silver (GMS) stain is probably the most widely used fungal stain, however, the somewhat capricious nature of silver staining may challenge even the most experienced of histology practitioners. Insufficient silver impregnation, overstaining that results in high background staining, or undesirable precipitate may all contribute to difficulties in finding stained fungal organisms. Proper determination of staining endpoint with a microscope, in many instances, is essential to a successful outcome.

As a result, some pathologists will routinely order a periodic acid-Schiff (PAS) stain in the hope of attaining a rapid and more foolproof stain. This method is among the most frequently employed techniques in histology laboratories. It is relatively simple to perform and reliable, even in inexperienced hands. The PAS stain will reliably demonstrate the polysaccharide-laden wall of most fungal organisms, with the exception of *Histoplasma capsulatum*.

A Common Mistake When Staining for Fungi

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Fungal stains remain an important tool in the histology laboratory’s diagnostic arsenal for identifying infectious microorganisms. Dermatomycoses, or superficial fungal infections involving the skin, are rarely a serious health problem in man; however, systemic mycoses with such organisms as *Cryptococcus neoformans* and *Candida albicans* can cause life-threatening infections, especially among those who are immune suppressed. Therefore, fungal stains are often ordered with some urgency by the surgical pathologist when hints of fungal infection are observed with the H&E stain.

Most fungi are fairly large and their walls are rich in polysaccharides (1,2-glycol groups), which can be demonstrated with Schiff’s reagent or hexamine (methenamine) silver solutions. Both techniques rely on the use of an oxidizer (periodic acid or chromic acid) to create aldehyde binding sites for the Schiff or silver ions. The Grocott’s methenamine silver (GMS) stain is probably the most widely used fungal stain, however, the somewhat capricious nature of silver staining may challenge even the most experienced of histology practitioners. Insufficient silver impregnation, overstaining that results in high background staining, or undesirable precipitate may all contribute to difficulties in finding stained fungal organisms. Proper determination of staining endpoint with a microscope, in many instances, is essential to a successful outcome.

As a result, some pathologists will routinely order a periodic acid-Schiff (PAS) stain in the hope of attaining a rapid and more foolproof stain. This method is among the most frequently employed techniques in histology laboratories. It is relatively simple to perform and reliable, even in inexperienced hands. The PAS stain will reliably demonstrate the polysaccharide-laden wall of most fungal organisms, with the exception of *Histoplasma capsulatum*.2

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One drawback to employing the PAS for fungal staining, however, is that it will stain polysaccharides wherever they appear in tissues. This method will stain many intracellular components in various types of normal cells, from a wide variety of animal species. Some of these intracellular components are listed below:

- glycogen
- neutral mucosubstances
- some epithelial sulfomucins and sialomucins
- colloid of the thyroid
- basement membranes
- reticular fibers
- paneth cell granules
- adrenal chromaffin cells
- neuronal glycolipids
- intranuclear inclusions in vas deferens
- afibrillar cells of arterioles of kidney
- granules within reticuloendothelial cells
- salivary glands
- gastric and Brunner’s glands
- mucin from respiratory tract
- acinar and endocrine glands, including the prostate in man

Staining of these elements in some tissues may be distracting when trying to find PAS-stained fungal organisms. As a result, pathologists who utilize the PAS for fungal staining will often request the stain with diastase digestion in an attempt to minimize staining structures of little or no interest.

Interestingly, few histology practitioners question the use of the oxidizer periodic acid in the PAS stain, yet we use the oxidizer chromic acid in the GMS stain for fungi. In fact, if we were to substitute periodic acid for chromic acid in the GMS stain, essentially we will have performed the Jones’ method, which specifically stains basement membranes and reticular fibers.

Yes, the Jones’ technique can demonstrate fungi; however, it will also stain many other structures, making the discovery of fungal organisms much more difficult. Whether we are using Schiff or silver stain, the weaker periodic acid oxidizer creates aldehyde binding sites in many more tissue structures. In this example, we can see how the selection of an oxidizing agent can significantly determine which structures will be stained. This choice will maximize the probability of achieving the desired results.

