Thermo Fisher Scientific Inc., Waltham, MA) for a period of 24 to 72 hours and then decalcified (Table 1). Decalcification endpoint was checked chemically for those samples placed in acid decalcifiers. This is done with the following method: 1 mL of 5% ammonium oxalate is combined with 5 mL of used decal solution. This combined solution is checked after 10 minutes for signs of white precipitate. Any precipitate present indicates that the sample needs further decalcification. Samples were removed from decalcifier solution and rinsed in phosphate buffered saline (PBS) when the chemical endpoint test was clear.

Following decalcification, tissues were chemically processed into paraffin and sectioned at 5 µm on a rotary microtome. Sections were then stained with H&E, toluidine blue, TRAP (TRAP kit 387-1a, Sigma-Aldrich, St. Louis, MO), and CD68 by immunohistochemistry. Photographs were taken at 10X and 40X on a Nikon Eclipse E600 upright microscope equipped with a Nikon DXM1200C digital camera.

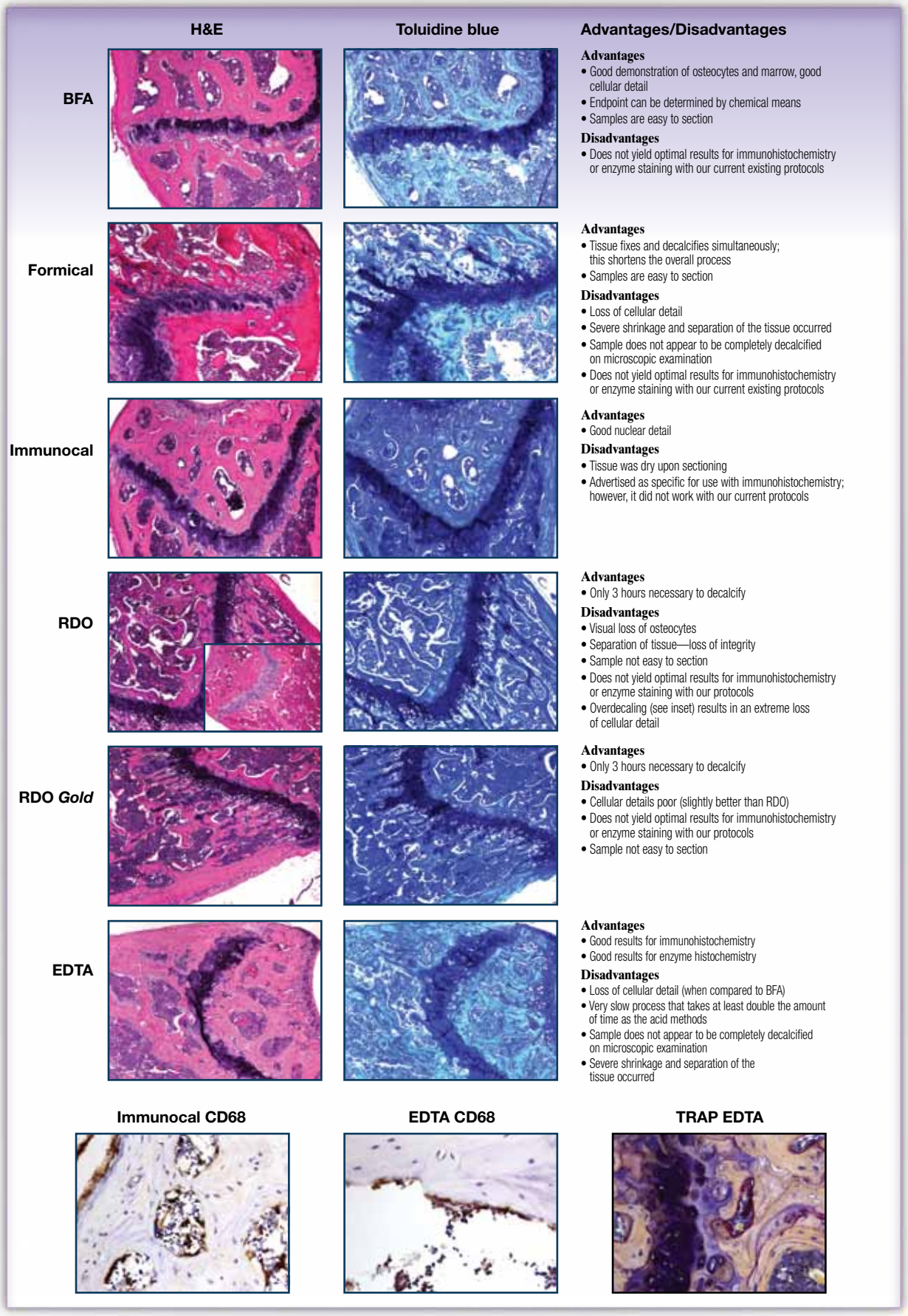

Fig. 1. Comparison of staining results for the decalcifying agents studied. 40X

Results

No one decalcification method is ideal; however, if immunohistochemistry is required, EDTA appears to yield results that are superior to the other methods we tested. If good cellular morphology is desired, then EDTA is not the optimal choice.

