HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor A Technical Bulletin for Histotechnology Vol. V, No. 3 - July, 1975

GOLDEN FORCEPS AWARD WINNER

We are pleased to announce that Louis W. Chang, Ph.D., University of Wisconsin Medical School, Madison, Wisconsin, has been selected as the recipient of the Golden Forceps Award for 1975. His paper, "A Silver Technique for the Study of Cellular Injuries," was selected from articles submitted to HISTO-LOGIC during the past year. Criteria for selection are clarity, originality, and scientific contribution. The Golden Forceps Award will be presented at the Symposium/Convention of the National Society for Histotechnology to be held in Silver Spring, Maryland, October 6-10, 1975. Reprints of Dr. Chang's paper, which appeared in HISTO-LOGIC in April, 1974, are available from Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 N. Aurora Road, Naperville, Illinois 60540.

Book Review

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Book Title: The Preparation of Decalcified Sections
Author: Edward B. Brain, B.Sc., F.I.I.P., F.R.P.S.
Publisher: Charles C. Thomas, Springfield, Illinois

"The Preparation of Decalcified Sections" is a very concise and thorough compilation of decalcification methodology information that makes it a very essential text for any Histotechnology Laboratory reference and teaching library.

The text begins with an historical review of the works of early researchers and their attempts to decalcify hard tissue. The author points out that it was as early as 1854 when Beale proposed the use of hydrochloric acid. He suggested that thin ground sections, which contained both hard and soft tissues, be soaked in a dilute solution of hydrochloric acid to render them soft and pliable prior to processing for examination.

An anatomical breakdown and distribution of the hard tissues in man is discussed; e.g., "Flat Bones. Examples of these are the ribs and scapula, and some of the bones of the skull which are perhaps thin rather than flat. These bones consist of cancellous bone sandwiched between two plates of compact bone." The text is a positive approach to the preparation of decalcified sections, and the author discusses and demonstrates the chemical as well as the physical processes involved. Choice and applications of various reagents along with accurate assessment on completion is provided.

Special features such as the use of a variable voltage x-ray unit for recording histological material prior to and during decalcification, and use of photography to demonstrate changes in the dimensions of the specimen which occur during processing, add to the educational interest of decalcification.

Easy as One, Two, Three (and Four)

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Having a complete description of various reagents and solutions with a note of their locations can be a great convenience in a busy histology department. Efficiency in this laboratory has been greatly increased by the preparation of four file boxes to be used for quick reference. Three contain 3 X 5 file cards which serve as a running inventory of all stains and reagents, while the fourth contains a description of the procedures to be used for stain and solution preparation.

The first box is labeled "STAINS." Here, in alphabetical order, are separate cards for each dry stain in stock. Each card also carries the color index, weight, date of purchase, manufacturer, and catalog number.

The cards in the second box, labeled "DRY REAGENTS," have a complete description of each product, as AR, ACS, TAC, CRYSTAL, POWDER, etc. The manufacturer, catalog order number, weight, and purchase date of each is noted.

File box three is "WET REAGENTS." Every liquid reagent in the laboratory is entered on a file card. This includes everything from concentrated acids to solutions made in the laboratory. Where, as in the case of ferric chloride, three different percent solutions are used, each is listed separately. The date, quantity prepared, and name of the technician who made up each reagent is entered.

A fourth, and larger, file holds two alphabetical files. The first is the "RECIPE" file. It contains, on individual cards, complete instructions for making up every stain or solution used in the department with the exception of single percent solutions. Behind this is another file labeled "BACK" because it is the backbone of all special stains done in the department.

On these cards are both instructions for preparation and special instructions written in red, for example: "MAKE FRESH," "FILTER BEFORE USING," "DISCARD IF CONTAMINATED." On every card the physical location of the reagent in the laboratory is noted, as "REFRIGERATOR" or "EMBEDDING CENTER." Also, at the bottom of each card is the source of the procedure and page number.

Although all of these procedures are in the department manual, these reference cards can be great time-savers. They are particularly advantageous for new department personnel. They can quickly learn our procedures, and at the same time find out the location of reagents, supplies, and equipment. In teaching institutions they enable the student to assemble all necessary reagents and stains in their proper order of use. This can eliminate last minute problems because of having forgotten a necessary item when a critical procedure is in progress.

