HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor A Technical Bulletin for Histotechnology Vol. III, No. 4 - October, 1973

Runners-Up
-- 1973 Golden Forceps Award

The following histotechnologists were in the final consideration for the 1973 Golden Forceps Award. Due to the significance of the technical contribution, their papers will be reconsidered, along with other papers submitted, for the 1974 Golden Forceps Award.

Sandra L. Green	"Automatic Staining Versus Hand Staining"
C. E. Potter	"Differential Staining Methods for Cells in the Central Nervous System"
Therese R. Ansman	"The Vacuum Infiltrator in Routine Fixation"
A. R. Villanueva	"Staining Fresh, Unfixed, Unembedded and Undecalcified Bone Sections"
Willa Mikowski	"A Simple Staining Procedure for Calcium Deposits"
Linda Marshall	"Preliminary Embedding in Agar-Agar"
William K. Gaines	"Gaines Iron Reaction"
Laurell Inman	"A New Technique for the Rapid Identification of Neoplastic Mast Cells"

Ziegler's Hematoxylin and Eosin Stain

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The most essential feature of this staining technique is the introduction of phosphotungstic acid as a self-limiting differentiating agent. The results are generally much sharper when this method of differentiation is used. In this paper, and as a result of much experience, we have been able to eliminate unnecessary data which appeared in a previous publication.[1] In addition, the general technical directions are more detailed and more explicit.

Important Considerations

Tissue slides, frozen section slides, and smears are made in the customary way. Slides are immersed in the solutions in the staining jars in the order listed below. Timing in most of the solutions is by *dips*. Each dip should be very slow, about one second per dip. Timing in the stains is longer, as indicated. Slides may be "rested" in the first and last xylene jars if convenient. *Alcohol*, as used in the technique, means absolute ethyl alcohol. This is an important feature for good results. *Slides should be dipped ten times (minimum) in each jar except for the staining jars.*

Staining Solutions

Normal Saline Solution

Sodium chloride	8.5 gm
Distilled water	1000.0 ml

2% Phosphotungstic Acid

Phosphotungstic acid	2.0 gm
Normal saline solution	100.0 ml

2% Sodium Citrate

Sodium citrate	2.0 gm
Normal saline solution	100.0 ml

Hematoxylins

Delafield's or Harris' preferred.

Eosin

Any conventional eosin may be used. (See remarks.)

Staining Procedure

1. Xylene.
2. Second xylene.
3. Alcohol.
4. Second alcohol.
5. Third alcohol.
6. Normal saline.
7. Hematoxylin (Delafield's or Harris'), 1 to 6 minutes.
8. Normal saline.
9. Phosphotungstic acid.
10. Normal saline.
11. Sodium citrate.
12. Normal saline.
13. Eosin, 1 to 2 minutes. (See remarks.)
14. Alcohol.
15. Second alcohol.
16. Third alcohol.
17. Xylene.
18. Second xylene.
19. Mount with resinous media.

Remarks

(1) It is recommended that alcohol and water soluble eosin be used. Also, that it be used in a concentration of from two to five percent. We prefer the stronger concentration. The timing in eosin is not critical. It can vary from one-half to two minutes depending on results desired. (2) Isopropyl alcohol cannot be used at it decolorizes the tissue sections. (3) Replace the solutions and stains as indicated by experience and by the

volume of work. (4) If the xylols and alcohols are used too long they will leave water or paraffin in the tissue sections. The sections will then be blurred and unsatisfactory. Run through a few pilot slides if there is doubt about solutions being too old. Change the normal saline washes frequently. (5) Keep staining jars and containers covered or closed when not in use. (6) Best results are usually obtained by fixing tissues in a neutral solution of formaldehyde. (7) After fixation, cytological smears may be started in container number six. (8) When fixed on the slide, frozen sections may be started in hematoxylin, container number seven. (9) The average time for the performance of this stain is only about ten to twelve minutes. In view of this fact, it is not feasible to adapt it to machine processing. (10) For frozen sections, timing can be cut a little if all of the solutions and stains are fresh enough. (11) This stain is an excellent differential stain. Practically all cells and intercellular substances will stain with different tints and densities of pink or blue-purple. (12) Deviations from the prescribed techniques usually give poor results. (13) After the original paper,[1] Tompkins and Miller developed an Iron-Hematoxylin-Phosphotungstic Acid stain for protozoa.[2] Lichtenstein[3] recommended the Ziegler H & E stain for neuropathologic work.

References

1. Ziegler, E.E.: Hematoxylin-Eosin Tissue Stain. Arch. Path., *37*:68-69, January, 1944.
2. Tompkins, V.N., and Miller, J.K.: Staining Intestinal Protozoa with Iron-Hematoxylin-Phosphotungstic Acid. Am. J. Clin. Path., *17*:755-758, September, 1947.
3. Lichtenstein, B.W.: *A Textbook of Neuropathology*, W. B. Saunders Co., pp. 427, 448, 449, 1949.

