

HistoLogic

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A Change in Work Patterns in the Histology Laboratory: An Explanation for an Increasing Incidence of Work-Related Health Problems

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Since the introduction of Tissue-Tek® Processing Embedding Cassettes around 1970, there has been quite a marked change of work patterns in histology laboratories. Before 1970, the histology worker was obliged to place tissue paper samples in metal capsules, together with tedious little paper labels with identifying data written in pencil. They were embedded by a variety of methods well known to anyone who has worked in histology before 1970.

The most common method was the use of "angle irons," which were simply two pieces of flat brass about 5mm thick, angled at 90 degrees, so that when fitted together and placed on a slab of glass or metal plate, they formed a rectangular mold into which the tissue samples were cast in wax blocks. The paper labels were affixed to the sides of the angle irons so that when the wax was poured, they were incorporated into the paraffin block. All surfaces had to be coated with mold release, usually an alcohol/glycerin mixture.

After the blocks had set, they were removed from the angle irons and mounted either onto wooden blocks with the aid of a heated spatula, or onto heated metal shanks. These then had to be faced and cut. Each block was differently aligned and necessitated an adjustment of the microtome chuck for each block. The paraffin blocks, after cutting, had to be demounted for storage and any subsequent recuts involved remounting of the blocks. With the introduction of efficient automatic knife sharpeners at about the same time as the introduction of process embedding cassettes, the burdensome hand-sharpening of microtome knives on

glass slabs was eliminated, thereby saving each technician at least one hour per working day.

All of this was, of course, very time consuming, and the number of slides stained and prepared per technician was much fewer than now. With the rapidity of embedding and cutting using process embedding cassettes and automated sharpening of microtome knives, much more time per technician is spent at the staining bench and hence greater exposure to xylene and toluene.

Lately there have been an increasing number of articles dealing with the effects of xylene on histotechnologists and cytotechnologists. Cytology has had a

INSIDE THIS ISSUE

A Change in Work Patterns in the Histology Laboratory: An Explanation for an Increasing Incidence of Work-Related Health Problems	227
Lead Poisoning Inclusion Bodies	229
"New Fuchsin for Old" — In Schiff's Reagent	230
Organizing Your Special Stain Solutions	231
Innovative Frozen Section Procedure	231
Automatic H & E Staining	232

No reader should strive or undertake procedures discussed in HISTO-LOGIC articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

very rapid growth over the last two decades and as the cytotechnologist does not embed or cut, but receives or prepares smears, the time spent staining and cover-slipping may be greater than the time spent in this activity by histotechnologists. It is plain to see, therefore, that there has been a work pattern change of some magnitude and that this may provide an answer to those who ask "why now?" when confronted with evidence of health problems due to chemical exposure. (I am indebted to Ms. Sharon Smuck for being the person who asked me the question "why now?")

There is still a primitive belief abroad that such risks are part and parcel of laboratory work. It is essential that inhalation of contaminated air does not result in adverse physiological effects, be it injury to a susceptible site in or on the body, or simply marked reversible discomfort. For all but a few contaminants reduction of the dose inhaled will, if carried far enough, lead to freedom from adverse effects in almost all exposed workers, and in general the most appropriate method of reducing the dose is to reduce the concentration of contaminant in the inhaled air. Wanton exposure to airborne chemicals should be avoided. Enlightened industrial hygienic practice maintains exposures well below rather than at the legislated standard. It is worth quoting from the Swedish *Arbete Och Halsa*.

"Scientific Basis for Swedish Occupational Standards" Abstract: With occupational exposure to toluene vapor, most body uptake is by inhalation. There is rapid and high retention during the first half hour, followed by gradual reduction to an uptake of about 50% of the inhaled amount. This proportion is maintained during the entire work day, reflecting a state of equilibrium between uptake and elimination. Under certain circumstances, toluene in liquid

LEE G. LUNA RETIRES

Mr. Lee G. Luna has recently retired from federal service after over thirty-five years as Chairman, Department of Histopathology at The Armed Forces Institute of Pathology. He has joined the staff of American HistoLabs, Inc., where he will continue his consultative service to the field of histotechnology.

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form can be taken up through the skin. Toluene is highly soluble in oil and accumulation of toluene in fatty tissues should therefore be noted in discussion of toxicity. The biological half-life of toluene in such tissues has been calculated to be about three days. This does not exclude the possibility of some day-to-day accumulation of toluene in the body fat, at least in the beginning of the exposure period.