These observations were actually quite well known a generation or two ago. However, this older, yet still important information can sometimes be overlooked. Modern histology practitioners can use this information today to guide surgical pathologists in selecting the most appropriate staining method available.

Bauer (1933) is credited with the earliest report that the oxidation of sugars (1,2-glycols) with chromic acid creates aldehydes that will bind the Schiff molecule. Many others, including Hotchkiss, McManus (1960), Spicer, Pearse, and Lillie continued to study the aldehyde-Schiff reaction using various oxidizers. It became well understood that the strength of the oxidizer, the duration of exposure, and the density of sugars in the tissue structures of interest determine the location where the Schiff molecule will bind.

Both chromic acid and potassium permanganate, which are stronger oxidizers than periodic acid, also produce aldehydes in polysaccharides, but they further attack and destroy those aldehydes if given sufficient time. “Because of the further oxidation of aldehyde by chromic acid, less density of aldehyde reaction is achieved and structures with fewer glycol groups are not demonstrated, such as basement membranes and collagenous and reticular fibers.”

This statement by Lillie tells us why chromic acid is the better oxidizer when staining fungi, since these organisms have a greater density of sugar (polysaccharides) in their cell wall.

While chromic acid continues further oxidation of all polysaccharides, complete conversion in the areas of heaviest concentration takes longer, allowing these to remain reactive with Schiff (or silver). Since the walls of fungi are particularly rich in 1,2-glycol groups, they stain more intensely than most other reactive components in the tissue section. The contrast between fungi and background is often more distinct than what is seen in duplicate sections stained by the PAS reaction (see Figs. 1 and 2). This discussion suggests that if fungi are present in your tissue section, they are less likely to be missed when a stronger oxidizer like chromic acid is used.

Work in our laboratory has convinced us that using chromic acid as oxidizer, followed by Schiff, yields results superior to PAS for fungal staining, as background staining is greatly decreased. This approach is as simple as doing a typical PAS, yet avoids the need to perform a diastase digestion, which adds unnecessary work and expense to fungal staining. The method reported by Casella utilizing potassium permanganate as oxidizer is another suitable alternative to periodic acid and yields similar results to chromic acid when staining fungi.

Interestingly, chromic acid-Schiff (CAS) is remarkably similar to the method by Gridley that can be
found in many histology texts except that Gridley’s technique also requires the use of aldehyde fuchsin following Schiff. This is done to achieve more intense staining. The aldehyde fuchsin acts as an aldehyde and occupies uninvolved linkages of the Schiff reagent, thus reinforcing the depth of the stain. While we have confirmed that aldehyde fuchsin does in fact intensify the stained organisms, we speculate that the Gridley method is not widely used today because the aldehyde fuchsin stain requires the use of paraldehyde, a controlled substance that may be problematic to obtain in some laboratories.

In conclusion, the next time your pathologist requests a PAS for fungus, we recommend that you suggest the CAS as a much better alternative. It makes little sense to utilize an oxidizer that will create aldehydes in undesirable locations only to have to use an enzyme to try to eliminate them later. Better to avoid creating them in the first place. A simple demonstration with parallel stained sections, one stained with PAS and the other with CAS, will convince even the most skeptical observer.

References

Fatty Tissue Fixation Using Microwave Technology

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Abstract
The microwave oven has been in use in the histology laboratory for many years. In recent years, laboratory-grade microwave instruments have become more widely adopted as standard equipment in many laboratories. These microwave units may be used to accelerate a number of laboratory procedures, including fixation, processing, decalcification, special staining, and immunohistochemical (IHC) staining.

Our histology laboratory has been using microwave technology for special stain procedures and antigen retrieval techniques in immunostaining procedures. Most recently, we investigated the use of the microwave to aid in fixation of fatty tissue specimens. These tissue specimens often do not process well during routine tissue processing. Tissue thickness and fat content are two main factors causing the problem of underprocessed tissue.