While organizing a complete running inventory of this type may seem like a major chore, it is well worth the effort. A couple of people, working together, can complete the file cards in a few hours. After that, keeping the files current is only a matter of minutes. You simply add a new card when a new procedure is instituted, or when a new stain or reagent is purchased. Or you write a new date on an already prepared card when a solution or stain has been remade.

These file boxes not only indicate when supplies are low, but simplify re-ordering. With the manufacturer and order number listed on each card, we save the time previously used paging through catalogs looking for them.

Stability of More Commonly Used Special Staining Solutions

An Editorial

The information contained in this handout concerning the stability of solutions used in the more common special stains is presented in answer to the many requests made by technicians throughout the country.

An attempt was made to include information for the more commonly used special stains. The staining procedures can be found in: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. (Luna, L.G., Ed), New York, McGraw-Hill, Blakiston Division, 1968.

General Remarks

1. Check stock solutions periodically for signs of precipitate. This can be done by holding the bottle in front of a strong light; for example, a gooseneck lamp.
2. All solutions should be labeled with date solution is prepared and initialed by histotechnologist.
3. Prevent cross solution contamination.
4. Use distilled water for making solutions.
5. Keep solutions well stoppered at all times.
6. Refrigerated solutions should be poured into staining dish, and the bottled solution returned to the refrigerator before it warms to room temperature.
7. Return all staining solutions to shelves soon after use to prevent mix-up.
8. Always use clean glassware.
9. Insure that scales, pH meter, etc., are in good working order.

Note **An Asterisk Preceding Solution Indicates Solution Is Used Once and Discarded.**

Hematoxylin and Eosin

1. Mayer's hematoxylin	stock	2 months
	working	2 wk/or 500 slides, whichever comes first.
2. Harris' hematoxylin	stock	6 months
	working	2 wk/or 500 slides, whichever comes first.
3. Alcoholic eosin	stock	4 months
4. Aqueous Phloxine B	stock	4 months
5. Eosin-Phloxine B	working	2 weeks

Remarks
Microscopic evaluation of H&E is essential to determine hematoxylin breakdown. This determination cannot be made, however, unless the technician is well experienced in recognizing good quality hematoxylin and eosin stained preparations.

McManus (PAS)

1. *Periodic acid		2 months
2. Coleman's or Schiff's	(refrigerate)	3 months
3. Mayer's hematoxylin		6 months
4. Light green		1 month
5. *Diastase of malt	(refrigerate)	1 week

Remarks
Discard light green solution if there is some evidence of mold growth. Mold strands will be deposited on microscopic slide if solutions containing them are used for staining.

Gridley (fungi)

1. Chromic acid		2 months
2. Coleman's	(refrigerate)	3 months
3. Aldehyde fuchsin	(refrigerate)	2 months
4. Metanil yellow		2 months

Remarks
Aldehyde fuchsin stains strongly when new, but weakens and deteriorates with age. Weakened or deteriorated solution can be recognized by poor staining of elastic fibers and mast cells. Fresh aldehyde fuchsin solution is a must for good demonstration of some substances such as pancreatic islet cells or sulfated acid mucosaccharides.

Grocott (fungi)

1. Chromic acid		2 months
2. *Sodium bisulfite		4 months
3. Silver nitrate	(refrigerate)	2 weeks
4. Methenamine	(refrigerate)	1 month
5. Borax		6 months
6. *Working solution (consists of solutions 3, 4 & 5		24 hours
7. Gold chloride		6 months
8. *Sodium thiosulfate		6 months
9. Light green		2 months

Remarks
Every effort should be made to prevent contamination of silver nitrate solution with any type of metal. Gold chloride solution should not be reused more than 3 times.

Ziehl-Neelsen (bacteria)

1. Carbol fuchsin	1 month
2. *Acid alcohol	6 months
3. Methylene blue	3 months

Remarks
Carbol fuchsin solution should be discarded if a precipitate is noticed adhering to the sides of the bottle.