Abstract: Xylene usually enters the body via the lungs. Of the amount entering the lungs, about 65% is absorbed during rest. Xylene's high solubility in lipids implies that its storage in fatty tissues should be considered in assessments of toxicity. The half-life of m-xylene in subcutaneous fat varies from one to about six days. Because of xylene's relatively long elimination time from subcutaneous adipose tissue, a certain accumulation will occur during the first weeks of normal occupational exposure (five-day work weeks). The possibility that long-term exposure to xylene may have neurotoxic effects cannot therefore be dismissed. Xylene, widely used not only in industry but also in biomedical laboratories, has been toxic in workers to the point of causing permanent disability.

The following report is from *Xylene Poisoning in Laboratory Workers: Case Reports and Discussion*, Lab. Med., Vol. 2, No. 9, Sept. 1980, by Roberta N. Hipolito.

At age 56, technician collapsed with severe chest pain. Testing revealed an abnormal ECG—a diagnosis of xylene poisoning was made.

Abstract: Patient sought medical attention because heart was "banging around like a ball on a string." One evening at home, patient belched and smelled xylene. The pathologist in charge of the laboratory agreed that xylene might be responsible for patient's physical problems.

From the medical records of this patient, typical symptoms included:

- solvent-induced encephalopathy with headache
- evidence of falling to the right and posteriorly
- distinct evidence of depression
- significant brain damage as a result of exposure to xylene.

Loyalty was part of the problem, in that patient put up with conditions too long.

As to the patient's hypertension, this is probably the result of frustration at being unable to achieve any type of relief. Patient has solvent-induced brain damage as a result of inhaling aromatic hydrocarbon solvents for many years in a poorly ventilated room.

Finally, workers should have that most basic of all rights, without question or the need to strike—the right to stop a job if they believe their life is endangered.

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Lead Poisoning Inclusion Bodies

An Editorial

Lead poisoning is a toxic condition caused by the ingestion or inhalation of lead or lead compounds. Children are especially vulnerable as the result of eating flaked lead paint. Poisoning also occurs from the ingestion of water from lead pipes, lead salts in certain foods and wines, the use of pewter or lead-glazed earthenware, and the use of leaded gasoline for cleaning. Inhalation of lead fumes is common in industry. Toxic effects include gastrointestinal distress, colic, pain in the joints, headaches, drowsiness, stupor, and convulsions and coma that may have a fatal outcome. In chronic lead intoxication, intranuclear inclusions may be found in the liver and especially in cells of the proximal convoluted tubules of the kidney. These inclusions vary in size and shape, but most are spherical or elliptical. They are distinct and not derived from nucleoli. They contain no DNA, but some (50%) are surrounded by a contracted rim, or granules of chromatin; see Figures 1 and 2.

Histologically, lead poisoning inclusion bodies can be demonstrated with an acid fast stain¹ followed by picric acid counterstain (Fig. 1). They can also be demonstrated with Emanuele's method² for RNA and DNA (Fig. 2). Both procedures require formalin-fixed, paraffin-embedded specimens.

References:

1. Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. McGraw-Hill, New York, 1968.
2. Emanuele, P.: Emanuele's Method for RNA and DNA. Personal communication.
3. Mosby's Medical and Nursing Dictionary. The C. V. Mosby Co., St. Louis, MO, 1983.
4. *Saunders's Dictionary and Encyclopedia of Laboratory Medicine and Technology*. James L. Bennington, Editor, W. B. Saunders, Co., Philadelphia, PA, 1984.

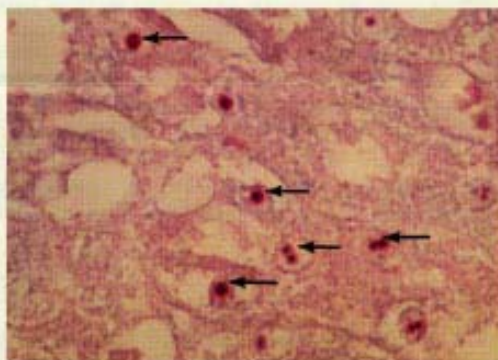


Figure 1. Section of kidney stained with Kiryouri's acid fast stain with a picric acid counterstain. Note the bright red staining inclusion bodies (arrows).