Conclusion

There are many different methods to decalcify samples. We set out to compare length of time in solution, ease of cutting, and quality of staining for several different methods. Based on our experiments, we have determined that there are several methods that yield acceptable results. For everyday routine use, we found BFA to be the best method in our lab. For use with immunohistochemistry and enzyme histochemistry, EDTA yields the best results with our current protocols; however, Immunocal also yielded acceptable immunohistochemistry results. Due to the length of time necessary to decalcify in EDTA, we will continue to evaluate possible alternatives when enzyme or immunohistochemistry is required.

Bibliography

Bancroft J, Stevens A. *Theory and Practice of Histological Techniques.* 4th ed. New York, NY: Churchill-Livingstone; 1996.

Carson F. *Histotechnology: A Self-Instructional Text.* 2nd ed. Chicago, IL: ASCP Press; 1997.

Acknowledgments

We would like to sincerely thank Dr. Lucy Phillips, Sue Ryan, Linda Suarez, Jennifer Johnson, Biomedical Media Services, Denise Griffiths, and John Lydon for their assistance with this study.

Colorization of Tissues With Methylene Blue as an Alternative to Eosin and Erythrosin B

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Introduction

Colorization of tissue with eosin or erythrosin B has primarily been used to assist in the proper placement of small tissue specimens in paraffin supporting medium, and cutting tissue from colorless paraffin blocks. However, high concentrations of eosin or erythrosin B within the tissue may cause increased tissue autofluorescence, hampering results from methods such as fluorescence in situ hybridization (FISH). FISH is a firmly established technique used in the diagnosis and assessment of lymphoid malignancies. It is widely used to assess HER2/neu status, a prognostic marker in breast carcinoma that predicts response, which aids in selecting patients who will benefit from Herceptin® treatment.

Materials and Methods

Twenty-one samples of breast tissue measuring 0.5 to 1.0 cm in length were collected over a period of 1 month. Twenty control tissue samples were collected at the same time. All samples were fixed over a period of 6 hours in alcoholic formalin.

Concentrations of methylene blue were used to colorize the tissue starting at 1%, 0.5%, 0.1%, and 0.01%. Each concentration of methylene blue was added to the 100% alcohol solutions on the Tissue-Tek® VIP® Processor (Sakura Finetek USA, Inc., Torrance, CA).

Each sample was embedded, cut, and stained with hematoxylin and eosin (H&E) and was submitted for HER2/neu FISH (Vysis PathVysion®, Abbott Molecular Inc., Abbott Park, IL).

Results

The tissue colorized with 1%, 0.5%, and 0.1% methylene blue demonstrated strong colorization, making the tissue difficult to orient during embedding. It also made it difficult to visualize the tissue while sectioning. The 0.01% solution (6.3 mL of 6% alcoholic methylene blue in 3800 mL of reagent-grade 100% alcohol) offered the optimal colorization of tissue, which allowed for ease in orienting, embedding, and sectioning small specimens. Control tissue and 0.01% methylene blue-stained tissues demonstrated similar FISH signals without evidence of autofluorescence (Figs. 1 and 2).

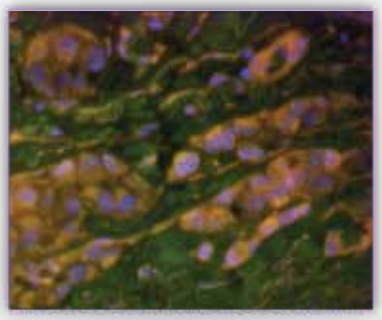

Fig. 1. Eosin dye is often used in tissue processor dehydrating solutions to make tissue samples more visible for accurate embedding and sectioning. Paraffin block contains breast tissue tinted with eosin during processing. Fluorescence in situ hybridization (FISH) for HER2/neu. 600X

Fig. 2. FISH preparation for HER2/neu in tissue that was colored with eosin. High background fluorescence makes it difficult to visualize the presence of the HER2/neu oncogene.

Conclusion

The addition of methylene blue in the 100% alcohol step on the tissue processor is a reliable and predictable method for obtaining adequate tissue colorization. Proper colorization facilitates embedding and sectioning by making small specimens easy to visualize. Methylene blue does not interfere with the results of fluorescence in situ hybridization studies performed on formalin-fixed and paraffin-embedded tissues.