Introduction
Diagnostically, the most critical fatty tissues are breast and lymph node specimens. Any delay in processing these specimens may affect patient care. We are continually faced with inadequate processing of fatty tissues, most often due to both the thickness of the sections when grossed in and their fat content. The use of alcoholic formalin in preprocessing and processing, often recommended as a solution to this problem, is not an option for some immunohistochemical procedures, especially those typically used for breast samples. We have found that formalin fixation done in a microwave oven offers a suitable alternative when preparing fatty samples.

Materials and Methods
For this study we collected a limited number of samples (n=20) of breast tissue from mastectomies and breast reductions. Parallel sections of these tissues were cut in at the grossing table at three thicknesses—approximately 3 mm, 5 mm, and 6 mm—and placed into plastic tissue cassettes.

Fig. 1. Routinely fixed and processed breast tissue stained with H&E. 200X
The cassettes were then placed and held in 10% formalin (Val Tech Diagnostics, Brackenridge, PA). One group of cassettes was processed routinely using a Tissue-Tek® VIP 3000 processor (Sakura Finetek, Inc., Torrance, CA), while parallel blocks were fixed in a MicroMED T/T™ microwave oven (Hacker Instruments, Winsboro, SC) followed by routine processing in the VIP 3000 processor. The cassettes to be microwaved were placed into the MicroMED T/T cassette holder in a large (400 ml) glass container that was filled with 10% formalin. A program for fixation was created that allowed for a 5 minute ramp-up of temperature of the formalin to 60°C, and this temperature was maintained with microwave stimulation for 55 minutes. The microwaved tissues were then transferred to the routine processor.

Tissue blocks were routinely embedded in paraffin and sectioned. Each sample was stained with H&E (Richard Allan Scientific, Kalamazoo, MI) and Masson’s trichrome (Poly Scientific R&D Corp., Bayshore, NY). In addition, IHC was performed for estrogen receptors (ER), progesterone receptors (PR), and HER-2/neu (Hercep Test, DakoCytomation, Carpinteria, CA).

Results
A preliminary study of 20 patient samples was conducted. These specimens were all extremely fatty tissues. Tissues of 3 mm thickness or less consistently processed well after microwave fixation, requiring no additional processing, while routinely fixed 3 mm sections did not consistently process well in the routine processor. Many required reprocessing. The tissues of greatest thickness, approximately 6 mm, did not process well, even after microwave fixation. The tissues of intermediate thickness, approximately 5 mm, were inconsistent in processing, irrespective of the method employed for fixation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>H&amp;E</th>
<th>Trichrome</th>
<th>ER</th>
<th>PR</th>
<th>HER-2/neu</th>
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</thead>
<tbody>
<tr>
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<td>Excellent*</td>
<td>Excellent*</td>
<td>Negative*</td>
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<tr>
<td>#1 MW</td>
<td>Excellent</td>
<td>Very Good</td>
<td>Excellent</td>
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<tr>
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<td>Very Good</td>
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<td>Negative</td>
</tr>
<tr>
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<td>Very Good</td>
<td>Very Good</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>#3</td>
<td>Excellent</td>
<td>Very Good</td>
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<td>Excellent*</td>
<td>Negative*</td>
</tr>
<tr>
<td>#3 MW</td>
<td>Excellent</td>
<td>Very Good</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Background staining observed. †Crisp nuclear detail. MW = microwave fixed tissue.
We chose 3 samples to evaluate staining consistency when comparing tissues fixed in the microwave to those fixed routinely. H&E and trichrome stains, as well as three immunostains, were compared for each (Table 1). With respect to nuclear detail, the microwave fixed tissue showed superior staining with H&E (Figs. 1 and 2). In many cases it was necessary to cut the routinely fixed tissue thicker in order to obtain a better section, due to the tissue’s fat content. Overall, microwave fixed tissue could be sectioned thinner, thereby yielding a higher quality sample to view microscopically. The trichrome stain displayed similar results, however, the nuclear staining appeared slightly paler but crisper in the microwave fixed tissue (Figs. 3 and 4).

