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

Volume XII, No. 4 - October, 1982

PROFESSOR C.F.A. CULLING
JUNE 11, 1918 - JULY 13, 1982
— A TRIBUTE —

Professor C.F.A. Culling passed away on July 13, 1982. There is no doubt the world of Histotechnology has lost a close friend and outstanding educator.

In this brief note, I would like to outline some of his accomplishments, which in a way will tell us something of the man and will also indicate, in small measure, the loss that all facets of laboratory medicine will experience.

From our first meeting in 1973 to the last time I talked with him in May of 1982, Charles continually reflected a desire to contribute as:

- **An educator,** which is verified by the books and chapters he wrote on Histotechnology and Histochemistry. His contributions in this area are recognized worldwide.
- **A researcher,** which is verified by the 93 papers he published in medical and laboratory journals as the results of original research.
- **A teacher,** by providing over 300 hours of lectures and seminars at the National Society for Histotechnology from 1974 to 1981. This does not take into account the many other lectures, workshops and other facets of teaching he participated in at various national and international meetings.
- **A friend to the Histotechnology world,** since he never met a stranger and was always willing to devote many hours of discussion to answer inquiries so technicians who sought him out could learn from his enormous storage of information. He fondly referred to them as "the kids."
- **A hard worker,** who never refused to take on any responsibility for the National Society for Histotechnology. He presented all-day workshops and many lectures at the Symposium/Convention for many years and served as Associate Editor of the *Journal of Histotechnology*, providing many hours of advice. He was a strong advocate of high quality journalism. He served the Society as representative on the Biological Stain Commission and Chairman of the Awards Committee. He was often asked and always accepted varied duties which he felt would further the profession of Histotechnology.

Charles was a very effervescent person who possessed a magnetic personality. This added to his many other outstanding attributes which will be missed by all of us.

We may miss Charles Culling, the person, but we will always have Charles Culling, the great contributor to our profession. This will be assured by his many contributions which no doubt will remain with us for decades. From a personal standpoint, I have lost a wonderful friend and strong motivator.

Technical Correction — Method for Juxtaglomerular Granules

M. Konopka
Histopathology Department
Royal Alexandra Hospital for Children
Camperdown, N.S.W. 2050, Australia

We encountered certain difficulties when applying for the first time Smith's method for juxtaglomerular apparatus as given in the *AFIP Staining Manual*.[1]

After oxidization with potassium permanganate there follows decolorization with sodium thiosulphate (hypo). The decolorization did not occur, which resulted in the sections remaining brown. On completion of the staining procedure on these sections, it was noticed that the brown produced by the permanganate masked the staining reaction of the Biebrich scarlet/Ethyl violet. Decolorization with oxalic acid was then performed, giving a marked improvement, but the results were not completely satisfactory.

Reference to the original article as published by Carlton L. Smith[2] showed the decolorizing agent to be 1% sodium metabisulphite ($Na_2S_2O_5$), not sodium thiosulphate as published by the Armed Forces Institute of Pathology.

Satisfactory results on human tissue were obtained using the correct method.

Another noteworthy observation was in the recognition of the neutralization end point when preparing the Biebrich scarlet/Ethyl violet complex. No abrupt color change from red to violet occurred as described by the author. A good guide signalling the end point was the formation of the precipitate on the side of the flask upon swirling.

References:

1. Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, Third Edition, McGraw-Hill, New York, 1968.
2. Smith, C.L.: Rapid Demonstration of Juxtaglomerular Granules in Mammals and Birds. Stain Technology, 41:291-294, 1966.

Practical Stain-Technology "Wet" Workshop and Seminar — March 6-11, 1983

Presented by
Lee G. Luna, HT (ASCP)
The Center for Histotechnology Training

This five-day extensive wet workshop and seminar will afford the registrants the opportunity to perform 20 special stains, demonstrating more than 25 pathologic entities.

