The Importance of Water Quality
in the Histology Laboratory

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Water is ubiquitous in histology laboratories. 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 ultraviolet (UV) lamp was used. The purified water obtained was used to prepare reagents and rinsing solutions for the common hematoxylin and eosin (H&E) stain, as well as for Grocott's Methenamine Silver (GMS) stain. The results obtained were all satisfactory, including those for the silver staining, which is known to be very sensitive to water quality. In conclusion, water purified with a combination of reverse osmosis (RO), electrodeionization and ultraviolet light is suitable for a wide array of histology experiments.

Introduction

Histology laboratories use water in various ways. Not only is it the main component of many of the reagents prepared in the laboratory (buffers, stains, rinsing solutions), but it is also used for tissue floatation baths, tissue processors and water baths. However, water purity is often taken for granted, and its potential impact on experimental outcomes overlooked.

Water contaminants

Aside from water molecules, tap water may contain many compounds:

- Inorganic ions: chlorides, nitrates, sulfates, sodium, calcium, iron, etc.
- Organic molecules: humic acids, phenols, tannins, pesticide residues, etc.
- Particles and colloids
- Microorganisms and their by-products
- Dissolved gases

Since water is the main component in many of the reagents prepared in the histology laboratory, these compounds may interfere with the quality of the final slides. For example, tap water may contain particulate material that may adhere to the tissue sections. If the water used in water baths and tissue floatation baths contains organic molecules, bacteria can proliferate rapidly by using these molecules as nutrients. These bacteria can then generate artifacts on slides.

Water hardness and silica may form deposits inside the lines of stainers and tissue processors and alter their functioning. These water contaminants may also be deposited on tissue and slides. If the water contains large amounts of organic molecules and other nutrients, bacteria and molds may grow inside stainer and tissue processor tubings and also may generate artifacts on slides. While all the steps of the histological process can be affected by water contaminants, the most sensitive step is undoubtedly the staining.

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Stain sensivity to water quality

The quality of the water used in preparing solutions used in staining may impact both the reagent quality and the staining process. Poor quality water may cause the precipitation of the stain (as in the case of silver stains), or may reduce its chemical stability. Staining reactions are based on a variety of basic chemical reactions, such as acid-base chemistry or oxidoreduction. Chemical contaminants that can interfere with these reactions may therefore alter the quality of the staining. The final staining may be lighter, or conversely, there may be a higher background or non-specific binding. The wrong color or specificity may also be obtained. This can be observed, for example, with stains that are highly sensitive to pH, such as alcian blue or trichrome. Ions, such as calcium and metals, are known to interfere with a variety of stains. Chlorine also can have a bleaching effect on various types of stains.

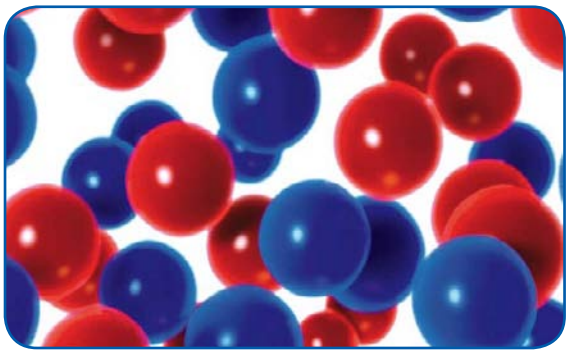
Many different stains are used in histology, and each has unique properties. Unfortunately, there is no precise description in the literature of the specific contaminants that may interfere with each and every type of stain. Therefore, most histotechnologists rely on serendipity and experience when troubleshooting staining problems. Using purified water can reliably reduce the risks of water contaminants interfering with the staining process. Most textbooks and published procedures recommend preparing the reagents and doing the rinsing steps in purified water.[1]

Water purification

Depending on the season and the location, tap water composition and pH may vary. However, in the laboratory it is important to obtain reproducible and reliable results. Using purified water from a reliable source not only reduces the risk of contaminant interference with the quality of the final slides, but also reduces experimental variability. Although deionized and distilled water are usually recommended in the literature as if they were interchangeable, the properties of these two types of purified water may be dissimilar, and can therefore affect histology procedures differently. Today, histotechnologists may also choose more sophisticated water purification technologies that together are capable of providing water of greater purity than distilled or deionized water.

