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IS YOUR HISTOLOGY LAB A TOXIC WASTE PARADISE?

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During the past several decades, the American public has become increasingly cognizant in regards to health awareness. We are concerned with the foods that we put into our bodies, the hazards of second-hand smoke and especially the degrees of environmental pollution and how it relates to us and future generations.

Being a histotechnologist, I have seen some of the health risks that are evident in the histology laboratory. During the past twenty years I have visited several histology laboratories and have been astounded by the strong odors of chemicals, dirty counter, open containers of solutions and rusty instruments laying around. When I was new at the profession, I thought that this situation was normal, but through my experience during the years, realize that this is far from true.

In the working day of the histology laboratory the technicians deal with potentially harmful solutions and their vapors. Many technicians will say they are unaware of any chemical fumes. This may be due to the fact that over the years, their sense of smell has become accustomed to these odors and thus, are unaware of their presence.

Many articles have been written lately on the possible long term affects of these chemicals. We know from studies, that people who work around formaldehyde are more prone to developing certain forms of nasal cancer.

Xylene fumes are shown to be neuro-toxic and possibly could have an affect on the liver in later life. Alcohol fumes are still being studied as to their adverse affects on humans. I would like to discuss some of the precautions that are followed in our laboratory to help eliminate the risk of handling and inhaling large quantities of potentially toxic chemicals.

Many histology technicians are aware that mercuric chloride is found in Zenker's fixative, Helley's solution and B5 fixative. These solutions are used for the fixation of lymph nodes, bone marrows and renal biopsies. First of all, none of these solutions should be poured down the sink. They are not biodegradable and thus can present a hazard to soil or drinking water. Anytime a solution containing mercury is handled, use protective gloves, as mercury can be absorbed directly into the body through the skin.

When we dispose of any of these solutions, we have a one-gallon plastic jug that is labeled "Mercuric Chloride For Disposal". When we receive a specimen in the laboratory containing mercury, we decant the solution into this jug.

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The specimen container should never be rinsed in the sink as it will continue to release mercury into the sewer. We simply cap the empty specimen container and place it in a special trash container that will be picked up by a toxic waste disposal company for incineration at a very high temperature, thus making it non-hazardous. By all means, do not place these containers in trash that will be delivered to the city waste disposal area, as the soil can become polluted and hazardous for future generations.

We use a solution called Bouin's fixative which is used in a trichrome stain. The solution is put into an oven at 56° C. The solution is made with formaldehyde, picric acid and glacial acetic acid. Be careful not to inhale the vapors when the lid is taken off the coplin jar; do this under a fume hood if possible. Always wear protective rubber gloves when doing special stains or making up chemicals. It is not known what kind of combinations of deadly chemicals will be produced on your hands from handling these substances.

Formaldehyde fumes are very irritating to both eyes and respiratory system. If you get formaldehyde into a cut, it burns very badly. Formaldehyde is used in embalming fluid because of its high degree of effectiveness as a preservative and disinfectant. Dead tissue responds well to its presence; however it is very caustic to living tissue. It must be treated with great respect. Use gloves when handling formaldehyde. Some form of approved eye goggles should be used, as serious burns can be caused if splashed into the eyes. When grossing tissue, always keep the water running in the sink, as this will keep the formaldehyde from pooling and causing vapors to escape. Don't pour formalin into the trash container (pour it into the sink) as this chemical is biodegradable. Place the cap back onto the empty specimen container before discarding, as this will help alleviate fumes arising from the trash container. Always have a damp sponge on hand for wiping spills of formaldehyde on the counter or dissecting table. Keep a lid on the basket of cassettes that are in formalin. Take the container lid off only to add another cassette. Make sure that you clean the grossing area and clean underneath the dissecting board, as this can harbor pooled formalin and thus cause fumes.

Much has been written about xylene and its affects on laboratory personnel. It may take many years before scientists see what kind of health problems arise from daily extended exposure to xylene. I recommend wearing gloves when coverslipping slides out of xylene. The routine surgical gloves will not work, as they melt in xylene. Most of the laboratory supply firms will carry a special "solvent resistant glove" in their catalogue which can be used when coverslipping. It may not be convenient to coverslip with gloves on your hands and may be a little slow, but with practice it becomes quite easy and safer. Our laboratory uses a fume hood when coverslipping with most of the xylene fumes being drawn into a charcoal filter. Don't leave xylene soaked gauze laying around on the counter when coverslipping. Dispose of them in a closed trash container – one that will be taken by the toxic waste disposal company.