Some of the entities which will be stained with the special stains performed are: Gram-positive and Gram-negative bacteria; Hepatic B surface antigen ($HBSA_g$); inclusion bodies; pancreatic islet cells; all pathogenic fungi; calcium; acidic and sulfated mucosaccharides; hematologic elements; neutrophilic myeloid and mast cells; Lepra 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; melanin. 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.

"My Way"

An Editorial

The current generation has been called the "me" generation because self-gratification has become a primary concern. We see a rebellion against authority and an emphasis on self-expression or "doing our own thing." Does this attitude also find expression in our professional life?

Do we carefully outline all technical procedures and establish quality control and safety programs for CAP or JCHA inspection and then continue to "do our own thing" between inspections? Are we losing touch with history and our pride in the past by not realizing that when we follow many procedures we are following procedures worked out by Nobel laureates such as Golgi and Cajal or by other great scientists such as Weil, Weigert, Lillie and Gomori. The list is endless and the history is fascinating. However, history does not demand total adherence. There is certainly room for experimentation and method modification, but carefully controlled studies should be done before permanent changes are made.

Are we guilty of the old adage "if all else fails — follow the instructions"? Many examinees are not awarded valuable points on the Board of Registry HT/HTL practical examination because they do not submit the requested tissue or follow the outlined criteria. This occurs in spite of underlining and/or capitalizing certain important aspects of the examination instructions. A checklist is also provided on the HT examination to insure that the examinee has fulfilled all requirements. As professionals we must ask ourselves why this occurs and whether this is symptomatic of the attitude in our laboratories. Is it due to a lack of involvement in our profession, carelessness, hurry or just doing it "my way"?

Quality assurance or assuring the patient of the best care possible demands that we be intently involved and have pride in our work, that we carefully follow all technical and quality control procedures, and that the patient is *always* uppermost in our mind. For a true professional, "my way" should be a job done well, with pride, care and total involvement.

Spirochete Staining — An Inquiry Response

Brenda Cuevas, HT (ASCP)
Zona 6
Panama, Rep. de Panama

In reply to the problem that Mr. Rob Bosma has for identifying spirochaetes, (HISTO-LOGIC, Vol. XI, No. 3, July 1981), I would like to suggest the Steiner Technic for Spirochaetes. This procedure works very well on tissues fixed in formalin and saves a great deal of time.

Fixation:
10% buffered formalin. Avoid any fixatives which contain heavy metals.

Microtomy:
Cut paraffin sections at 3 or 4 microns.

Solutions:

5% Gum Mastic (Stock)

Gum mastic powder*	5.0 gm
Absolute alcohol, ethyl	100.0 ml

Let the solution stand but shake several times over a period of 2 days, then filter through a double layer of filter paper. The resultant filtrate should be clear and have a yellow color.

Uranium Nitrate — Gum Mastic Solution

Uranium nitrate	0.5 gm
Absolute alcohol, ethyl	40.0 ml
5% gum mastic (stock)	10.0 ml

This solution may be used not more than twice.

0.1% Silver Nitrate Solution

Silver nitrate (C.P.)	1.0 gm
Distilled water	1000.0 ml

This is a stable solution if kept in an amber glass stoppered bottle.

5% Catechol Solution

Catechol† (C.P.)	5.0 gm
Distilled water	100.0 ml

Remarks:
All glassware must be acid-cleaned. Use paraffin or teflon coated forceps. A control slide must be included.

Staining Procedure:

1. Deparaffinize slides in 2 changes of xylene.
2. Place slides in 2 changes of absolute alcohol.
3. Transfer slides from absolute alcohol to the uranium nitrate-gum mastic solution for 5 minutes.
4. Wash slides in 4 or 5 changes of distilled water, making sure milky appearance of the slides disappears.
5. Impregnate slides in freshly prepared 0.1% silver nitrate solution in a 60°C oven for 14-16 hours (overnight).
6. Prepare 5% Catechol aqueous solution and place in 60°C oven to pre-heat (see step #10).
7. Remove slides from the oven and wash 4 times in distilled water.
8. Dehydrate through fresh 95% ethyl alcohol and 2 changes of absolute alcohol.
9. Transfer slides for 5 minutes to a 2% gum mastic solution prepared as follows:

Gum mastic alcoholic solution 5% (stock)	20.0 cc
Absolute alcohol, ethyl	30.0 cc

10. Place slides in preheated 5% Catechol aqueous solution for 1 hour in a 60°C oven (see step #6).
11. Rinse slides 4 times in distilled water.
12. Wash slides in running water for at least 10 minutes.
13. Dehydrate slides slowly through 95% ethyl alcohol, absolute alcohol and clear in xylene.
14. Mount coverglass with resinous media.