Deionization

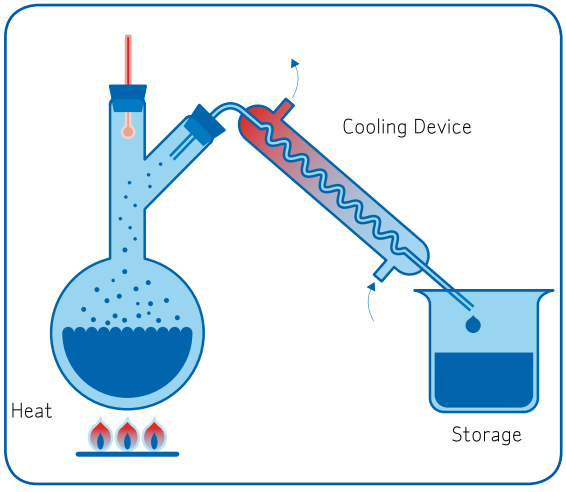
The deionization process removes ions from water via ion-exchange resins. Although this process is very efficient at removing charged molecules, neutral organic molecules are not removed. As a result, deionized water may be free of ions but may contain some organic contaminants. Exhausted resins can be regenerated, but repeated resin regeneration cycles lead to broken resin beads and may release particles and organic molecules into the water. In addition, resins are prone to bacterial growth.



Ion-exchange resin beads

Distillation

Distillation removes a wide range of contaminants. The water contained in a boiler is heated, the vapor produced is condensed and the purified water is collected in a receiving flask. This process 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. However, a number of organic molecules may be carried along with the water vapor, or may co-distill with water. In addition, the recipient vessel may contribute to re-contaminate the distilled water. Finally, maintaining a distillation apparatus may be cumbersome, especially in hard water areas.



Distillation apparatus

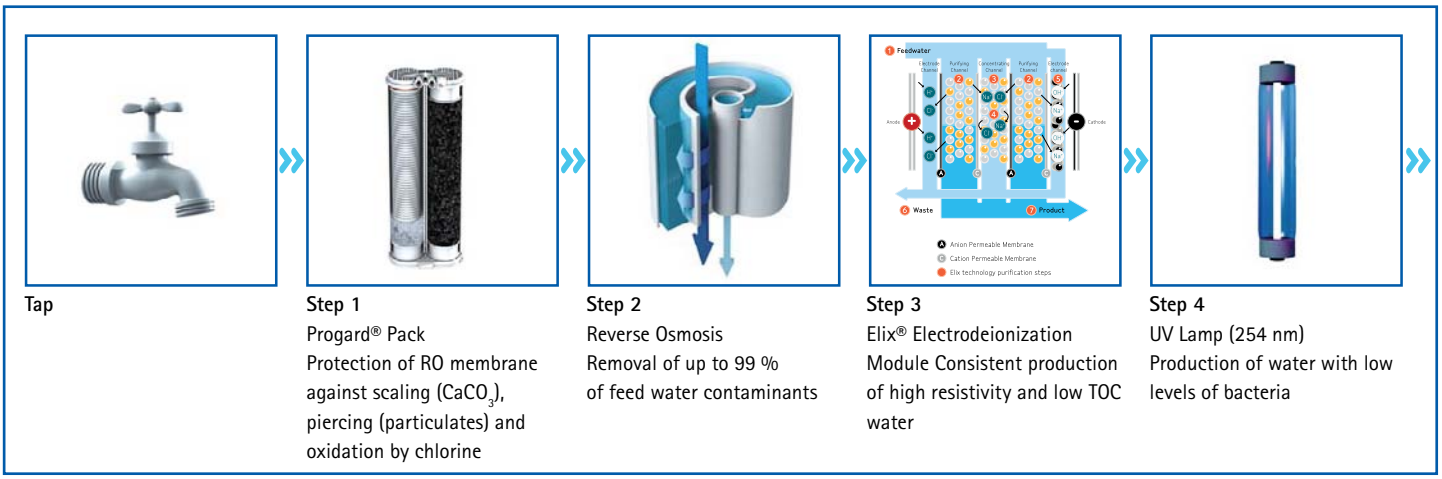


Figure 1. Schematic representation of the purification technologies included in the Elix® UV water purification system