Immunostaining analysis of ER and PR receptors and HER-2/neu showed comparable staining (Figs. 5-8). Nonspecific background staining seen in the routinely fixed tissue was completely absent in the microwave fixed tissue. Only one sample showed very rare PR staining in the routinely fixed tissue but not in the microwave fixed tissue. This discrepancy may be attributable to sampling but warrants further study. None of the tissues studied was found to be positive for HER-2/neu, preventing us from evaluating the quality of immunostaining with this marker. Although the tissues were negative with this antibody, nonspecific background staining commonly seen in the routinely fixed tissues was virtually absent in the microwave fixed tissues.

**Conclusion**

Historically, we have found that routine fixation and processing of even the thinnest of fatty tissue sections frequently requires reprocessing in our laboratory. Even though the number of
specimens observed in this study is very small, we believe that formalin fixation of fatty tissue specimens processed using microwave technology, especially with tissues sectioned at a reasonable thickness, helps to prevent the need for reprocessing of these tissues.

Microwave fixation of fatty tissues does not appear to create any undesirable artifacts with H&E, trichrome, or immunohistochemical staining. On the contrary, it appears to help decrease nonspecific IHC background staining seen in some routinely prepared sections and improve the nuclear detail of the cells overall.

References
marker Ki-67. Ki-67 is a nuclear protein that is expressed in all active phases of the cell cycle (G₁, S, G₂, and M-phases) but is absent in resting cells (G₀-phase).² The second section was stained by standard methods with hematoxylin and eosin (H&E). Proliferating cells were immunohistochemically stained with a monoclonal mouse anti-human Ki-67, clone MIB-1 (DakoCytomation, Carpinteria, CA). A pretreatment of heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0 was utilized prior to the primary antibody incubation. The primary antibody was incubated at 1:150 for 30 minutes at room temperature then visualized using EnVision+ mouse (DakoCytomation, Carpinteria, CA) followed by DAKO Liquid DAB+ (DakoCytomation, Carpinteria, CA). The sections were counterstained with hematoxylin and coverslipped prior to assessment.

Analysis Methods
Four different parameters were used to determine the proliferative effects of various treatments: overall thickness of the epithelium, basal cell layer thickness, number of basal cell nuclei, and number of Ki-67 positive nuclei. The H&E stained slides were used to measure the overall thickness of the specimens and the thickness of the basal layer. The outer edges of the specimen were not evaluated since these were not representative of the entire specimen. Specifically, we found the edges to be much thicker than the center of the specimens and the concentration of Ki-67 positive cells was much higher at the periphery than in the center of the specimen. We believe that this was primarily due to culture conditions. The thickness of the specimen was measured at 20X magnification on a Zeiss Axioskop 2 Plus microscope (Fig. 2A). Measurements were taken at three equidistant points along the length of each of the two specimens using an ocular micrometer (10 mm scale with 100 divisions, KR-207 Klarmann rulings), totaling six measurements per specimen (Fig. 3). The raw results of these measurements were then converted to microns by taking into account the magnification factor using...
the following equation: ocular micrometer measurement/objective used x 1000 = microns.

In a portion of the EpiGingival specimens there was an artificial separation of the keratinous layer that was due to processing. The separation was excluded from the measurement of the thickness (Fig. 2B). The thickness of the basal cell layer was measured at 40X magnification (Fig. 2A).

The number of Ki-67 positive nuclei and basal cell nuclei were counted at 20X magnification using the Ki-67 stained section. The entire specimen, excluding the outer edges, was used to count the Ki-67 positive nuclei. All cells that had any nuclear staining were counted as positive for Ki-67 (Fig. 4). This figure demonstrates the variable intensity of staining with this antibody. In some cells there is focal staining of the nucleus, whereas in other cells the entire nucleus is stained. This conforms to previous observations and reflects the short half-life of the protein. Each bisected portion was counted, so a total of two numbers were generated per specimen. The total number of nuclei in the basal cell layer was estimated by counting three representative lengths of 1 mm that were evenly spaced along the entire construct, totaling 6 counts per specimen (Fig. 3).

The data generated from the individual measurements and cell counts were entered into a Microsoft Excel spreadsheet to tabulate individual and group means (Fig. 5). Graphical representation of the data is shown in Fig. 6.

