HISTO-LOGIC®

No reader should utilize materials and/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.

Editor, Lee G. Luna, D. Lit., H.T. (ASCP) | Technical Bulletin for Histotechnology Published: January, April, July, October | Vol. XI, No. 2 - April, 1981

Professional or Stone Age Histotech?

An Editorial

The illustration provided below may be considered somewhat comical, but if studied from a different perspective it can pose a rather serious question. That is, are you and I performing our duties in a professional or stone age manner? Each of us will have to answer that question individually, but let me implore you to conduct all facets of Histotechnology in a productive, innovative and professional manner. Always work toward providing better support to your pathologist and the patient, by cooperation and high-quality performance. In the long run you will contribute to the patient's well-being, improve the status of Histotechnology, and gain immense self-satisfaction along with the respect of your supervisors and peers. **Let's work together to eliminate stone age Histotechnology.**

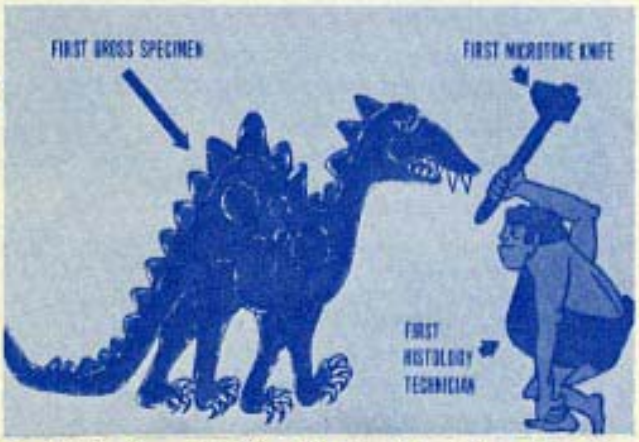

Illustration drawn by Richard Verfuerth, President of American HistoLabs, Inc.

Quick and Easy Slide Albuminizing

J. Ziaja, V. Granat and E. Gruebbel
Lutheran Medical Center
St. Louis, Missouri 63118

Pre-albuminized slides are easily prepared by filling a "Press-to-Seal" Moistener (of the type used by secretaries to moisten envelope flaps) with egg albumin. The egg albumin flows into the sponge at the end of the moistener and is then applied on a clean glass slide. Slides may be albuminized ahead, stored in a slide box and used as needed to pick up paraffin sections from the floatation bath.

This applicator saves having to finger spread individual drops of egg albumin on slides and eliminates the constant necessity of washing or wiping the egg albumin from your hands.

A Modified Aldehyde Thionin Method for Demonstrating Hepatitis B Surface Antigen (HBsAg)

Charles Churukian, B.A.
University of Rochester Medical Center
Rochester, New York 14642

Two widely used methods for demonstrating HBsAg are Shikata's[1] orcein method and Luna's[2] modification of Shikata's[1] aldehyde fuchsin technique. Shikata[1] reported that Paget's[3] aldehyde thionin procedure would also produce satisfactory staining results in demonstrating HBsAg, but this method has not come into widespread use. Problems have been reported with all of these staining methods. An awareness of these problems can help prevent most of the difficulties encountered with these methods.

Luna[2] listed four problems which have occurred with the orcein procedure. He reported that the orcein solution tends to break down sooner than aldehyde fuchsin, that there is difficulty in securing a satisfactory orcein, poor staining, and lack of contrast between HBsAg and background staining. Mowry[4] and Churukian[5] discuss problems which occur with aldehyde fuchsin methods. The majority of these problems are caused by using rosanilin (C.I. 42510) instead of pararosanilin (C.I. 42500) in preparing aldehyde fuchsin, not using fresh paraldehyde in preparing aldehyde fuchsin, insufficient ripening of aldehyde fuchsin, and using aldehyde fuchsin beyond its shelf-life. Paget[3] reported that some thionins made in England failed to produce satisfactory staining results in his aldehyde thionin method. It would seem reasonable to assume that by using thionin certified by the Biological Stain Commission in preparing aldehyde thionin, the possibility of unsatisfactory staining would be greatly reduced.

This modified aldehyde thionin method produces results as good as, if not slightly better than, the orcein and aldehyde fuchsin methods. There is less background stain; therefore, a nuclear counter stain can be used which gives good contrast and background detail.

Fixation:
10% buffered neutral formalin

Microtomy:
Cut paraffin sections at 6 microns

Solutions:

0.3% Acidified Potassium Permanganate (Stock)

Potassium permanganate	0.3 gm
Distilled water	100.0 ml
Sulfuric acid, concentrated	0.2 ml

For use, dilute with equal parts of distilled water. The shelf-life of the stock solution is about five days.