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 has advantages over distillation or resin deionization: the various technologies are complementary and remove a wider range of water contaminants than distillation or deionization alone. Reverse osmosis is a membranebased technology that removes a large spectrum of contaminants. Electrodeionization is a self-regenerating process and is comprised of ion-exchange resins, semi-permeable membranes and an electrical current. Ultraviolet light at 254 nm inactivates bacteria. Elix® UV water purification systems combine these three purification technologies and typically deliver water with low levels of organic contaminants (Total Organic Carbon, or TOC, < 30 ppb in-line), low levels of ions (resistivity > 5 MΩ·cm) and low levels of bacteria.

Pratical illustrations

We performed a study to assess the suitability of water purified with the Elix® UV water purification system when performing commonly used staining procedures, and to investigate which common water contaminants might have an effect on these procedures.

Hematoxylin and Eosin (H&E) stain

The H&E stain is the fundamental stain for all pathology tissue samples. Hematoxylin stains cell nuclei in blue; eosin stains the cytoplasm and most connective tissue various shades of red, pink or orange. After the differentiation step, the slides are placed in a dilute alkaline "bluing" solution in order to cause the nucleus to turn a deep purplish blue color. Tap water may be used in areas where it is slightly basic, but the time needed for bluing may vary. In areas where tap water is acidic or fluctuates, and to facilitate a faster and more reliable bluing step, it is better to use a mildly basic solution. Tap water also may contain elements that can destain hematoxylin, such as iron (which acts as a mordant), chlorine (which bleaches the stain) and sulfur (which can influence the pH).[2]

The H&E staining was performed using a Leica Multistainer™ Workstation (Leica Microsystems, Bannockburn, IL). A regressive staining was performed. This stain procedure almost always employs running tap water for the rinse steps performed before and after the hematoxylin, differentiation and bluing steps. In the present study, all the rinsing steps, as well as the preparation of the bluing solution (Scott's tap water substitute, Surgipath Medical Industries, Richmond, IL), were performed entirely with either tap water or water purified using an Elix® 5 UV system (EMD Millipore Corporation, Billerica, MA).

Figure 2. Photomicrographs of tonsil tissue stained with H&E. Rinsing steps and Scott's tap water substitute prepared with tap water (A) or Elix® purified water (B). *Pictures courtesy of E. Macrea and W. Lange.*

Tap water is commonly used for the rinsing and bluing steps of the H&E staining, and the manufacturer of Scott's tap water substitute recommends preparing the reagent with tap water. However, tap water quality may vary and at times negatively affect the staining. When substituting tap water for Elix® purified water, the quality of the H&E staining was comparable to that of the staining done with tap water (Figure 2). Using purified water minimizes the risk of contaminant interference with the staining and provides consistency without compromising staining quality.

Grocott's Methenamine Silver (GMS) stain

Silver stains are well known for their sensitivity to water contaminants. The GMS stain is commonly used to demonstrate the presence of fungus, yeast and Pneumocystis. Most fungi are fairly large and their cell walls are rich in polysaccharides. The GMS stain uses chromic acid to oxidize the polysaccharides to form aldehydes, which can then react with silver ions.

Sections of lung tissue infected with Pneumocystis carinii (Pneumocystis jiroveci) were mounted on Superfrost® slides (Erie Scientific Co., Portsmouth, NH), and a commercial GMS kit was used (Grocott's Method for Fungi, Poly Scientific R&D Corp, Bay Shore, NY). Solutions of methenamine silver nitrate were prepared with Elix® purified water, distilled water, or deionized water.

Water purified with the Elix® system (C) gave satisfactory results: good staining of P. carinii and no background staining (Figure 3). Distilled water (D) lightened the staining of the organisms, but did not contribute to background staining. Deionized water (E) appeared to intensify background staining as well as staining of P. carinii. Water purified with an Elix® system can therefore be substituted for the distilled or deionized water called for in the GMS stain procedure.