Results and Discussion
The appearance of EpiOral and EpiGingival cultures is shown in Fig. 1. The most notable difference between them is that one is non-keratinizing and the other is keratinizing. In Fig. 2B, the separation of the keratinized layer from the underlying epithelium can be seen. This separation was created during processing. The EpiGingival cultures were highly susceptible to this artifact. Great care must be taken in the handling of these specimens from grossing to embedding, to decrease the potential for this artifact. Figs. 7B and 7D show the increased numbers of cells that are positive for Ki-67 following application of an agent that induces proliferation, and Figs. 7A and 7C demonstrate epithelium that is relatively inactive. The cells that are positive for Ki-67 tend to be in the basal cell layer, as would be expected. There is an increased number of cells in the basal cell layer and increased thickness of the basal cell layer associated with the increased number of Ki-67 positive cells. In addition, the overall thickness of the epithelium was increased.

The results generated from the analysis of several groups of cultures under different conditions indicate that the parameters measured are capable of elucidating differences in the cultures caused by the agents being tested. In some cases, however, there was considerable variability between individual specimens (perhaps due to varying culture conditions) that made statistical analysis difficult. We recommend using groups of more than 5 specimens per group to avoid this potential problem.

Conclusion
Measurement of epithelial thickness, basal cell layer thickness, number of cells in the basal cell layer, and number of Ki-67 positive cells provides a robust method for assessment of proliferation in EpiOral and EpiGingival cultures.

References
Specimens A and C are normal, untreated, mature EpiOral and EpiGingival constructs. Specimens B and D are treated constructs, which demonstrate increases in Ki-67 positive cells, basal cell nuclei, basal cell layer thickness, and epithelial thickness.

<table>
<thead>
<tr>
<th>Group</th>
<th>Specimen Number</th>
<th>Section A - Total Thickness (µm)</th>
<th>Section B - Total Thickness (µm)</th>
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<tr>
<td></td>
<td>Specimen Number</td>
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<td>2</td>
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<tr>
<td>Group 1</td>
<td>A</td>
<td>105</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>130</td>
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</tr>
<tr>
<td></td>
<td>C</td>
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</tr>
<tr>
<td></td>
<td>MEAN</td>
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<td>118.33</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>8.33</td>
<td>3.33</td>
</tr>
</tbody>
</table>

Mean + SE Cell Counts

Mean + SE Thickness Measurements (µm)

Graph A

Graph B

Fig. 5. Example of a portion of the Excel spreadsheet.

Fig. 6. Graphical representation of the data generated.

Fig. 7. Specimens A and C are normal, untreated, mature EpiOral and EpiGingival constructs. Specimens B and D are treated constructs, which demonstrate increases in Ki-67 positive cells, basal cell nuclei, basal cell layer thickness, and epithelial thickness.
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Rapid Detection of Lipid in Livers for Transplantation

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Abstract
There is a substantial increase in the demand for organs available for transplantation due to the increased use of transplantation as a method to treat end-stage liver disease. Increasing the donor pool has resulted in the inclusion of both older donors and those donors who have some high-risk conditions. Retransplantation or death may result from transplanting a liver with 30% or greater fat content. As a result, donor livers with greater than 30% fat content are deemed unsuitable. Time is of the essence in transplanting harvested organs—a liver is only viable for 12-16 hours after removal from the donor. Therefore, a rapid method for the detection of lipid is necessary to determine the viability of donor livers.