One problem I have seen in histology laboratories relates to the staining of microscopic slides. How many times does one see the lids off the staining dishes? Why allow these fumes to permeate the laboratory? Gauze soaked with xylene and alcohol should not be left on the counters. That is simply asking for trouble. Keep the lids on the containers at all times when staining is taking place. Take the lid off only to move to the next container. Many people will say this is too time consuming and slows you down. But how important is your health? Xylene should never be poured down the sink. Treat xylene the same as mercuric chloride. It is disposed of in plastic gallon jugs (the kind that will accept xylene without melting) and taken by the toxic waste disposal company.

Our laboratory is fortunate to have a ventilation system that takes away most of the fumes in the grossing and routine staining area. If you are not as fortunate, you can minimize the vapors in your laboratory by simply being aware of how to handle these substances. Keep your laboratory clean; do not create a "toxic waste paradise". Most of the things I have stated in this article should be known by histotechnologists. This article is for those who are not aware of these problems. We have to think of our future and approach our later years with good health. If you have questions about the chemicals you work with from day to day, don't be afraid to ask your hospital infectious control staff, your engineering staff or your public health department. These people can provide you with the information you require. "Become more aware and don't let your laboratory become a toxic waste paradise"!

QUALITY CONTROL FACTOR

"THERE IS NOTHING CONSISTENT IN THE SCIENCE OF HISTOTECHNOLOGY. WHAT PERFORMS WELL TODAY MOST LIKELY WILL NOT TOMORROW. THEREFORE, ONE MUST USE TODAY'S RESULTS TO DETERMINE TOMORROW'S QUALITY. THIS SHOULD BE PRACTICED DAILY ON ALL FACETS PERFORMED IN THE HISTOLOGY LABORATORY."

LEE. G. LUNA

An Alternate Counterstain for the Grocott-Gomori Methenamine Silver Procedure

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Experience in our laboratory has shown that the most commonly encountered problem with the Grocott-Gomori methenamine silver (GMS)[1] procedure for fungi in tissue sections is overstaining (overimpregnating) in silver-methenamine solution. When there are few fungal elements, overstaining of tissue components such as elastic fibers may obscure important tissue structures with the potential for significant inaccuracy in interpretation. In addition to the problem of overstaining tissue structures, silver precipitation may be so intense that the internal details of fungi are totally obscured. Further, erythrocytes staining dark brown to black may mimic yeast form cells of fungi in infected tissue.[2] Overstained, crenated erythrocytes in alveolar spaces of the lung can be mistaken for cyst forms of *Pneumocystis carinii*.

To solve this problem, we attempted to use various counterstains that would overlie the silver in erythrocytes. One counterstain that effectively solves this problem by differentiating the erythrocytes from fungal elements is Halmi's light green-orange G.[3] This staining solution has been modified in our laboratory and is used in place of the usual or standard light green for GMS counterstaining. The original formula[4] and our modification are as follows:

Original Formula	Modified Formula
Light green - 1.8 gm	Light green - 0.5 gm
Orange G - 9.0 gm	Orange G - 1.0 gm
Distilled water - 900.0 ml	Distilled water - 225.0 ml
Phosphotungstic acid - 4.5 gm	Phosphotungstic acid - 2.2 gm
Acetic acid - 9.0 ml	Acetic acid - 4.5 ml

All of the reagents combine readily and do not need to be mixed in any particular order. The intensity of the background stain depends largely on the consistency or density of the tissue to be stained and on the staining time. Fifteen to thirty seconds in modified Halmi's light green-orange G is probably optimal. In addition to being an effective diagnostic aid, this counterstain for the GMS procedure is esthetically pleasing under the microscope and provides superior contrast for photomicrography. (Fig. 1)

References

1. Grocott RG. A stain for fungi in tissue sections and smears using Gomori's methenamine-silver nitrate technic. Am J Clin Pathol 1955;25:975.
2. Swisher BL, Chandler FW. Grocott-Gomori methenamine silver method for detecting fungi: practical considerations. Lab Medicine 1982;13:568-570.
3. Koski JP. Silver-methenamine borate (SMB) cost reduction with technical improvement in silver nitrate-gold chloride impregnations. J Histotechnology 1981;4:115-120.
4. Halmi NS. Two types of basophils in the anterior pituitary of the rat and their respective etophysiological significance. Endocrinology 1950;47:289-299.