Additional methenamine silver nitrate solutions were prepared with Elix® water and one of the following contaminants in order to assess their impact on staining: potassium chromium sulfate (1 mg/l, or ppm), cupric sulfate (1 ppm), nickel sulfate (1 ppm), ferrous sulfate (1 ppm), sodium hypochlorite (4 ppm), sodium triphosphate (100 ppm), sodium silicate (10 ppm), endotoxin (1000 EU/ml), humic acid (1 ppm). The remainder of the procedure was the same for all slides. All slides were prepared in duplicate and were evaluated using identical lots of all applicable reagents for each set of slides. Humic acid solution was obtained from Biocontrol Network (Brentwood, TN). All other supplies were purchased from Sigma-Aldrich (St. Louis, MS), and were of the highest purity available.

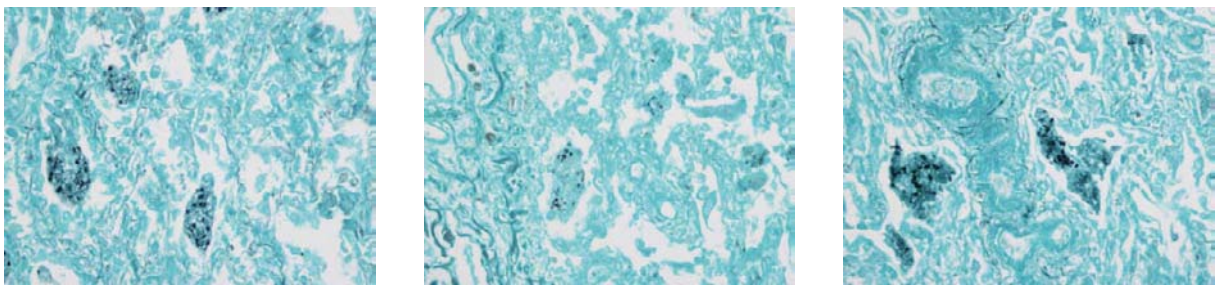


Figure 3. Photomicrographs of GMS staining using solutions of methenamine silver nitrate prepared with Elix® purified water (C), distilled water (D), or deionized water (E). *Pictures courtesy of E. Macrea and W. Lange.*