Introduction
A core biopsy from a donor liver was brought to the frozen section room, sections cut, and slides given to the technician in the special stains lab. We had recently changed the oil red O procedure from one using isopropanol to another using propylene glycol. Even though the isopropanol method was quick (15 minutes), the results were often dirty. Droplets of the dye solution would be deposited on and around the tissue, making interpretation difficult. The lack of a differentiation step in the method probably accounted for this. Switching to the propylene glycol method allowed for some control of differentiation, producing a cleaner, crisper stain. However, the lengthy fixation of the tissue in 10% neutral buffered formalin specified in the method was a drawback for rapid diagnosis of liver steatosis. Formalin fixation is desired in order to fix the tissue proteins, which aids in the retention of lipids. It does not fix the lipid, but traps it while maintaining the architecture of the tissue. The total time for this staining procedure was 90 minutes. With a transplant surgeon and his transplant team pacing in the hallways, and an impatient pathologist and a resident waiting for this slide, it was obvious that adjustments had to be made. Some steps were shortened before the slides were given to the pathologist. The stain looked good—clean and crisp. Fig. 1 is the result of the stain performed on the liver allograft. As the photo depicts, the fat content is greater than 30%.

Fig. 1. Donor liver biopsy demonstrates fat content that is more than 30%. Oil red O stain was performed as described in this article. 400X.

Fig. 2. Donor liver biopsy, with no dye droplets to interfere with interpretation. Oil red O stain was performed using the propylene glycol method described below. 600X.
requirements for the solvent used in fat staining were suggested by Chiffelle and Putt (1951):

1. The solvent should not extract any lipids from the tissue section.
2. The solvent should dissolve sufficient amounts of pigment to yield brilliant staining results.
3. The stain should be easy, rapid, and economical to prepare, and stable in solution.

Of the two common solvents used for oil red O, propylene glycol is preferred because it removes no lipid, whereas minimal amounts of lipid are removed with isopropanol. Introduced by French in 1926, oil red O (Sudan red B, solvent red 27) is classified as one of the Sudan dyes, which have been in general use since the late 1800s. Degenerating material containing fat, such as cell membranes or myelin, may coalesce into fat droplets that can be seen with fat-detecting stains, differentiating between fine lipid droplets and fine glycogen droplets. Tumors with unusual morphology, such as liposarcomas, can be differentiated from other types of tumors (rhabdomyosarcomas) using the oil red O stain.\(^5\)

**Method**

**Purpose:** Oil red O stain can demonstrate neutral lipids in frozen tissue sections, determining the viability of donor livers, as well as find fat that appears in abnormal places, such as in the kidneys or brain. It is also useful in verifying fat embolism as a cause of death, as well as demonstrating fat emboli that may develop and enter the circulatory system after a bone fracture or a crushing injury in a fatty body area. Degenerating material containing fat, such as cell membranes or myelin, may coalesce into fat droplets that can be seen with fat-detecting stains, differentiating between fine lipid droplets and fine glycogen droplets. Tumors with unusual morphology, such as liposarcomas, can be differentiated from other types of tumors (rhabdomyosarcomas) using the oil red O stain.\(^5\)

**Principle:** The basis for staining lipids with an oil-soluble dye is its greater solubility in lipid substances than in the usual hydroalcoholic dye solvents. This is a physical method of staining. The dye must not be water soluble, must be strongly colored, and must act with tissue constituents only in solution. The solvent choice is critical.

**Fixation:** Air dry 2 frozen section slides. Place one in 10% neutral buffered formalin (NBF), in case there is an adhesion problem resulting in the tissue washing off the slide. Proceed with staining the second slide.

**Specimen:** To ensure the preservation of all lipids, freezing the tissue is preferred. Routine processing removes all but a few protein-bound lipids. Frozen sections are cut at 5 microns.

**Quality Control:** Previously cut frozen sections of fatty liver or skin are used as control slides.

**Precautions:**
- Wear suitable protective clothing.
- Lab coats, gloves, and safety glasses are required.
- Use propylene glycol under a fume hood.
- Gloves are necessary to avoid absorption of dyes into skin.
- Dispose of hazardous waste in appropriate receptacles.

**Solutions:**

**Mayer’s Hematoxylin**

0.5% Oil Red O

Oil red O powder ...............0.5 g
Propylene glycol ...............100.0 ml

Add a small amount of propylene glycol to the oil red O and mix well. Gradually add the remainder of the propylene glycol, stirring periodically. Heat gently until the solution reaches 95°C. (DO NOT ALLOW THE SOLUTION TO GO OVER 100°C.) Stir while heating. Filter through coarse filter paper while still warm. Allow to stand overnight at room temperature. Filter again before use.