2% Oxalic Acid

Oxalic acid	2.0 gm
Distilled water	100.0 ml

Aldehyde Thionin

Thionin, C.I. 52000	0.5 gm
Alcohol, 70%	92.0 ml
Hydrochloric acid, concentrated	1.0 ml
Paraldehyde	7.0 ml

Allow to ripen in a tightly stoppered container at room temperature for three to five days. Store in a tightly stoppered container at room temperature.

Nuclear Fast Red (Kernechtrot)

Dissolve 0.1 gm nuclear fast red (C.I. 60760) in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, and add a few grains of thymol as a preservative.

Staining Procedure:

Use control slide.

1. Decerate and hydrate to distilled water.
2. Place in diluted acidified potassium permanganate for 5 minutes.
3. Rinse in 2 changes of distilled water.
4. Place in 2% oxalic acid for 5 minutes.
5. Wash in running tap water for 5 minutes.
6. Rinse in 2 changes of distilled water.
7. Place in 70% alcohol for 30 seconds.
8. Aldehyde thionin for 1½ to 8 hours. Longer staining times are necessary, as the solution gradually deteriorates.
9. Rinse in 2 changes of 70% alcohol.
10. Wash with 4 changes of distilled water.
11. Nuclear fast red for 5 minutes.
12. Rinse with 3 changes of distilled water.
13. Dehydrate in graded alcohols.
14. Clear in xylene, 3 or 4 changes.
15. Mount with resinous media.

Results:

HBsAg* — blue (diffuse cytoplasmic)
Copper-binding protein — deep blue, finely granular intracytoplasmic
Elastic fibers — blue
Erythrocytes — pink
Kupffer cells — sky blue
Lipofuchsin — sky blue
Nuclei — pink to reddish purple
Pituitary gonadotrops and thyrotrops — blue

Discussion:

The staining properties of aldehyde thionin are quite similar to those of aldehyde fuchsin. In addition to staining HBsAg, both solutions will stain copper-binding protein, elastic fibers, Kupffer cells, lipofuchsin, and pituitary gonadotrops and thyrotrops. Aldehyde fuchsin is also useful in staining pancreatic beta cells, but we have not been successful in demonstrating beta cells in formalin-fixed pancreas with the aldehyde thionin method. According to Paget[3] aldehyde thionin will only stain beta cells in chromate-fixed pancreas.

Copper-binding protein and lipofuchsin granules are similar in size and appearance when stained with aldehyde fuchsin and aldehyde thionin. Aldehyde fuchsin stains copper-binding protein and lipofuchsin with about the same intensity, which makes it difficult to distinguish the substances from one another. Aldehyde thionin stains copper-binding protein deep blue and lipofuchsin sky blue, therefore aldehyde thionin might be useful in demonstrating copper-binding protein.

Paget[3] reported the shelf-life of aldehyde thionin to be ten to fourteen days if stored in a tightly stoppered container. We have found its shelf-life to be about three weeks and have used it for up to seven weeks in staining HBsAg by increasing the staining time to eight hours. We have observed that aldehyde thionin keeps better if stored at room temperature.

Gomori[4] reported that aldehyde fuchsin had a shelf-life of about four weeks at room temperature and over six months if stored in the cold. Mowry[4] stated that he was not aware of anyone except Elftman[5] reporting that aldehyde fuchsin lasted longer if stored at room temperature than in the cold (5 °C). Because of our observation that aldehyde thionin keeps better at room temperature than in a refrigerator, we decided to see if this might also be true of aldehyde fuchsin. To our surprise we observed that aldehyde fuchsin keeps better at room temperature than in the refrigerator. We also observed that aldehyde fuchsin stored at room temperature forms much less precipitate with age than does aldehyde fuchsin stored in a refrigerator.

References:

1. Shikata, T., et al: Staining Methods of Australia Antigen in Paraffin Sections. Jap. J. Exp. Med., 44:25-36, 1974.
2. Luna, L. G.: Recommended Procedure for Demonstrating Hepatic B Antigen (HBAg) in Paraffin Sections. Histo-Logic, 8:119-121, 1978.
3. Paget, G. E.: Aldehyde Thionin: A Stain Having Similar Properties to Aldehyde-Fuchsin. Stain Technol., 34:223-226, 1959.
4. Gomori, G.: Aldehyde-Fuchsin: A New Stain for Elastic Tissue. Am. J. Clin. Path., 20:665-666, 1950.
5. Mowry, R. W.: Aldehyde Fuchsin Staining, Direct or After Oxidation: Problems and Remedies with Special Reference to Human Pancreatic B Cells, Pituitaries, and Elastic Fibers. Stain Technol., 53:141-154, 1978.
6. Elftman, H.: Aldehyde-Fuchsin for Pituitary Cytochemistry. J. Histochem. Cytochem., 7:98-100, 1959.

*Stains only HBsAg, not core Ag.