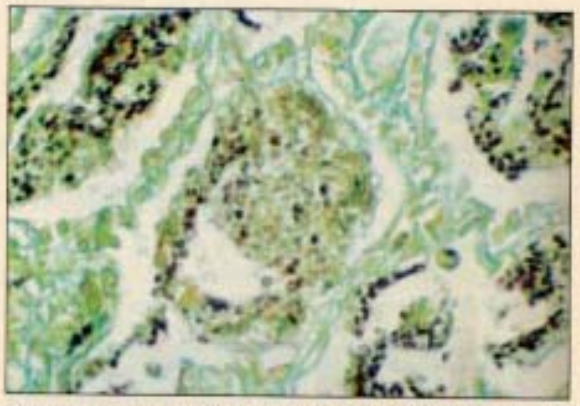

Figure 1: Pneumocystis carinii of an AIDS case stained with Grocott's method for fungi and the modified Halmi's counterstain. Note the pleasing clear background which prevents potential slide misinterpretation.

Rabies

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Rabies[1,2] is an acute viral disease of the central nervous system that affects mainly wild animals, including skunks, bats, foxes, dogs, raccoons, cats and may also infect humans. The most common source of the infection worldwide are dogs, followed by cats. In the United States, skunks and bats are currently the most common reservoir. The infection is almost never found in rodents. The disease is transmitted from the saliva of an infected animal to a new host, through bites or the licking of an open wound. After introduction into the body, the virus travels along nerve pathways to the brain and later to other organs. The etiologic agent of rabies is the rabies virus, which is a bullet shaped double stranded ribosenucleic acid virus, belonging to the rhabdovirus group. The incubation period for the virus in humans varies from 10 days to 1 year, depending upon the size of the inoculum and the site of infection. The incubation period is followed by fever, malaise, headache, sore throat and abnormal sensation around the site of the infection. If the disease progresses, there are spasmodic contractions of the throat, hydrophobia, convulsive seizures, paralysis and eventually death.

The rabies virus antigen may be demonstrated in tissue specimens by using the fluorescent antibody staining technique. Histologically, the neurons demonstrate intracytoplasmic inclusion bodies known as Negri bodies (named after the Italian physician Adelchi Negri). The viral inclusion is seen in approximately 70% of brains affected with rabies, particularly in the cytoplasm of the pyramidal cells of the hippocampus, or the Purkinje cells. The most useful method for formalin fixed, paraffin embedded tissue, is Luna's[1] method for Negri bodies (Fig.1). For tissue fixed in Zenker's, the method of choice is Schleifstein's[1] method for Negri bodies (Fig. 2).

References:

1. Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw Hill, New York, p. 109, 1968.
2. Saunders Dictionary and Encyclopedia of Laboratory Medicine and Technology. Edited by J. L. Bennington, W. B. Saunders Co., Philadelphia, PA. 1984.
3. Mosby's Medical and Nursing Dictionary. C. V. Mosby Co., St. Louis, 1983.

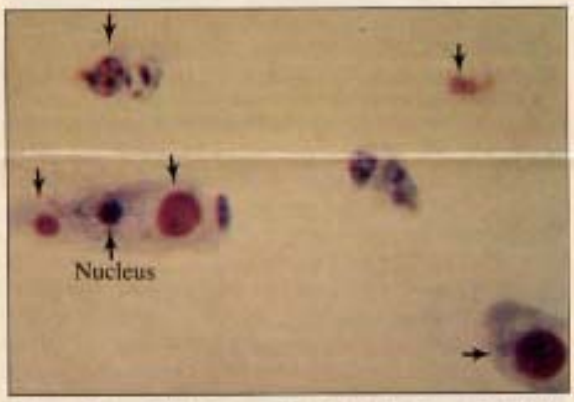

Figure 1. Section of brain demonstrating red stained rabies inclusion bodies (arrows) with the Luna stain, following formalin fixation.

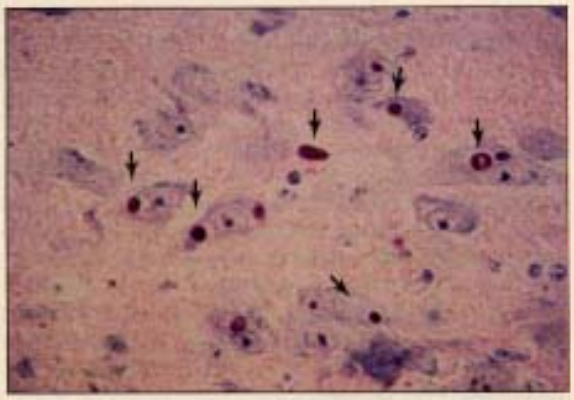

Figure 2: Section of brain demonstrating rabies inclusion bodies (arrows) stained with Schleifstein's method.