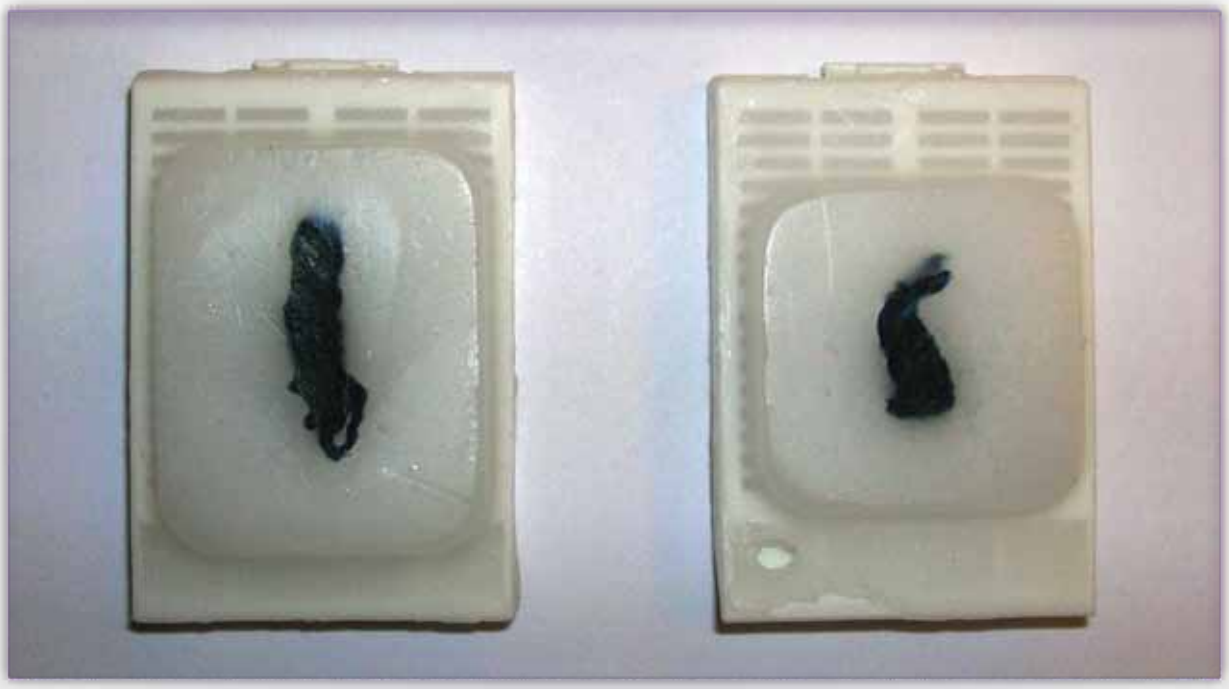

Fig. 3. Paraffin blocks containing tissue that was colored with methylene blue. At concentrations greater than 0.01%, the color is too dark to allow structures to be easily seen.

Fig. 4. When using methylene blue dye at 0.01%, tissues are lightly colored, allowing for accurate orientation at embedding.

Your Paper Towels Can Do What?

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Abstract

Histologists are challenged to get as much information from their samples as possible. This requires taking a 3-dimensional structure and presenting it in a 2-dimensional arrangement on a glass slide. This arrangement is created with tissue(s) embedded in a paraffin block in a single plane. So how do we do this?

Tissue handling *before* processing is the key to achieving maximum information from each specimen. This article discusses the differences we found in orienting tissue before processing by using common paper towels found in the lab. Paper towels can be used to keep tissues supported in a way that allows for more effective embedding and sectioning. Without support during fixation and processing, many tissues tend to curl up, making orientation during embedding difficult. The proper placement of tissues in cassettes makes embedding and sectioning more efficient. Optimal orientation in the paraffin block can reduce the number of slides needed by the pathologist for analysis. We used our paper towel technique on mouse and rat tissue, including skin, paws, lungs, arteries, and nerves, to demonstrate just what paper towels can do.

Materials and Methods

Mouse and rat tissues were collected and fixed in 10% neutral buffered formalin (NBF) (Thermo Fisher Scientific Inc., Waltham, MA) for 24 hours before processing. C-fold paper towels were utilized during grossing; corresponding samples from the same tissues were fixed and processed without paper towel support.

The paws were decalcified in a buffered formic acid solution (cat #516 9K, buffered formic acid trisodium citrate-formic acid [Poly Scientific R&D Corp., Bay Shore, NY]). Tissues were processed overnight using the Sakura Tissue-Tek® VIP® Processor (Sakura Finetek USA, Torrance, CA). After processing, tissues were embedded in paraffin (Paraplast® X-tra™, McCormick Scientific, St. Louis, MO) blocks. Blocks were sectioned at 5 µm, and sections were stained with hematoxylin and eosin (H&E) stain (Harris Hematoxylin, cat #7221; Eosin-Y with phloxine, cat #71304, Thermo Fisher Scientific Inc., Waltham, MA).