Finger Cots for Protection in the Histology Laboratory

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A constant hazard in histopathology work is the possibility of contracting some form of dermatitis from prolonged exposure to routinely-used chemicals such as xylene, formalin, picric acid, etc. The author can attest to this danger from personal experience. After 15 years as a histopathology technician, a condition developed noticeable as a deformation and discoloration of the fingernails of the right hand. Only the little finger was unaffected (Fig. 1). The dermatitis fortunately appears to be remediable.

The author believes that bare finger contact with xylene, an aromatic hydrocarbon, in the procedure of cleaning freshly cover-slipped slides was the most probable cause of the fingernail damage.[1] Because rubber is rapidly attacked by xylene, surgical (or other rubber) gloves are not suitable as a safeguard against this reagent. Introduction of a flexible, form-fitting glove or finger sheath with high resistance to xylene would be a welcome contribution to occupational safety and health in the laboratory.

In this laboratory a non-toxic, close-fitting, disposable glove made of polyvinyl chloride (PVC)[2] was tried. Although this plastic is not recommended for use with aromatic hydrocarbons in general, the PVC utility glove appears to provide a limited measure of protection, depending on length of exposure, against the action of xylene in histologic procedures. The glove, especially the fingers, should be examined and tested periodically in use and changed at the first sign of perforation.

Experience has shown that, for certain procedures, the most comfortable way to wear hand protection is in the form of finger cots, made by snipping off the fingers of the glove. With the PVC material, it was found that a second cot worn over the first provided better protection against xylene than a single cot, without undue loss of sensitivity.

If only the distal part of the thumb and fingers makes contact with an injurious chemical in a procedure, protective cots need be only long enough to terminate just before the middle joint of the fingers, leaving them easy to bend. If only two fingers and the thumb are required in an operation, as in cleaning a slide, only these need be covered (Fig. 2).

Finger cots are advantageous as a covering for ointment-smeared fingers. They effect closer contact of ointment and skin and prevent the medication from drying rapidly. The waterproof cots allow working in water with ointment-smeared or damaged fingers, and they make prolonged wearing of a protective cover more tolerable.

1. The U. S. Occupational Safety and Health Act of 1970 makes it a federal offense to risk life and health on the job in disregard of safety and health regulations and practices.
2. Available from laboratory supply houses.

Fig. 1: Contact dermatitis of fingernails showing disfigurement.

Fig. 2: Abbreviated PVC cots afford flexibility and short-term protection in various histologic procedures.

Marking Tissue with Mercurochrome

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Since mercurochrome survives processing but does not show up in stained slide, specimens can be marked for embedding as follows. Using an applicator stick, put a red dot on surface of tissue to be cut, blot on paper towel and process as usual. Embed red side down.

Multiple Helpful Hints

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1. The upper dome portion of a goose neck type lamp is helpful in the removal of the wrinkles from paraffin sections. The wrinkles are removed by gently touching the underside of the slide to the top of the lamp.
2. The rim of the flotation bath is used to smooth out wrinkles and convolutions in bone and cartilage sections.
3. Three helpful hints when cutting fragmenting tissues and blood: Wetting the block with warm water; exhaling on the block; cutting at a slower pace.
4. To prevent sections from sticking to forceps while separating sections on the water bath, place forceps on ice tray while cutting.
5. When cutting difficult sections or fragmenting sections, "subbed" slides are helpful in keeping the sections from floating off. Dip in the gelatin-potassium alum solution used in autoradiography. (0.5 gm gelatin and 0.05 gm chrome alum in 100 ml distilled water.)
6. Place blocks on ice trays to keep them chilled while cutting.
7. Wiping the knife bevel frequently with a xylene saturated gauze pad prevents artifacts.
8. Icing of the paraffin block before taking a ribbon assists in obtaining good thin sections and often obviates poor tissue impregnation.
9. An applicator stick can be used to aid in picking up and stretching ribbons on the water bath.
10. To prevent artifacts from collecting under sections, clean flotation bath frequently with paper towel.
11. The "diapering" of the microtome knife holder with gauze is helpful to catch paraffin debris and absorb water. The gauze can be changed as needed throughout the day.
12. Flotation bath should be scrubbed daily to prevent artifactual bacterial growth on microscopic slides.
13. To aid in shorter, more thorough fixation, a beaker of heated fixative (40-45° C) can be used; especially good for small biopsies that have been received late in the day.
14. To prevent tissue drying subsequent to sectioning, seal blocks by dipping in hot paraffin.
15. Use of a microscope to aid in differentiation of slides is essential to maintain high staining quality.
16. Keep an adequate supply of timers on hand for use with each staining procedure to prevent errors in staining.
17. Boxes of unstained control slides can be kept in the cutting area so that when special stains are cut, a control can be put in the same staining rack.
18. Stock chemical or dye solutions should be kept in alphabetical arrangement and labeled with percentage and content.
19. A central dehydrating and clearing area which is used after all special stain techniques is more economical and results in a saving of bench space.
20. Prolonged alcoholic storage of tissue causes excessive hardening and lyses of RBC.
21. Agar (0.25%) solution acts as a good flotation bath section adhesive and eliminates background staining.
22. A 5% solution of potassium ferricynide bleaches overstained silver nitrate reactions.
23. To cut an eye lens, soak cotton in warm water and place on cut surface of lens for 30-60 seconds.
24. Have you tried using Tetrahydrofuran (THF) after the last absolute alcohol in staining? The dehydrating and cleaning results appear to be improved.
25. Wrapping aluminum foil around the tops of alkali and acid containing bottles prevents fumes from spreading to other chemicals and stains.
26. To help remove difficult wrinkles from tissue, float tissue in 20-30% ethanol instead of plain water.
27. When tissue specimens are to be stored for long periods in 10% formalin, add 10 ml of glycerin to each 100 ml of 10% formalin. This will keep tissue soft and pliable.
28. To soften keratin, soak tissue specimen for 1 hour in either permanent wave lotion or depilatory lotion.
29. The addition of 1 drop of Tergitol #7 to every 100 ml of hematoxylin solution will greatly increase the speed of staining.
30. White karo syrup (blue label type) is good as a temporary mounting media.