PLEASE NOTE

Final program for the 1981 NSH Symposium/Convention will appear in the June, Journal of Histotechnology and the July issue of Histo-Logic. Complete registration information will be included. If details are required before these dates, call Roberta Mosedale, 301/552-9678.

Microtomy and Staining of Cataract Lens

Susan Duncan
Good Samaritan Hospital
Pottsville, Pennsylvania 17901

By definition a cataract is a loss of transparency of any degree and for any reason in the lens of the eye. The lens must be clear to allow light to reach the macula in the retina; any imperfection to the lens affects sight. How badly it affects sight depends on the cataract. Although cataracts cause blurred vision, they do not cause excessive pain, tearing or redness. The lens has no blood supply and is the densest concentration of protein in the body. The lens retains its old cells as new cells form on its periphery.

Age is perhaps the biggest factor in the development of *senile* cataracts. Generally they will occur in both eyes. *Secondary* cataracts are caused by diabetes, glaucoma, retinal problems, ultraviolet light, x-ray, infrared, or a sharp blow to the eye (traumatic cataract). *Congenital*, the third type, occurs in newborns because the mother contracted German measles during the first three months of pregnancy.

Treatment of cataracts is usually surgery. It involves waiting until the cataracts are mature or ripe (finished with the four stages of development: incipient; intumescent; mature; hypermature). Under local or general anesthesia an incision is made at the limbus, lifting out the iris, and a small cut is made. The cataract lens is then removed. Phakoemulsification or ultrasound is another method where the lens is dissolved and suctioned out of the eye.

Removal of cataracts artifically makes the eye farsighted. Either cataract spectacles, contact lenses or intraocular lens are prescribed or implanted.

After numerous experiments, I have found the following procedure to produce the best results in attempting to section eye lens for microscopic study (Figs. 1 & 2).

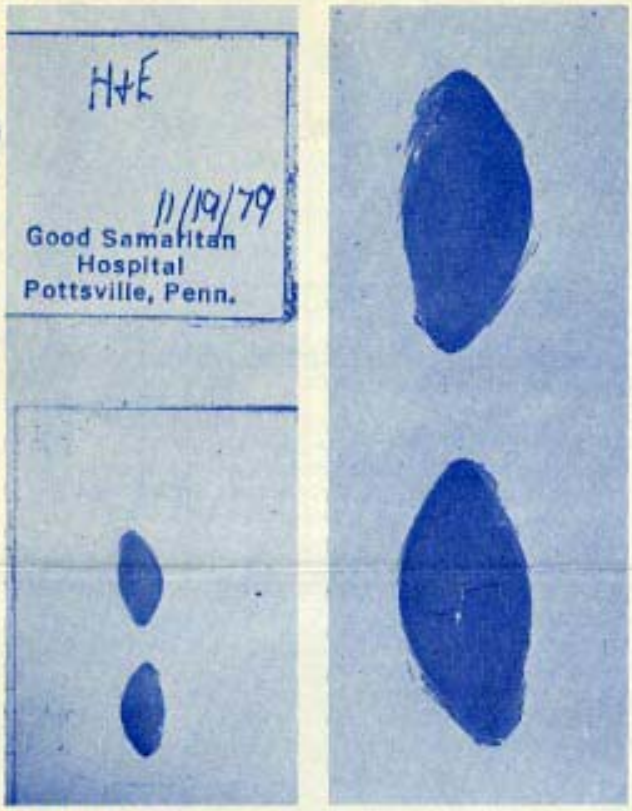

FIGURE 1 (left):
Picture shows a microscopic slide containing two lenses illustrating the quality of the procedure presented herein (X2).

FIGURE 2 (right):
Higher magnification of Figure 1, H&E (X10).

Fixation:
10% buffered formalin

Microtomy:
1. After routine tissue processing, the bisected cataract is embedded on its cut edges, then sectioned at 5 microns.
2. Temperature of the floatation bath may be slightly higher than the normal 48°C.
3. The use of gelatin and egg albumin will help sections adhere to the slide.
4. Rough cut the block slowly, then dampen a piece of gauze with warm water and hold firmly on the block surface for approximately 10 seconds. Move the knife to a clean area to produce sections free of wrinkles and fragmentation.

Stain sections with routine hematoxylin and eosin. Since cataracts are composed of protein which in turn are amino acids, iron and sometimes copper can be demonstrated with Perl's method for iron.

Perl's Method for Iron

10% potassium ferrocyanide solution (stock)
10% hydrochloric acid solution (stock)

Potassium Ferrocyanide-Hydrochloric Acid Solution (Working)

Potassium ferrocyanide (stock) ..... 70.0 ml
Hydrochloric acid (stock) ..... 30.0 ml
Mix just before use.