Results

Our results were consistent for both the rat and mouse lungs (Figs. 1 and 2). The crumpled paper towel technique was used before fixation and during tissue processing for these tissues. A crumpled paper towel was placed in the center of the tissue, and as soon as the cassette lid was shut, pressure was applied to keep the tissue stable. Before embedding the tissue, the paper towel was removed from the tissue and discarded. The tissue was embedded dorsal side down, enabling a full image of the lung to be maintained on one slide. The crumpled paper towel also helped to expand the lobes, compared to the lungs without the paper towel, which tended to curl and showed fewer histological features.

The results for both the mouse and rat paws were also consistent (Figs. 3 and 4). The crumpled paper towel technique was again used before fixation and during tissue processing. The paper towel was crumpled and added on top of the paws, and then some pressure was applied to flatten them by closing the lid of the cassette. Before embedding the tissue, the paper towel was removed from the tissue and discarded, and then the paw was embedded flat. As a result, all joints were obtained in one section, and good orientation as well as good tissue architecture was maintained throughout the histology process. In comparison, the paws that did not receive the paper towel treatment remained curled up, resulting in the need for multiple levels to get a good end result.

The flat paper towel technique was used to keep skin tissues from warping or curling (Fig. 5). This technique was used before fixation and during tissue processing for rat skin samples. A flat piece of paper towel was placed underneath the tissue as well as on top of the tissue, and pressure was applied when the cassette lid was closed. This technique enabled the skin sample to remain flat, so it could be embedded on edge. Again, before embedding the tissue, the paper towel was removed and discarded. The skin samples that didn't receive the paper towel treatment were warped, making it harder to see all the layers of the skin on one slide.

The rolling method technique was done by wrapping the spinal cord in a paper towel and rolling it gently until enough pressure was maintained within the tissue and paper towel. Then it was placed into a cassette where more pressure was maintained to keep the tissue straight. As a result, a full section was obtained on one slide, compared to free-floating tissue that tends to bend or roll up, making it harder to see the full section on one slide (Fig. 6).

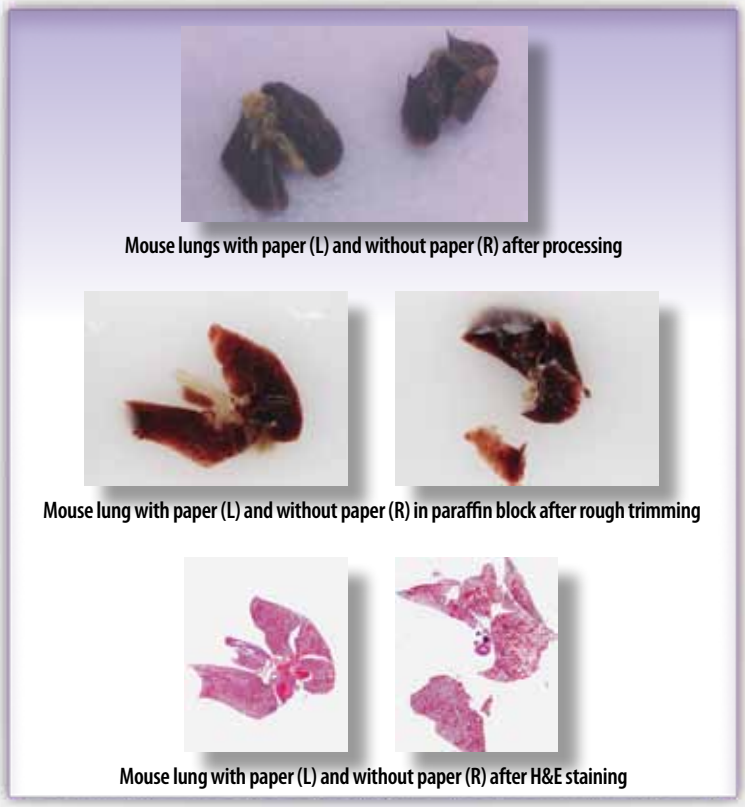

Fig. 1. These images demonstrate good orientation and maintenance of the tissue architecture, while using less time throughout the histology process, with the crumpled paper towel technique. When not using the paper towel technique, the histology shows tissue that is more spread apart with pieces missing.