Brown and Brenn (bacteria)

1. Crystal violet	3 months
2. Sodium bicarbonate	6 months
3. Gram's iodine	2 months
4. Ethyl ether-acetone	1 month
5. Basic fuchsin	1 month
6. Picric acid-acetone	1 month

Remarks
All solutions are discarded after use. Ethyl ether is extremely volatile and flammable. Be sure all open flames and cigarettes are extinguished when using this solution.

MacCallum-Goodpasture (bacteria)

1. Goodpasture's	(refrigerate)	4 months
2. *Formalin		6 months
3. *Saturated aqueous picric acid		6 months
4. *Stirling's gentian violet		2 months
5. *Gram's iodine		6 months
6. *Aniline-xylene		24 hours

Giemsa

1. *Giemsa's solution	working	3 months
2. *Rosin alcohol	stock	3 months
	working	24 hours

Mowry Colloidal Iron (mucopolysaccharides)

1. *Glacial acetic acid, 3%		6 months
2. *Colloidal iron	working	24 hours
3. *HCl + $K_4Fe(CN)_6 3H_2O$		24 hours
4. Van Gieson's		4 months
5. *Hyaluronidase	(refrigerate)	2 weeks

Remarks
The hydrochloric acid potassium ferrocyanide solution must not be contaminated by introduction of any metal substance. Hyaluronidase crystals should be stored in the refrigerator.

Alcian Blue (acid mucopolysaccharides)

1. *Glacial acetic acid, 3%	4 months
2. Alcian blue	2 months
3. Kernechtrot	2 months

Manuel (reticulum)

1. *Uranium nitrate		1 month
2. *Fontana's silver	(refrigerate)	1 month
3. *Formalin		6 months
4. Gold chloride		6 months
5. *Sodium thiosulfate		6 months
6. Kernechtrot		2 months

Mallory (iron)

1. *Hydrochloric acid	stock	1 month
2. *Potassium ferrocyanide	stock	1 month
	working	24 hours
3. Kernechtrot		2 months

Remarks
Potassium ferrocyanide solution must not come in contact with metal of any kind. Most frequent problem arises when metal capped containers are used for storage of this solution.

Fontana Masson

1. *Fontana's silver	(refrigerate)	1 month
2. Gold chloride		6 months
3. *Sodium thiosulfate		6 months
4. Kernechtrot		2 months

Mayer (mucin)

1. Weigert's hematoxylin	stock	4 months
	working	1 week
2. Mucicarmine	stock	4 months
	working	1 month
3. Metanil yellow		2 months

Remarks
Weigert's hematoxylin (working) can be reused at least 3 times with satisfactory results. Mucicarmine solution is very stable but on occasion mold will form in the bottom of the container. This mold will deposit on stained microscopic slides.

Mallory (PTAH)

1. Zenker's	stock	2 months
	working	2 weeks
2. Alcoholic iodine		2 weeks
3. *Sodium thiosulfate		6 months
4. Phosphotungstic acid hematoxylin (PTAH) solution		Indefinite

Remarks
PTAH solutions are generally considered to have a long shelf life, but one should always keep close check on the solution. The best way to do this is to use a piece of cerebrum as a control. Nerve trunks, axons, dendrites, etc., stain deep bluish-purple. Other tissue elements stain salmon color and light purple.

Masson (trichrome)

1. Bouin's fixative		2 months
2. Weigert's hematoxylin	stock	4 months
	working	1 week
3. Biebrich scarlet-acid-fuchsin		2 months
4. *Phosphomolybdic-phosphotungstic	working	1 month
5. Aniline blue		2 months
6. *Glacial acetic acid		6 months

Additional Information for Prestaining Small Specimens

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The recent edition of HISTO-LOGIC (January, 1975) emphasizes the need for more frequent and continuing communication between histotechnologists.

We have been using a prestaining method for small tissue specimens for several years. For the past year we have gone one step further. Once a month we put 0.5 grams Eosin Y in the last dehydrant and found that specimen gross structural details show up much better in the paraffin block and subsequent ribbon. This simple step has eliminated the problem of cutting incomplete tissue sections since one can readily see the tissue margins. This suggestion also helps to locate small fragments of tissue during the embedding process.