**85% Propylene Glycol**

Propylene glycol ...............85.0 ml
Distilled water ...............15.0 ml

NOTE: Store the oil red O solution and 2 coplin jars of 85% propylene glycol in a 60°C oven.

**Staining Procedure:**

1. Dip briefly in distilled water.
2. Dip slides in undiluted propylene glycol.
3. Stain in oil red O solution for 1 minute at 60°C.
4. Differentiate by dipping twice in each of 2 changes of 85% propylene glycol at 60°C.
5. Rinse in 2 changes of distilled water.

---

Fig. 3. Donor liver biopsy showing dye droplets that make interpretation difficult. Oil red O stain used with propanol. 400X
6. Counterstain in Mayer’s hematoxylin for 30 seconds. Be sure to filter hematoxylin prior to use if not filtered daily.

7. Rinse in distilled water.
- **Do not use acid alcohol.**
- **Do not dehydrate through alcohols.**

8. Mount in an aqueous mounting medium.

**Results:**
- Fat …………………….. Intense red
- Nuclei ………………….. Blue

**Procedure Notes:**
1. Synthetic resinous media contain organic solvents that will dissolve lipids; therefore, the use of an aqueous mounting medium is vital. **Permanent aqueous mounting media are NOT suitable.**
2. Do not exert any pressure on the coverglass, as this may displace the fat. Do not try to press air bubbles out if they are present in the section, as the fat in the section is liquid and mobile. If necessary, remove the coverglass by soaking the slide in water.
3. Oil red O is more soluble in lipid than in propylene glycol; the 60°C oven keeps the solution warm enough to liquefy lipids that are solid at room temperature, thereby allowing the dye to enter the lipid more readily.

**Procedure Limitations:**
Simple lipids may be removed by any fixative or solution containing alcohol or organic solvents. As a result, sections that are paraffin embedded cannot be used. Only frozen sections may be used for this procedure.

**References**

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To B-5 or Not To B-5
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Abstract
Despite years of controversy due to hazards associated with its use, B-5 fixative has found wide acceptance as a fixative for hematopoietic (bone marrow) and lymphoreticular (lymph node) tissues because tissue fixed in this solution will demonstrate beautiful nuclear detail. The purpose of this study was to explore whether a safer alternative fixative with similar advantages could be used to replace B-5 in our laboratory. Using lymphoid tissue, sections were split and fixed in B-5, neutral buffered formalin (NBF), and several products marketed as B-5 substitutes. The specimens were routinely processed and then stained with hematoxylin and eosin (H&E). Immunohistochemical (IHC) staining with the markers CD30, BCL-2, and Cyclin D1 was also assessed. The slides were evaluated under blind study conditions by several pathologists for comparison.

Materials
AZF™ Fixative: Newcomer Supply, Middleton, WI

B-5 Stock Solution:
Mercuric chloride ...............133.4 g
Sodium acetate ............... 27.8 g
Distilled water ...............1400 ml

Dissolve mercuric chloride in distilled water while heating. After it is dissolved, add sodium acetate and mix. Filter the solution.

B-5 Working Solution: Add 3 ml of full-strength formalin to 30 ml of stock solution before use.

B-Plus™: BBC Biochemical, Stanwood, WA

B-5 fixative | Buffered zinc formalin | B-Plus
---|---|---

Fig. 1. Tonsil tissue, H&E. 400X. All comparable with good nuclear detail, clarity, and contrast.
Slides for IHC were stored overnight in a 37°C incubator. Prior to staining, the slides were placed in a 60°C oven for 30 minutes. Heat induced epitope retrieval (HIER) pretreatment was done using citrate buffer (pH 6.0) in a microwave pressure cooker as per our usual protocol for these markers (CD30, BCl-2, and Cyclin D1).