Let's Get Our Facts Straight

An Editorial

Many hematoxylins are oxidized into hematein by the action of mercuric oxide. The question whether oxide red or oxide yellow is used is often asked. Since they are chemically identical, either can be used satisfactorily:

Mercuric oxide red is a red precipitate. HgO, Mol. Wt. 216.61. Hg 92.61%, O 7.39%.

Mercuric oxide yellow is a yellow precipitate. HgO, Mol. Wt. 216.61. Hg 92.61%, O 7.39%.

The main difference between the two is the size of the crystal. Mercuric oxide red crystals are larger than oxide yellow and therefore appear red. Mercuric oxide red turns yellow when the crystals are finely powdered. Mercuric oxide yellow is slightly more reactive due to the more finely powdered crystals. However, this in no way affects hematoxylin solutions. It has been proven, experimentally, that mercuric oxide red or yellow can be used in the compounding of hematoxylins with no noticeable chromatic variable in the hematoxylin staining reaction.

Simplifying Special Stains

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The method of labeling special stain bottles suggested herein has proven to be extremely efficient, practical, and has reduced the possibility of error. The method can be used with all special staining procedures. The PAS procedure is used as an example below.

Label bottles to include the following information: Method, step number, solution contained, exposure time required in that solution, and step which follows.

P.A.S. #1	*P.A.S. #2*	*P.A.S. #3*
0.5% Periodic Acid	Schiff's	Harris' Hematoxylin
5 minutes	15 minutes	3 minutes
distilled water	tap water	tap water
(Bottle 1)	(Bottle 2)	(Bottle 3)

The labels serve as an immediate reference to each staining step, eliminating the necessity of using staining manuals and/or staining procedures.

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Editor's Corner

New Jersey Seminar

The New Jersey State Society of Histotechnologists will present their Fall Seminar at Atlantic City, New Jersey, November 10-11, 1973. For information contact: Mr. Robert M. Bingman, Histology Laboratory, Atlantic City Hospital, Atlantic City, New Jersey 08401.

Minnesota Second Annual Conference

The Minnesota Society of Histotechnologists will hold their 2nd Annual Conference, November 1-4, at the Radisson South Hotel, Minneapolis, Minnesota. For information contact: Mr. Don Hammer, 12805 - 13th Avenue, North, Minneapolis, Minnesota 55441.

ASCP Good Histology Workshop

The all-day "Good Histology Workshop" will be presented at the ASCP-CAP meeting on October 25, 1973, in Chicago. For information contact: Joint ASCP-CAP Meeting Registration, P.O. Box 8013, Chicago, Illinois 60680.

International Histology Seminar

The First International Histology Seminar co-sponsored by Lab-Tek Products Division, Miles-Sankyo and Sakura Seiki will be held in Tokyo November 14, 1973. The theme of the symposium will be "How to Rationalize Histotechnology in Japan." The guest speaker will be Mr. Lee Luna, HT (ASCP) Editor of HISTO-LOGIC. One of the topics of discussion will revolve around the role and function of the histotechnician in the Japanese laboratory service. Mr. Luna will also discuss new developments in histopathology and the use of special stains and staining techniques. The entire project is under the direction of Dr. Akira Hirayama, M.D., Department of Pathology, Tokyo Women's College, Kawada-cho, Shinjuku, Tokyo.

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, write: Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 N. Aurora Rd., Naperville, Illinois 60540.