Notes on the Tissue Section and Debri Bonding Question

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In 1979 Luna[1] stressed the need to understand "the mechanisms of the strong bond between floating debri and tissue sections." In response, Golick[2] suggested the role of water mobility, and conformational changes of macromolecules in tissue sections exposed to debri, should be investigated for a better understanding of the attachment mechanisms. Recent developments may bring us even closer to the answer.

Kellenberger et. al.[3] discussed studies involving "low denaturation embedding for electron microscopy of thin sections" with Lowicryl embedding resins. These resins infiltrate and cure at temperatures below 0° C, and are available in either a non-polar hydrophobic form or a very polar hydrophilic form. This allows experimenters to control variables involving hydration shells, which influence hydrophobic bonding and conformations of proteins. One may then do electron microscopic and X-ray diffraction studies to determine the fate of water in tissue section macromolecules undergoing conformational changes.

Researchers have revealed that dehydration and infiltration with solvents manipulated water in tissue sections, and caused conformational changes of macromolecules. Substantial structural effects occurred during sectioning and staining with no effects by polymerization.

Conformational changes in macromolecules, associated with debri, most likely involves the displacement of organized associated water molecules similar to what has been indicated by experiments with low denaturation embedding. Further investigation with this new technology will provide a clearer understanding of how structure and water interactions occur in processed tissue, and then bring us closer to the mechanisms of debri bonding as well as other phenomena we employ with tissue sections.

References:

1. Luna, L.G.: An Intriguing Question in Search of an Answer. Histo-Logic, Vol. IX,p. 125 1979.
2. Golick, M.L.: Possible Causes of the Strong Bond Between Floating Debris and Tissue Sections. Histo-Logic, Vol X,p. 151 1980.
3. Kellenberger, E., Carlemalm, E., Villiger, W., Roth, J., and Garavito, R.M.: Low Denaturation Embedding for Electron Microscopy of Thin Sections. Chemische Werke Lowi, Federal Republic of Germany.

Rapid Microwave Modification of Mayer's Mucicarmine and Bennhold's Amyloid Methods

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Microwave bombardment has been increasingly applied in recent years to accelerate the staining times of metallic stains[1-5]. Microwave modifications of stain techniques increase technical efficiency and hasten diagnosis. Its application to numerous other stain procedures is currently utilized for trichrome mordanting in this laboratory[4] and trichrome staining of plastic embedded sections[3].

Our laboratories have found that two commonly used special stains, Mayer's mucicarmine and Bennhold's amyloid methods, are amenable to microwave heating with concomitant reduction in staining times of approximately one hour each. Equipment: Our laboratories use Samsung RE5151 and Sanyo EM3621 microwave ovens. Each oven will vary in efficiency, hence it is important to establish the exact time frame in which the oven will bring a given volume of water to 90° C.

All microwave procedures must be carried out in loosely covered plastic coplin jars or in plastic staining trays, in plastic staining dishes. Both methods described are modified from techniques detailed in the AFIP Staining Manual[2] as follows:

MAYER'S MUCICARMINE METHOD

Modification A: Deparaffinized and hydrated slides are placed in Weigert's hematoxylin solution and heated in the microwave oven for 30 seconds and immediately transferred to tap water.

Modification B: Slides are placed in mucicarmine working solution and heated in the microwave oven for 60 seconds. The hot stain jar is removed from the oven and allowed to remain at room temperature (22° C) for 5 minutes. Slides are then transferred to distilled water.

Results:
Mucin and cryptococci stain a deep red hue. Nuclei are black. The background stains yellow.

BENNHOLD'S METHOD FOR AMYLOID

Modification A: Deparaffinized and hydrated slides are placed in 1% aqueous congo red solution and heated in the microwave oven for 60 seconds. The hot stain jar is removed from the oven and allowed to stand for 60 seconds at room temperature (22° C). Slides are immediately transferred to distilled water.

Modification B: Mayer's hematoxylin counterstain is carried out with microwave heating for 30 seconds.