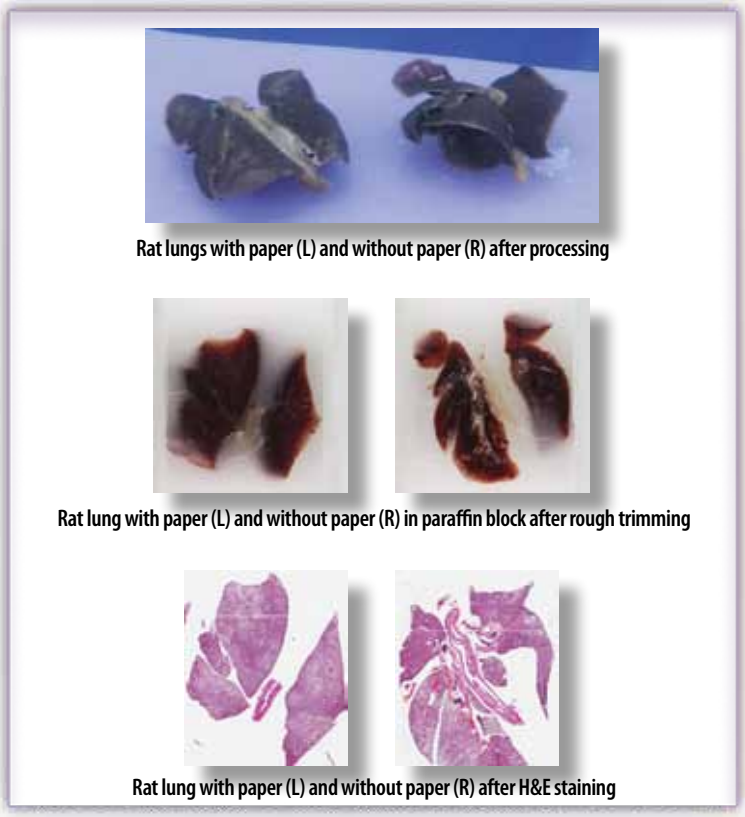

Fig. 2. These images demonstrate good orientation and maintenance of the tissue architecture, while using less time throughout the histology process, with the crumpled paper towel technique. When not using the paper towel technique, the histology shows tissue that is more spread apart with pieces missing.

Discussion

Orientation of the tissue prior to processing is as easy as using a piece of paper towel that is readily available in the laboratory. This is also a valuable technique to reduce time/effort at the embedding station. Tissue is often grossed and placed in a cassette with too little consideration for the final product, the stained slide. During fixation and processing, tissue tends to shrink, warp, bend, curl, and may become hardened into an undesirable position or shape. When tissue is placed in the cassettes freely without support, it tends to float or bend, making it difficult to embed on the same plane. The challenge for the histologist is to be able to embed the sample so it can be cut quickly, all on the same plane, yielding critical information in as few slides as possible.

We have demonstrated with several types of tissue the benefit of using paper towels both for support and for orientation.

Depending on the type of tissue, paper towels can be used in several ways. Flat pieces of towel can be cut to the size of the cassette and used above and below the tissue to prevent curling. Keeping skin flat is an ideal use for this technique. Sometimes, a crumpled piece of paper towel helps to give more volume and support for concave samples, such as lungs and paws. Another use is to envelope or sandwich the tissue to prevent distortion and maintain a desired form. This has been useful in our laboratory when working with samples containing biomaterials, such as gels. Rolling specimens in paper towels can help to retain shape, especially when working with arteries, nerves, and the spinal cord; without support, they tend to shrink into a ball. In this study, we took samples of many types of tissue, including paws, skin, spinal cord, kidneys, lungs, colon, aorta, sciatic nerves, and stomach. The tissues with the most significant results are presented on the next page.

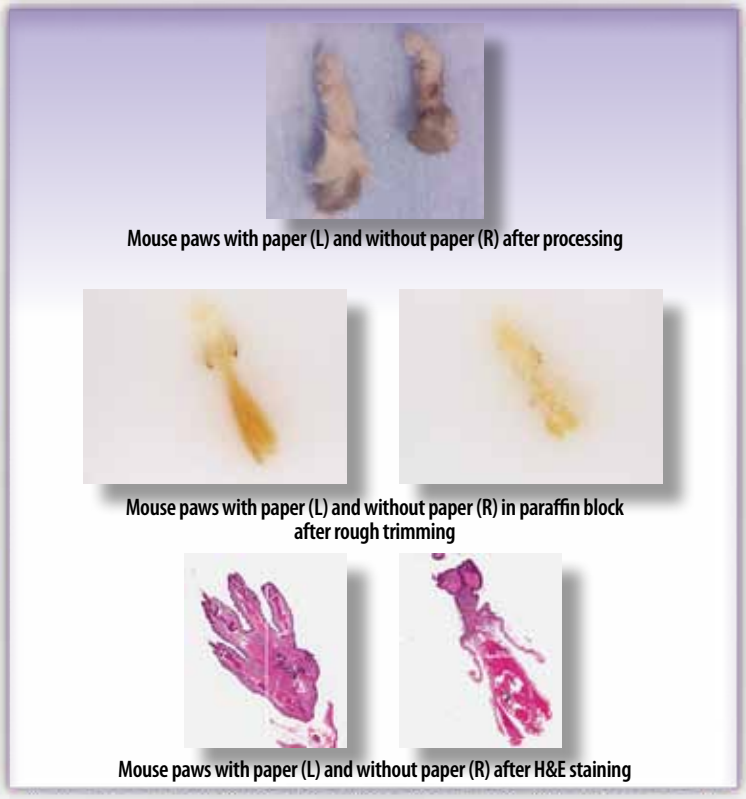

Fig. 3. These images, prepared by the crumpled paper towel method, demonstrate good orientation, tissue architecture, and the ability to see all the joints on one slide, compared to processing without paper towels. Multiple levels of slides would be needed to obtain a full joint section if prepared without paper towels.