**Discussion**

The first written records noting the hazards of mercury date back to the first century AD. In the 1500s, the vapor hazards associated with mercury distillation prevented workers from working more than 1 month per year. The phrase “mad as a hatter,” linked to the madcap hatmaker in Lewis Carroll’s classic *Alice in Wonderland*, relates to the disease found in the hatmaking industry of the 19th century. A mercury solution used in the process of turning fur into felt to make hats produced toxic fumes, which the workers inhaled; this eventually led to accumulation of mercury in the workers’ bodies. The symptoms included trembling, loss of coordination, slurred speech, loosening of teeth, memory loss, depression, irritability, and anxiety. The mad hatter syndrome is still used today to describe the effects of mercury poisoning. We now know that mercury poses a serious environmental hazard, which will persist in a bioactive form that can contaminate the food chain. Fish in many of the nation’s waterways have been found to be contaminated with mercury. Today, there is considerable pressure from regulatory agencies to remove mercury from use in laboratories. This is to reduce the waste stream that could lead to environmental contamination, as well as to eliminate the hazards for laboratory staff. As a result, several manufacturers now offer substitute fixatives that are touted to provide the benefits of mercury fixation without the hazards associated with exposure to this heavy metal. However, resistance to eliminating mercury-based fixatives is due to the failure of many of these products to achieve the performance and results expected from fixatives such as B-5.
But now the American Hospital Association (AHA) is working with the Environmental Protection Agency (EPA) to get all hospitals to voluntarily agree to eliminate all mercury by 2005. The College of American Pathologists (CAP) has included a new standard in its accreditation checklist (Laboratory General Checklist) asking, “Does the laboratory have a written plan to reduce or eliminate mercury?”

Interestingly enough, the title of the article parodies the famous line from Shakespeare’s *Hamlet*, where the main character manages to feign, or not to feign, his own madness. Can we, after reviewing all the facts and knowing what mandates are in our future, make a sane decision to use B-5 or not to use B-5?

References

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On behalf of *HistoLogic*, I would like to express my appreciation to all of you who have supported this publication through the submission of manuscripts over the past 34 years. *HistoLogic* was created in 1971 in an attempt to keep histology practitioners around the globe connected to one another through the sharing of relevant, practical scientific and technical information. Since that time, it has been read worldwide by more than 1,000,000 individuals. It is my sincere hope that the information that has appeared within these pages during my editorship has been of benefit to you in your work.

We understand that many of our colleagues may not have the opportunity to further their professional development through attendance at symposia and workshops. For this reason, the appearance of sound scientific information in your mailbox becomes all the more important to aid you in keeping your theoretical knowledge complete and up to date. As a result, we at *HistoLogic* remain committed to providing you with the highest caliber publication possible.

Few things contribute more to the advancement of science and technology than the sharing of knowledge and ideas with our colleagues. Indeed, this communication is the very foundation upon which new ideas are stimulated and new developments and discoveries are built. I believe that as scientists we have a responsibility to share what we know. It matters not whether you publish your work in *HistoLogic*, the *Journal of Histotechnology*, or any other scientific publication, only that you do, in fact, share your knowledge. Knowledge shared in print will always remain one of the most effective tools for reaching large numbers (often tens of thousands) of individuals.

This is where you come in. If you have never considered submitting your work for publication, I urge you to consider the opportunity. I know that some may be daunted by the perception that it is very difficult to get one’s work published. *HistoLogic* is interested in any manuscript that is scientifically valid. I’ve conversed with some colleagues who believe that they don’t write well enough to successfully publish an article. However, as the scientific editor, I am available to assist you in any way possible. I am happy to help you construct your article, so please do not allow this concern to discourage you from coming forward with your ideas.

For those who are interested in submitting a manuscript to *HistoLogic*, I urge you to contact me directly at dellav@musc.edu, or you may correspond with me via the mailing address included on the back of this issue. I prefer that manuscripts be prepared using word processing software such as Microsoft® Word or Corel® WordPerfect.®

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Most important of all, please be sure to include the most expedient way for me to communicate with you. E-mail is preferred over postal mailing, but if e-mail is not available, please include a telephone number where you can be reached.

I hope that you will seriously consider making a contribution to the histology community by submitting a manuscript to *HistoLogic*. I look forward to hearing from you.

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