Nuclear Fast Red Solution

Dissolve 0.1 gm nuclear fast red in 100.0 ml of 5% solution of aluminum sulfate with aid of heat. Cool, filter, and add a grain of thymol as a preservative.

Staining Procedure:
1. Deparaffinize and hydrate to distilled water.
2. Stock potassium ferrocyanide solution for 5 minutes.
3. Working potassium ferrocyanide-hydrochloric acid solution for 20 minutes.
4. Rinse well in distilled water.
5. Counterstain in nuclear fast red solution for 5 minutes.
6. Wash well in running water.
7. Dehydrate in graded alcohols, then clear in xylene and mount with resinous media.

Results:
Ferric iron — bright blue
Nuclei — red
Cytoplasm — light pink
Copper — red
Uranium — brown
Nickel — greenish white or brown

References:
1. Luna, L. G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Edition, McGraw-Hill, New York, 1968, pp. 184-186.
2. Eden, J.: *The Eye Book.*
3. Berland, T., M.D., and Peritt, R. A., M.D.: *Living With Your Eye Operation.*

Can You Help?

Mequita D. Praet
Dept. of Pathology
Susie Parker Stringfellow Memorial Hospital
Anniston, Alabama 36201

I have a problem with Gram-positive and Gram-negative stains. For safety reasons we cannot keep ether in the hospital. My pathologist does not like the MacCallum-Goodpasture method for Gram-positive/Gram-negative bacteria. Is there any way to do the Brown and Brenn method without ether? Or, is there another method that gives similar results? It would be greatly appreciated if any readers of **Histo-Logic** can help with this problem.

Editorial Comment:
The Brown-Hopp's method does not require the use of ether. Secondly, we at the Armed Forces Institute of Pathology find this technique superior to either the MacCallum-Goodpasture or the Brown and Brenn. As a matter of fact, I personally have never demonstrated Gram-negative bacteria with the Brown and Brenn. The Brown-Hopp's procedure can be found in: Amer. J. Clin. Path., Vol. 60, No. 2, pp. 234-240, 1973.

Any help or advice may be forwarded directly to Ms. Praet with a copy of suggestion to the Editor of **Histo-Logic**.

Dr. John J. Duda
Technical Director of Laboratory
Brownsville General Hospital
125 Simpson Rd.
Brownsville, Pennsylvania 15417

I have been trying to find a reputable commercial laboratory to assay the room air in our histology laboratory for levels of formaldehyde and xylene to no avail. I did collect some samples for one firm, but after a prolonged period I received only a tentative report. Perhaps some of the **Histo-Logic** readers are having more success than I and if so, would they please share this information. Any information on the techniques or names of commercial laboratories performing these assays would be most appreciated.

Any help and advice may be forwarded directly to Dr. Duda with a copy to the Editor of **Histo-Logic**.

A Technical Bulletin for Histotechnology

HISTO-LOGIC®

Coming soon
Special Issue
10th Anniversary

LAB-TEK DIVISION
Miles Laboratories, Inc.
30W475 North Aurora Rd.
Naperville, IL 60540
Address Correction Requested

MOVED? NEW NAME?

Please print corrections below and return with old label to Lab-Tek. Mailing list updated quarterly. Allow 90 days for address corrections.

Name _____

New Address _____

City/State _____ Zip _____

Histopathology Staining Control Slide Program

This program was initiated in 1979 on the recommendation of the Board of Members of the American Registry of Pathology, a non-profit corporation established by Public Law 94-361 in 1976. The Board of Members consists of representatives of 30 professional societies that sponsor 27 registries at the Armed Forces Institute of Pathology.

The following Histopathology Control Slides are now available. Each box of 25 slides contains one stained slide and 24 unstained slides. A copy of the recommended staining procedure is included with each box of slides. These slides are not sold separately.

M. Tuberculosis	Spirochetes
M. Leprae	Cryptococcus
Gram-Negative Bacteria	Copper
Gram-Positive Bacteria	Hepatitis B Surface Antigen
Fungus	Legionnella Pneumophila
Amyloid	

The cost is $45.00 per box of 25 slides (check or money order) sent with the order, or $50.00 per box if a purchase order is sent. Shipping costs will be paid by the ARP within the U.S.A. and Canada. For shipments to other countries, an additional $7.50 will be required for airmail shipment.

NOTE: Make checks payable to the American Registry of Pathology.

Address inquiries to: Histopathology Control Slide Program; American Registry of Pathology (ARP); Armed Forces Institute of Pathology (AFIP); 14th Street at Alaska Avenue, N.W.; Washington, DC 20306.

If you have any questions, call: (202) 576-2043; (202) 576-2978; or (202) 576-2980.

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 Division, Miles Laboratories, Inc., 30W475 North Aurora Rd., Naperville, Illinois 60540.

Printed in U.S.A.

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.