Utilizing this procedure we discovered a serious problem: The embedding center used in our laboratory incorporates a vacuum system within the paraffin dispenser. We have actually seen small pieces of stained tissue come out of the spigot, contaminating the paraffin in the embedding mold. We have corrected this problem by inserting a polyfoam pad, covered with filter paper, on the bottom of the reservoir.

Editor's Corner

Did You Know

. . . that both sides of a microtome knife can be used for sectioning. Ed. Note: This is in reply to a question sent by Linda M. Clark, High Point, North Carolina.

. . . that Hexamethylenetetramine $(CH_2)_6N_4$, which is used in combination with silver nitrate in Gomori's methenamine silver, is also known as:

Methenamine
Hexamine
Hexamethyleneamine
Formin
Aminoform
Urotropine

. . . that better cell block preparations can be obtained by (1) spinning fluid, (2) pouring off supernatant, and (3) adding a small amount of melted agar to the sediment. At this point mix preparation, and allow to harden. Process specimen in conventional manner. Suggestion submitted by Gordon Mann, Victoria General Hospital, Winnipeg, Manitoba R3T 2E8.

. . . that it is necessary to neutralize free aldehyde groups after glutaraldehyde fixation, to produce positive mucosaccharide staining.

Tissue specimens fixed in glutaraldehyde produce two distinct problems when mucosaccharide procedures are performed: The mucosaccharides are less vividly stained with the alcian blue and colloidal iron staining procedure. Secondly, the periodic acid Schiff reaction produces a more intense, generalized diffuse staining of all tissue structures. Application of Schiff's reagent without oxidation with periodic acid yields similar intense staining, indicating that the glutaraldehyde treated specimens provide free aldehyde groups to react with the Schiff's reagent. Glutaraldehyde produced reactivity may be neutralized by the use of the following method.

Solution

88% Aniline Oil

Aniline oils	88.0 ml
Acetic acid, glacial	12.0 ml

Neutralizing Procedure

1. Decerate slides and run through absolute alcohol, 95% alcohol, 3 changes each.
2. Place slides in aniline oil solution for 1 hour.
3. Rinse slides quickly in 2 changes of 95% alcohol.
4. Wash slides in running tap water for 10 minutes.
5. Perform periodic acid Schiff procedure in the usual manner.

Remarks
Sections should be celloidinized if glycogen digestion with diastase of malt is to be performed. Celloidin (0.5 gm celloidin in 50 ml ether and 50 ml absolute alcohol), can be applied after the absolute alcohol used in step one. Slides are dipped in celloidin solution and allowed to dry on a clean dry surface for 45 minutes. Slides are then dipped in 80% alcohol and transferred to the aniline oil solution. Continue with procedure outlined above.

Reference
Janoff, M., et al: Am. J. Clin. Path., 44: No. 2, 167-171, 1965.

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The Publication That's Written by Its Readers

Now in its fifth year of publication, HISTO-LOGIC remains unique in its field. With the exception of material contributed by its Editor, Lee G. Luna, HISTO-LOGIC is entirely written by its readers. We know of no other news publication that can say the same.

The success of HISTO-LOGIC has always depended on its readers in two ways. First, they keep us informed about new and improved techniques they have developed in the laboratory. And second, they keep us informed about their particular reading interests in this highly specialized field.

HISTO-LOGIC has carried information about new procedures, changes in old procedures, improvements in staining or block preparation, new and more economical methods and techniques. It also published announcements of symposiums, workshops, and elections of officers in the various local societies. It pointed out the availability of special educational material — films, books, audio-visual aids. All have been widely read. All have been helpful in generating closer lines of communication throughout the profession.

Now once again, we want to remind you that your manuscripts are not only welcome, but essential for the continued success of the journal that serves your needs. Submit them to: Lee G. Luna, Editor, HISTO-LOGIC, P.O. Box 36, Lanham, Maryland 20801. Unless accompanied by a written request when submitted, no articles, photographs, etc., will be returned.

HISTO-LOGIC

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 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.

To receive your own personal copy of HISTO-LOGIC, or to have an associate added to the mailing list, submit home address to: Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 N. Aurora Rd., Naperville, Illinois 60540.