Results:
Amyloid stains rose to red. Nuclei are blue.

Summary:
Two frequently applied non-metallic special stain procedures for mucin and amyloid have been successfully adapted to microwave acceleration with identical results to those of conventionally applied methods. There is a saving of approximately 1 hour in stain time for each method.

References:

1. Brinn N.T.: Rapid metallic histological staining using the microwave oven. J. Histotechnology 6(3)125-129. 1983.
2. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. Luna L.G.(ed) Third Ed. McGraw Hill, New York pgs. 153-154, 161-162. 1968.
3. Maynard J.H.: A trichrome stain in glycol methacrylate that works. Lab. Med. 17(8) 471-473. 1986.
4. Shapiro S.H.: Rapid trichrome mordanting using microwave heating. Am. J. Med. Technol. In press.
5. Sohn L. and Shapiro S.H. Rapid Dieterle stain with microwave heating. Histologic 15(3) 232. 1985.

The Use of Colored Tattoo Ink During Specimen Grossing[1]

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There are many instances when identification of a given side of a tissue specimen is essential for proper microtomy. For example, proper sectioning of a piece of skin to ensure the epidermis is included, proper sectioning of biopsies and many other situations, which may determine the final microscopic results.

In our laboratory, we use colored tattoo ink available from tattoo supply firms[2] A variety of colors come in 2 oz. and 4 oz. bottles that can be used with cotton tipped applicator sticks, or the spout that comes with the bottle. The colors do not run and can be blotted with a paper towel. Tissue processing and slide staining does not interfere with the color. It will remain on the surface of the tissue specimen to provide an excellent marker for orientation of the specimen and many other uses.

References

1. Schmidt, W.A.: Principles and Techniques of Surgical Pathology. Addison-Wesley, Menlo Park, CA p. 153, 1983.
2. Source: Spaulding Rogers-Tattoo Supplies. Rt. 85, New Scotland Road, Voorheesville, NY 12186.

METHODS FOR SEALING AND MOUNTING PARAFFIN BLOCKS

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Editor's Note: This article is being presented since Histo-Logic is distributed to more than 50 countries and the procedure contained herein, may be of significant value to histotechnologists with limited resources. It should be noted that Ames Division, Miles Laboratories, Inc., now manufactures an updated, modern tissue embedding center. Further, it is interesting to see that histotechnologists, whether in Nigeria or the United States, possess a flair for innovation. Mrs. Lillian Antonio has a B.Sc. in medical technology, a Master's in Public Health and is employed by the Nigerian Ministry of Health. Her article is being published with limited editing.

I have been using products manufactured by Lab-Tek Products (now Ames Division, Miles Laboratories, Inc.) for the past eight years and admire the ingenuity of how laboratory equipment has been modeled and brought into reality for use. Let me share my experience in using the Tissue Tek II Embedding Center. I have found that the "hot platform" of this apparatus serves two other purposes, as follows.

SEALING TISSUE BLOCKS BEFORE STORAGE

After sectioning, tissue blocks may be sealed using the hot platform of the Tissue Tek II Embedding Center.

1. Place a small piece of paraffin on the hot platform. Allow it to melt enough to cover the ungrooved portion of the platform.
2. Place the sectioned tissue surface of the block on the melted paraffin for a few seconds until the surface is completely covered.
3. Allow the block to solidify before storage.

MOUNTING TISSUE BLOCK ON THE WOODEN BLOCK

In a few occasions, there is need for sectioning larger specimens which cannot be accommodated in the larger metal base molds. These specimens are embedded in molds such as the Leuckhart, or paper boats. In this case, paraffin tissue blocks have to be mounted on wooden or fiber blocks before sectioning. This is most often done with a hot spatula. The Lab-Tek II Embedding Center's hot platform has proven effective for this purpose also.

1. Trimmed tissue paraffin block and the wooden block are placed side by side on the hot platform. (This allows the paraffin block to melt, creating a flat surface while simultaneously heating the wooden block.)
2. Quickly place the two heated surfaces together, applying enough pressure to ensure good adhesion.
3. Allow melted paraffin from the embedding center spout to run along the sides of attachment.
4. Let the paraffin completely solidify before sectioning. This can be facilitated by immersion in ice cold water.

NOTE: A small paper tag bearing the case number may be affixed to one side of the block by again touching the side of the paraffin block to the heat platform and applying the tag to melted paraffin.

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20706. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.