

# Water for Histology Staining

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## Abstract

Water purified with a combination of reverse osmosis, electrodeionization, and ultraviolet light was used to prepare reagents and rinsing solutions for the hematoxylin and eosin (H&E) stain, as well as a special stain, Grocott's Methenamine Silver (GMS), known to be very sensitive to contaminants, and gave reliable and high quality results.

## Key words or phrases

Pure water, distilled water, deionized water, histology, pathology, hematoxylin and eosin, H&E, special stains, Grocott's Methenamine Silver stain, GMS

## Introduction

Histology is an important tool for pathologists. It plays a key role in the diagnosis and monitoring of cancer and other diseases, as well as in the detection and identification of pathogens. Water is the main component of many histology reagents and buffers<sup>1</sup>, and is also used for rinsing and in floatation baths. Poor quality water may alter the quality of staining by generating artifacts, affecting color intensity or specificity, or reducing the chemical stability of dyes.<sup>2</sup> Tap water composition may vary from one season to another (its pH may change, as well as the ions and organics it contains), which may have an impact on the reproducibility of results. Deionized water and distilled water, whether purchased in bottles or produced in-house from tap water, may also vary in quality, and may degrade with time. Today, histotechnologists can rely on more sophisticated water purification technologies delivering reagent water of constant and reliable quality.

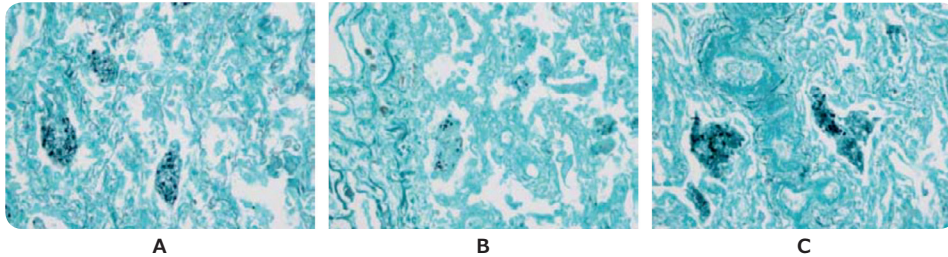


## Results and discussion

In the present study, we tested whether water purified with an Elix® purification system combining reverse osmosis, electrodeionization, and ultraviolet light, could be successfully used for histology staining.

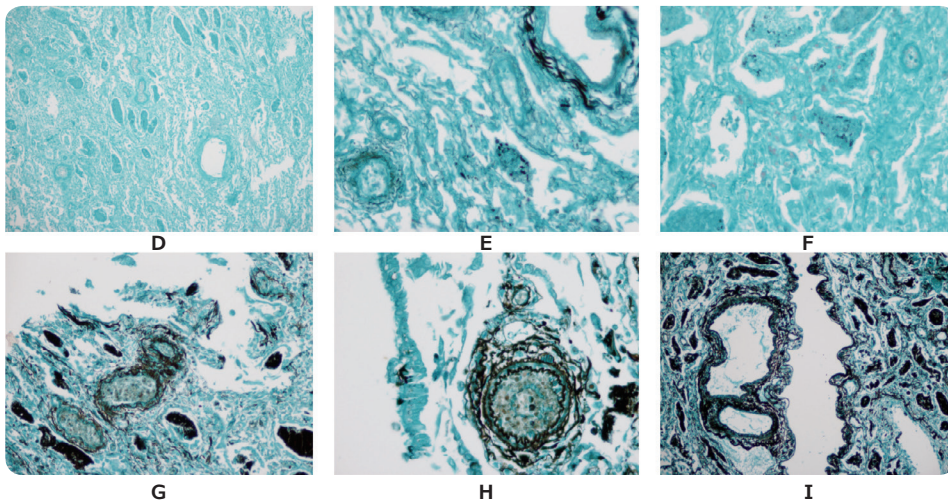
### 1. Special Stains

Some special stains, such as silver stains, are well known for their sensitivity to water contaminants.<sup>3</sup> Grocott's Methenamine Silver (GMS) staining of *Pneumocystis jirovecii* in lung tissue was used as proof of principle. High quality results were obtained, without any background staining, when water from an Elix® system was used to prepare reagents (**Figure 1**). Water purified with an Elix® system can therefore be substituted for the distilled or deionized water called for in silver staining procedures.