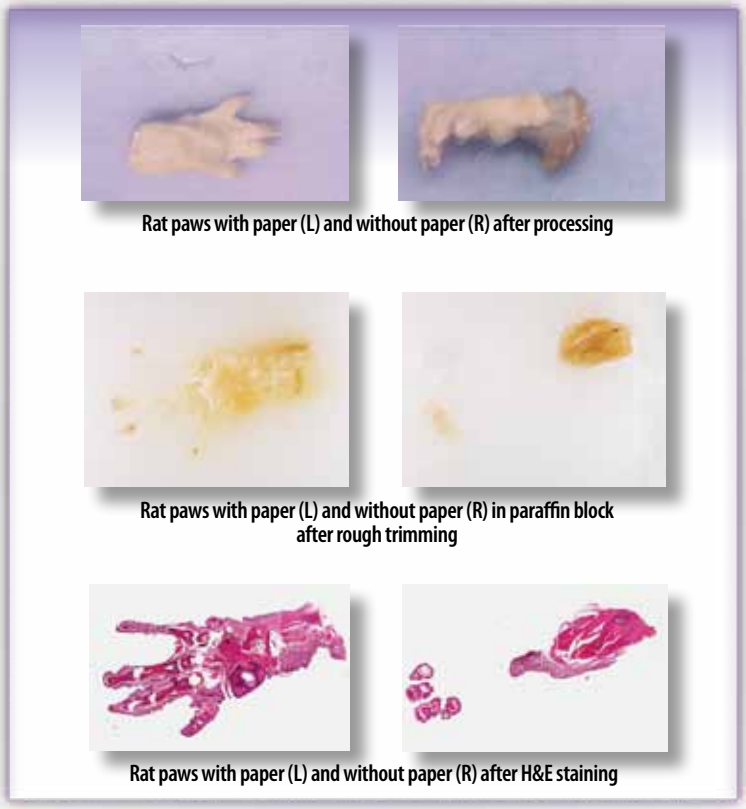

Fig. 4. These images show that tissues processed by the crumpled paper towel method showed the same results as the mouse paws in Figure 3.

As can be seen from the photos, there are some noticeable differences in the tissues supported with paper towels versus those without. (Figs. 1-6).

Although there are many commercially available products for tissue support during grossing, we had several reasons for choosing paper towels. Since paper towels are within arm's reach in the lab, they are easy to access and don't need to be special ordered. We noticed that basic laboratory paper towels have a fine texture, which helps to hold the tissue in place. This is an advantage over other supports, such as sponges, which can cause artifacts and processing carryover. Finally, paper towels are versatile. They can be crumpled, rolled, shaped, or kept flat. There are many other possibilities; we encourage you to see what will work best for you.

Conclusion

The paper towel technique has made it easier for our laboratory to increase speed and quality, while reducing the number of slides per study. By maintaining specimen shape and orientation in this manner during tissue processing, we were able to achieve high-quality sections of desired structures in less time with fewer slides, which translated into a cost savings both in labor and materials.

Fig. 5. These images show tissues processed by the flat paper towel method, which kept the skin tissue flat. Embedding was perpendicular, which helped maintain the correct ratio of tissue layer thickness, so the end result was a quality section.

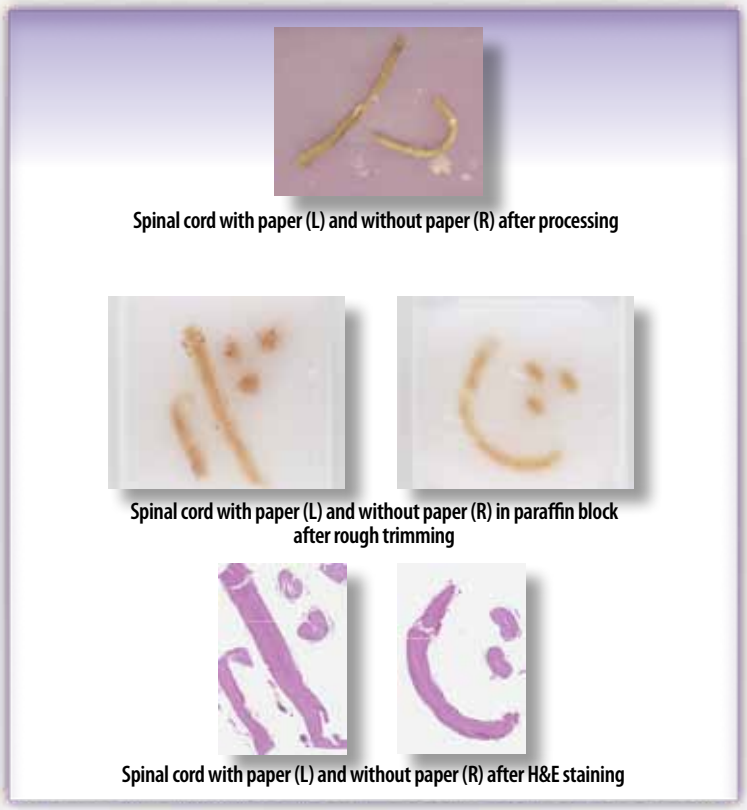

Fig. 6. These images show how the tissues processed by the rolling method achieved good orientation and tissue architecture compared to those tissues that weren't processed with paper towels, resulting in a tendency to bend or roll up, making it harder to achieve a full section.