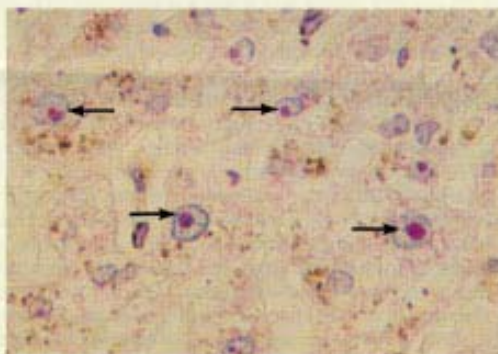


Figure 2. Section of kidney demonstrating lead inclusion bodies seen in red, surrounded by a contracted rim of chromatin. Section has been stained by Emanuele's method for RNA and DNA.

"New Fuchsin For Old"—In Schiff's Reagent

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Because of the problems associated with the preparation of Schiff's reagent from standard recipes using Basic Fuchsin, it was decided to try New Fuchsin (Gurr's Biological Stains) as an alternative dye. The reagent was prepared as follows:

- (1) Distilled water 200.0 ml
New Fuchsin 1.0 gm
Potassium metabisulphite 2.0 gm
Hydrochloric acid, concentrated 2.0 ml

The components were mixed in the order given, mixing thoroughly at each addition, and allowed to stand for a minimum of thirty minutes after which 0.4 ml of concentrated sulphuric acid was added as a stabilizer. Finally the solution was decolorised with 5 grams of activated charcoal, filtered and stored at 4°C. The optimum times for staining 3µ paraffin sections were found to be:

- Periodic acid 4 minutes
Schiff's reagent 8 minutes

The results were comparable to those of the Basic Fuchsin-based Schiff's solutions. The reagent worked well on 2µ glycolmethacrylate-embedded sections, although extended times of eight minutes and sixteen minutes respectively were required.

The advantages of this reagent are threefold; first, no heating is required to dissolve the dye; secondly, the reagent may be prepared and ready to use in forty minutes if required, although the amount of charcoal used to decolorise may have to be increased slightly; and thirdly, different batches of reagent were found to be consistent.

Reference:

J.D. Bancroft and A. Stevens: *Theory and Practice of Staining*, p. 148, Churchill Livingstone, London, 1977.

Practical Stain Technology "Wet" Workshop & Seminar

Presented by: Lee G. Luna,
Center for Histotechnology Training

This five-day extensive wet workshop and seminar will afford the registrants an opportunity to perform 20 special stains demonstrating more than 35 pathologic entities. Some of the entities which will be stained with the special stains performed are: Gram positive and Gram negative bacteria; hepatitis B surface antigen; elastic fibers; pancreatic islet cells; all pathogenic fungi; calcium; acidic and sulfated mucosaccharides; hematologic elements; mast cells; Lepa and tubercle bacilli; nucleic acids—RNA and DNA; amyloid; silver reactive cell granules from the entire neuroendocrine system; copper; connective tissue; cross striations in Rhabdomyosarcoma and fibrin; mucins; spirochetes; Legionnaires' disease bacilli. Also, the proper tinctorial qualities of a good hematoxylin and eosin (H&E) will be discussed in great detail.

In addition to the practical special staining aspects, lectures will be presented daily (to include evenings) on: Chemistry of Staining; Staining Mechanisms; Tissue Identification for Histotechnologists; Shelf Life of Solutions; Preferred Controls; Introduction to Immunoperoxidase; Decalcification; and various other subjects directly related to the production of high-quality microscopic slides.

National Society for Histotechnology Continuing Education Credits have been applied for. For program and related information contact:

Registrar
Center for Histotechnology Training
P. O. Box 2453
Rockville, Maryland 20852
(301) 330-1200

Organizing Your Special Stain Solutions

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Organization is the key to getting a job done well and rapidly. We applied this policy to the special stain setup in our laboratory and found it much easier to do a stain if all dyes and chemicals for that stain are grouped together, the stains are listed alphabetically, solutions used are listed under each stain, each bottle is numbered accordingly, and the assigned number is marked on the staining procedure.

EXAMPLE

A. Acid Fast

Kinyoun's:

carbol fuchsin	A-1
acid alcohol, 1%	A-2
methylene blue, working	A-3
methylene blue, stock	A-4

A & R Fluorescent:

auramine and rhodamine	A-5
acid alcohol, 1%	A-2
potassium permanganate	A-6

B. Acid Mucopolysaccharides

Alcian blue:

acetic acid, 3% aqueous	B-1
alcian blue, 0.1%	B-2
...and so on	

The solutions are grouped on the shelves alphabetically according to stain and a procedure manual is always nearby. The solutions can also be placed in a different box for each stain and put away alphabetically. Include a card with the stain procedure inside each box.