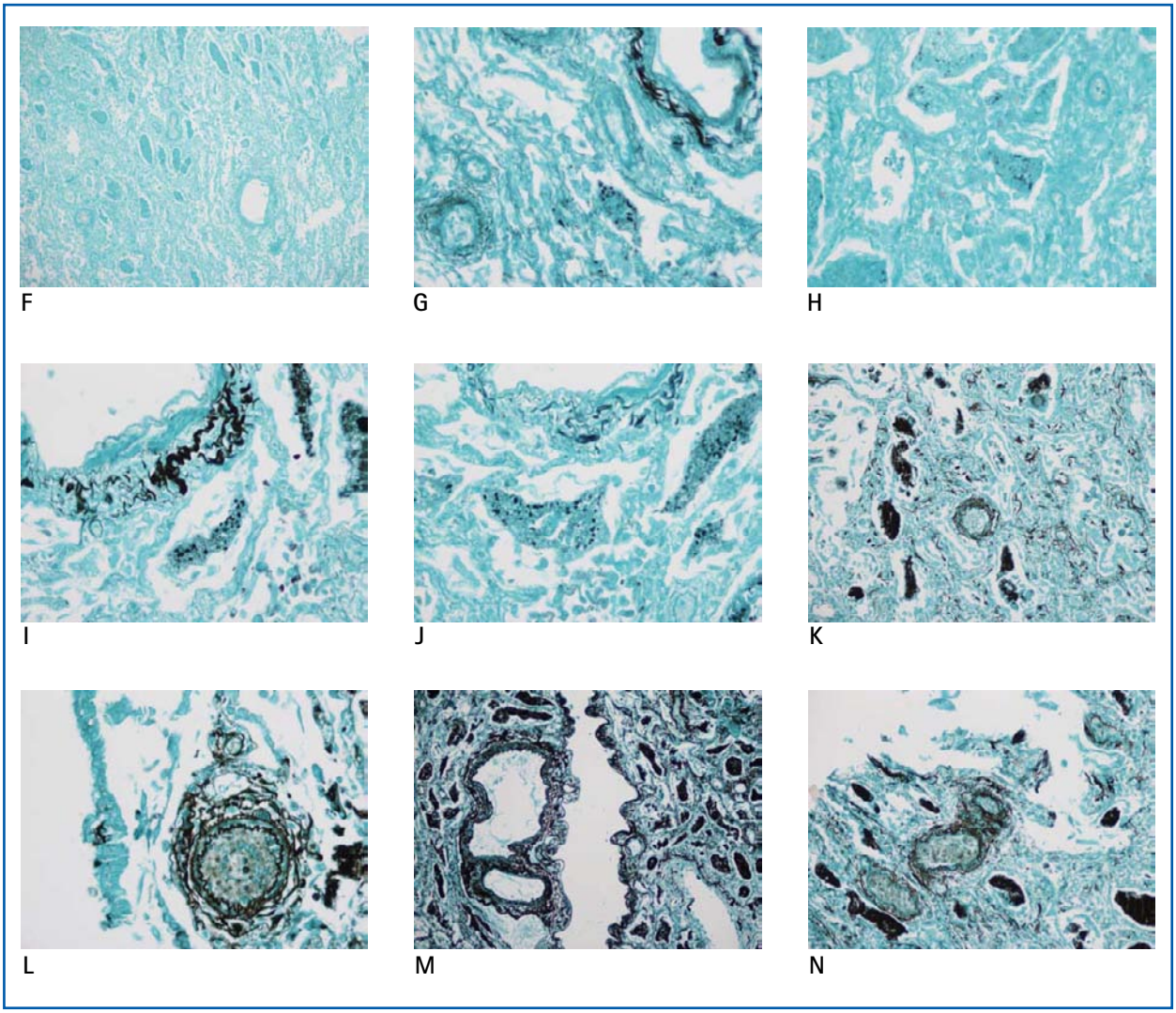


Figure 4. Photomicrographs of GMS staining using solutions of methenamine silver nitrate prepared with Elix® purified water and one of the following contaminants: potassium chromium sulfate (F), cupric sulfate (G), nickel sulfate (H), ferrous sulfate (I), sodium hypochlorite (J), sodium triphosphate (K), sodium silicate (L), endotoxin (M), humic acid (N). *Pictures courtesy of E. Macrea and W. Lange.*

All four of the metal sulfates tested reduced the intensity of the P. carnii staining, when compared to solutions prepared with Elix® purified water (Figure 4). Copper and iron also led to the staining of elastic fibers. Sodium triphosphate, sodium silicate and endotoxins suppressed the P. carnii staining and led to excessive background staining. Humic acid reduced the intensity of the P. carnii staining, and led to excessive background staining of the elastic fibers, reticulum and collagen.

Conclusion

Water purified with a combination of reverse osmosis, electrodeionization and ultraviolet light 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 place of distilled or deionized water and gives similar results, while reducing the risk of interferences due to water contaminants. Purified water produced by Elix® systems can be used throughout the laboratory. These water purification systems are easy to use and maintain, and they consume significantly less energy and water than conventional distillation equipment. In addition, the systems' UV lamp minimizes the risk of bacterial contamination of the water from bacterial byproducts, such as endotoxins and alkaline phosphatase,[3] which can lead to high background staining. Using a water purification system provides constant and reliable water quality, which is important at a time when an increasing number of histological procedures are automated and quality management systems are being implemented in histology laboratories.[1]

References

1. *Theory and Practice of Histological Techniques*, John D. Bancroft and Marilyn Gamble, Churchill Livingstone Elsevier, 6th edition, 2008.

2. NSH guidelines for H&E staining (1973), http://www.nsh.org/PDF/Guidelines_For_Hematoxylin_ and_Eosin_Staining.pdf

3. Bole J. and Mabic S., "Utilizing ultrafiltration to remove alkaline phosphatase from clinical analyzer water," *Clin Chem Lab Med, 44 (2006) 603–608.*