Acknowledgments

We would like to thank Jennifer Johnson, Genzyme DCM, Biomedical Media Services, Bill Weber, Histology and Pathology Departments, and Helen Chim for their assistance with this study.

✔ Mark Your Calendar!
Educational Opportunities in 2009

JANUARY

16 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Making Everyone Happy: Prostate and Bladder Biopsies**
Speaker: Dean Troyer, MD
Professor of Pathology and Urology
University of Texas Health Science Center
San Antonio, TX

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Working with Difficult People**
Speaker: Amy Dixon, MS
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060
Email: histo@nsh.org

FEBRUARY

20 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Creutzfeldt-Jakob Disease and the Laboratory**
Speaker: Jennifer L. Hofecker, HT(ASCP)
Vanderbilt University Medical Center
Nashville, TN

20 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Breast Cancer and Immunohistochemistry**
Speaker: David Tacha, HTL(ASCP)HT, PhD
Biocare Medical Inc
Concord, CA
Phone: (443) 535-4060
Email: histo@nsh.org

MARCH

13-14 **Indiana Society for Histotechnology**
Site: Marriott East
Indianapolis, IN
Contact: Corrie Toler
Phone: (317) 277-1075

20-22 **Georgia Society of Histotechnology**
Site: Sea Palms Resort
St. Simons Island, GA
Contact: Shirley Powell
Email: powell_sa@mercer.edu
Website: www.histosearch.com/gsh

20 **ASCP Teleconference 12:00 pm Central Time (800) 267-2727, option 2**
Title: **Tissue Identification for the Histotechnologist**
Speaker: Thomas S. Haas, DO, FASCP, Chairman
Dept of Pathology, Mercy Health System
Janesville, WI

20 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Safety in the Histology Laboratory**
Speaker: Clifford Chapman, MS, HTL(ASCP), QIHC
Children's Boston Hospital
Boston, MA

25 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Microwave Processing for the Lean, Green Laboratory**
Speaker: Donna Willis, HTL(ASCP)HT
Milestone Medical
Grand Prairie, TX
Phone: (443) 535-4060
Email: histo@nsh.org

APRIL

TBD **Histotechnology Society of Delaware**
Site: Wilmington, DE
Contact: Michelle Hart
Phone: (302) 733-3657
Email: mhart@christianacare.org

12 **Nebraska Society for Histotechnology**
Site: Nebraska Medical Center
Omaha, NE
Contact: Konnie Zeitner
Phone: (402) 552-3132
Email: kzeitner@nebraskamed.com

APRIL (Cont.)

17 **ASCP Teleconference 12:00 pm Central Time (800) 267-2727, option 2**
Title: **The Many Faces of Melanoma**
Speaker: Barbara McGahey Frain, MS, CT(ASCP)
Education Coordinator
Cytotechnology Program
Indiana University
Indianapolis, IN

17 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Histology Process Improvement: Workload Recording**
Speaker: William DeSalvo, BS, HTL(ASCP)
Sonora Quest Laboratories
Gilbert, AZ

17-19 **Histology Society of Ohio**
Site: Holiday Inn French Quarter
Perrysburg, OH
Contact: Mary Abbuhl
Phone: (216) 844-3834
Email: mary.abbuhl@uhhs.com

18-19 **Region 1 Symposium**
Site: Newport Harbor Hotel & Marina
Newport, RI
Contact: Bruce Bouchard
Email: president@rihistology.org

22 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Controlling Immunohistochemistry: Theory and Practice**
Speaker: Tim P. Morken, HTL(ASCP)
ThermoFisher Scientific/LabVision
Fremont, CA
Phone: (443) 535-4060
Email: histo@nsh.org

24-25 **New York State Histotechnological Society**
Site: Fishkill, NY
Contact: Mary Georger
Phone: (585) 259-6248
Email: mgeorger@gmail.com
Website: www.nyhisto.org

29-May 1 **Tri-state Conference (Iowa, Minnesota, Wisconsin)**
Site: The Concourse Hotel
Madison, WI
Contact: Jean Mitchell / Dave Cavanaugh
Phone: (608) 263-9184 / (515) 239-4493
Email: jmitchell@uwhealth.org / dcavan55@yahoo.com