**Figure 1.** Photomicrographs of lung tissue with *Pneumocystis jirovecii*, stained with GMS stain. Methenamine silver nitrate solutions were prepared with (A) Elix® purified water, (B) distilled water, or (C) deionized water. Pictures courtesy of E. Macrea and W. Lange.

**Figure 2** illustrates how water impurities such as metals, organic molecules (humic acid, commonly found in tap water), silica, and bacteria by-products (endotoxins) affect GMS staining. For optimal results, it is therefore recommended to use pure water free of these impurities.

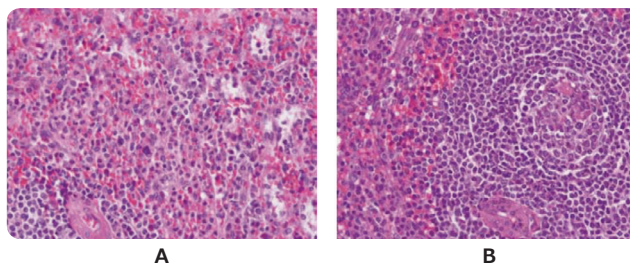


**Figure 2.** Methenamine silver nitrate solutions were prepared with Elix® purified water and added contaminants: (D) chromium, (E) copper, (F) nickel, (G) humic acid, (H) silica, (I) endotoxin. Pictures courtesy of E. Macrea and W. Lange.

### 2. Hematoxylin and Eosin Stain

H&E stain is probably the most widely used stain in histology laboratories. Tap water may not give consistent bluing, due to fluctuations in its pH, or may contain contaminants that can destain hematoxylin (iron, sulfur, or chlorine).<sup>4</sup>

Elix® purified water was substituted for tap water for all the rinsing steps, as well as for preparing the bluing reagent, and gave equivalent staining specificity and color (**Figure 3**).



**Figure 3.** Photomicrographs of tonsil tissue stained with H&E. Rinsing steps and Scott's tap water substitute prepared with (A) Elix® purified water or (B) Tap water. Pictures courtesy of E. Macrea and W. Lange.

## Experimental section

### Grocott's Methenamine Silver (GMS) Stain

A commercial GMS kit was used. In a 1<sup>st</sup> experiment, solutions of methenamine silver nitrate were prepared with distilled water, deionized water, or water from an Elix<sup>®</sup> system. In a 2<sup>nd</sup> experiment, solutions were prepared with Elix<sup>®</sup> water spiked with some common water contaminants (metals: 1 mg/L of potassium chromium sulfate, cupric sulfate or nickel sulfate; organic molecules: 1 mg/L of humic acid; 10 ppm of sodium silicate; or bacteria by-products: 1000 EU/mL of endotoxin).

### Hematoxylin and Eosin (H&E) Stain

The H&E staining 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), were performed entirely with either tap water or water from an Elix<sup>®</sup> water purification system.

## Conclusion

The techniques used in modern biomedical laboratories rely on high quality reagents in order to yield reliable and reproducible results. Water may contain contaminants that interfere with histology results: ions and metals, which may affect pH and interact with dyes; organics and endotoxins, which may affect the staining process; as well as bacteria and particles, which may deposit on slides and create artifacts. The combination of several water purification technologies (reverse osmosis, electrodeionization, and ultraviolet light) yields pure water of consistent and reliable quality that is suitable for a wide array of stains, from commonly used and robust ones, such as H&E staining, to more delicate ones, such as silver staining. This water can be used whenever distilled or deionized water is called for in histology protocols, and reliably gives high quality results, while reducing the risk of interferences due to water contaminants. Finally, a water purification system with online water quality monitoring as well as an embedded e-solution<sup>5</sup> data management tool, ensuring data reliability and traceability, is a great asset for laboratories seeking accreditation to standards such as ISO<sup>®</sup> 15189: 2012.<sup>6</sup>



For more information about hematology and histology, please visit <http://www.sigmaaldrich.com/life-science/cell-biology/hematology-and-histology.html>

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6. ISO<sup>®</sup> 15189: 2012 Medical laboratories – Requirements for quality and competence, <http://www.iso.org>.

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