Some of the solutions used in our laboratory are not specifically for any special stain. These are listed alphabetically as "Miscellaneous Solutions" and assigned consecutive numbers.

EXAMPLE:

Fixative for frozens	1
Hematoxylin, Harris'	2
Iodine, 0.5% alcohol	3
Toluidine blue, 1% aqueous	4
Zenker's solution	5

These are stored in a separate place from the special stain solutions (i.e., on a different shelf) in numerical order. Be sure bottles are numbered clearly.

A master list is then composed, listing all ready-made solutions in stock. Their assigned number follows so that any solution can be found rapidly. You can also check the master list to see where any given solution

is located or to see if that solution is in stock.

The example list below represents solutions listed in above stains:

Acetic acid, 3% aqueous	B-1
Acid alcohol, 1%	A-2
Alcian blue, 0.1%	B-2
Auramine-rhodamine	A-5
Carbol fuchsin, Kinyoun's	A-1
Fixative for frozens	1
Hematoxylin, Harris'	2
Iodine, 0.5% alcohol	3
Methylene blue, working	A-3
Methylene blue, stock	A-4
Toluidine blue, 1% aqueous	4
Potassium permanganate	A-6
Zenker's solution	5

Lists are posted inside the staining shelf doors next to where the solutions are kept. A copy of the list is also kept in the histology staining manual. Lists are updated continuously as new procedures and/or solutions are added.

This system has worked quite well in our laboratory during the past ten years and has simplified both performing and teaching special stains.

Innovative Frozen Section Procedure

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Canada

The following is a quick and simple method for fat-staining formalin fixed tissue, when neither a freezing microtome nor a cryostat are available. I developed this method when our cryostat was out of commission and found it very effective.

The fixed tissue is embedded in Tissue-Tek®O.C.T. compound using standard Tissue-Tek®II cassettes and molds. Chill the mold first on your embedding center cooling plate (this will make it easier to position the tissue in the O.C.T.), then freeze the block in the freezing compartment of your refrigerator. Quickly cut sections at 10 microns using a standard rotary microtome and a chilled knife. Float the sections out on a water bath; the O.C.T. will melt instantly, but it will still be possible to pick the sections up with a microslide. It is best to use albuminized slides and to dry them longer than usual in the 60°C oven. Stain with Oil Red O, or other fat stains as desired.

Automatic H&E Staining

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I would like to share my technique for staining H&E slides using a dual Auto-Technicon.

Specimens are fixed with 10% buffered formalin, processed in the conventional manner and embedded in paraffin. After cutting at 3-5 microns, slides are placed on a slide warmer for a minimum of 15 minutes. I might add that I have not used any slide adhesive, such as gelatin or albumin, for at least five years and I rarely lose a section. After heating, the slides are cooled quickly with a fan and then stained with the following procedure, using the top level of the Auto-Technicon.

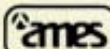
The basic idea for this procedure was taken from Technicon's method for staining, but the changes in solutions and timing are from my experience. This procedure has been used since 1977 with very good results.

STAINS-SOLUTIONS	POSITION	TIME
1. Xylene	Container on counter	3 min.
2. Xylene	Near Technicon	5 min.
3. Absolute alcohol	1st position on Technicon	2 min.
4. 95% alcohol	2nd position on Technicon	2 min.
5. Deionized water	3rd position on Technicon	1 min.
6. Gill-2 hematoxylin	4th position on Technicon	2 min.
7. Deionized water	5th position on Technicon	1 min.
8. 0.01% lithium carbonate	6th position on Technicon	2 min.
9. Deionized water	7th position on Technicon	1 min.
10. Eosin	8th position on Technicon	3 min.
11. Deionized water	9th position on Technicon	1 min.
12. 95% alcohol	10th position on Technicon	1 min.
13. Absolute alcohol	11th position on Technicon	1 min.
14. Abs. alcohol-xylene	12th position on Technicon	1 min.
15. Abs. alcohol-xylene (equal parts)	On the counter near Technicon under fume hood	10 dips
16. Xylene	On the counter near Technicon under fume hood	10 dips
17. Xylene	On the counter near Technicon under fume hood	10 dips
18. Mount coverslip with resinous media		

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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.



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