30-May 2 **North Carolina Society of Histopathology Technologists**
Site: Doubletree at Biltmore House
Asheville, NC
Contact: Wanda Grace Jones
Email: wanpto@aol.com

MAY

8-9 **Colorado Society for Histotechnology/NSH Region**
Site: Cheyenne Mountain Resort
Colorado Springs, CO
Contact: Stacey Langenberg
Email: slangenberg@summitpathology.com
Website: www.coloradohisto.org/

14-17 **California Society for Histotechnology**
Site: Westin San Francisco Airport
San Francisco, CA
Contact: Lydia Figueroa
Phone: (800) 725-8723 ext 7863
Email: Lefigueroa90501@yahoo.com

14-17 **Florida Society for Histotechnology**
Site: Bahia Mar Beach & Yacht Resort
Fort Lauderdale, FL
Contact: Susan Clark
Phone: (954) 987-2000 ext 5371
Email: sclark@fshgroup.org

✔ Mark Your Calendar!
Educational Opportunities in 2009

MAY (Cont.)

15-16 **Michigan Society for Histotechnology**
Site: Livonia, MI
Contact: Paula Bober
Email: pbober@dmc.org
Website: www.mihisto.org

15 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Increased Effectiveness of Work Processes in Histopathology with Principles from Process Excellence and the Toyota Production System**
Speaker: Susan F. South
Ortho Clinical Diagnostics
Scottsdale, AZ

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Paraffin Embedding and Process Improvement**
Speaker: Joelle Weaver, HTL(ASCP)
Blanchard Valley Hospital
Findlay, OH
Phone: (443) 535-4060
Email: histo@nsh.org

28-30 **Missouri Society for Histotechnology**
Site: Cruising on the Lake Resort at Port Arrowhead
Lake of the Ozarks, MO
Contact: Amanda Kelley
Phone: (314) 609-9194
Email: kelleypathl@charter.net

29-31 **Texas Society for Histotechnology**
Site: Hyatt Regency Austin
Austin, TX
Contact: Veronica Davis
Email: veronida@baylorhealth.edu

JUNE

19 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Bringing FISH Testing In-House**
Speaker: Bonnie Whitaker, HT(ASCP)QIHC
Ohio State University Medical Center
Columbus, OH

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Troubleshooting IHC**
Speaker: Jim Burchette, HT(ASCP)
Duke University Health System
Durham, NC
Phone: (443) 535-4060
Email: histo@nsh.org

25-26 **Tennessee Society for Histotechnology**
Site: Doubletree Hotel
Memphis, TN
Contact: Charlene Henry
Email: Charlene.henry@stjude.org

JULY

17 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Melanomas: A Diagnostic Approach Using Immunohistochemistry**
Speaker: Elizabeth Sheppard, MBA, HT(ASCP)
Ventana Medical Systems, Inc.
Tucson, AZ

22 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Staining and Identification of Pigments and Minerals in Tissue**
Speaker: Debra Wood, MS, HT(ASCP)
Indiana University School of Medicine
Indianapolis, IN
Phone: (443) 535-4060
Email: histo@nsh.org

AUGUST

21 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Microwave Technology: When, Where, Why and How**
Speaker: Donna Willis, HTL(ASCP)HT
Milestone Medical
Grand Prairie, TX

26 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Role of Immunohistochemistry in the Diagnosis of Lymphoma**
Speaker: Ann Marie Blenc, MD
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060
Email: histo@nsh.org

SEPTEMBER

18 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Small Specimen Management in a Large Volume World**
Speaker: Herbert Skip Brown, HT(ASCP)
Leica Biosystems LLC
St. Louis, MO

OCTOBER

3-8 **National Society for Histotechnology Symposium/Convention**
Site: Birmingham, AL
Contact: Aubrey Wanner, NSH Office
Phone: (443) 535-4060
Fax: (443) 535-4055
Email: Aubrey@nsh.org

16 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Histology Process Improvement: Defect/Error Tracking**
Speaker: William DeSalvo, BS, HTL(ASCP)
Sonora Quest Laboratories
Gilbert, AZ

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Getting Through CAP Inspections**
Speaker: Christine 'Charlie' Dorner
Celerus Diagnostics
Flippin, AR
Phone: (443) 535-4060
Email: histo@nsh.org

NOVEMBER

18 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Muscle Case Studies**
Speaker: Jon D. Wilson, MD
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060
Email: histo@nsh.org

20 **Teleconference Network of Texas/UT Health Sciences Ctr**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Process Improvement: A Beginner's Guide**
Speaker: Tim Webster
Seacoast Pathology, Inc.
Exeter, NH

DECEMBER

16 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Handling the Big "C's" in Histology**
Speaker: Maureen Doran, HTL(ASCP)
SIU School of Medicine
Carbondale, IL
Phone: (443) 535-4060
Email: histo@nsh.org

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Vinnie Della Speranza, ***HistoLogic***® Editor, 165 Ashley Avenue, Suite 309, Charleston, SC 29425. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.

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