Results:
Spirochaetes, bacteria and fungi — brown to black
Mast cell granules — yellow-brown
Background — yellow

Reference:
Steiner, G.: Modified Silver Stains for Microorganisms in Tissue. Amer. J. Clin. Path., 20:489-490, 1950.

*Gum mastic powder, N.F.; S.B. Penick and Company, New York
†Pyrocatechin or Orthodioxybenzene

Solution to Eosin Staining Problems

Brian Moore, B.S., R.T.
Veterinary Laboratory
Abbotsford, British Columbia, Canada V2S 4N8

Editor's Note: The following articles are in response to an inquiry regarding eosin staining which appeared in the Tenth Anniversary issue of HISTO-LOGIC.

The Tenth Anniversary issue of HISTO-LOGIC contains an article on eosin staining and pH dependence. I was very interested in this article as we also have faced this problem of inadequate staining of sections and rapid color leeching on differentiation.

We found that altering the pH had a considerable effect on the staining quality of different eosin brands. A literature search produced nothing to suggest a remedy. Our answer has been a buffered eosin solution as follows:

Solution One

Glacial acetic acid	5.75 ml
Distilled water	1000.0 ml

Solution Two

Sodium acetate	8.2 gm
Distilled water	1000.0 ml

Mix 295 ml of solution one with 705 ml of solution two. Add 5 grams eosin (C.I. No. 45380, dye content 82% or better). Add 2 crystals of thymol to prevent molds. The final pH should be 4.98. This gives a consistently reliable staining quality with a shelf life of several months.

Our H&E staining schedule is as follows:
1. Deparaffinize to water.
2. Harris' hematoxylin (with added glacial acetic acid), 1.5 minutes.
3. Wash in water.
4. Blue in base (1½% $NaHCO_3$ in H_2O).
5. Wash in water.
6. Differentiate for 5 seconds using 1% concentrated HCl in 95% alcohol.
7. Wash in water.
8. Blue in base.
9. Wash in water.
10. Buffered eosin, 20 seconds.
11. Wash in water.
12. Dehydrate in 95% absolute alcohol, clear in xylene.
13. Mount coverslip with resinous media.

We have used buffered eosin for three years and it gives excellent staining differentiation of muscle, collagen and R.B.C.

Tissue Surface Decalcification

Penelope Ferre, HT (ASCP)
West Coast Laboratory Services, Inc.
St. Petersburg, Florida 33705

Culling[1] states that bone specimens that have no previous decalcifying can be processed right along with soft tissue specimens, blocked in paraffin, trimmed with a scalpel, placed face down in 0.5% hydrochloric acid for a specified period (depending on the size of the bone), and sectioned with ease on the microtome.

I have used this technique with much success on tissue blocks containing unsuspected calcium deposits, improperly decalcified bone specimens, and bone marrow biopsies that were not decalcified beforehand. I have developed the following modification:

Place several 4" x 4" gauze pads in a glass staining dish. Saturate them with decalcifying solution of choice. After trimming (macrosectioning) the paraffin block and exposing the bone, place the block face down on the gauze pads saturated with decalcifying fluid. Tiny pieces of bone, such as a bone marrow biopsy chip, require approximately 20 to 30 minutes. Blocks of decalcified bone which are too hard to section require 10 to 20 minutes. Blocks with unsuspected calcium deposits take approximately 15 to 20 minutes.

If these blocks are trimmed first and placed on the decalcifying solution, they will usually be ready to section by the time one is finished sectioning the regular daily load of surgical blocks.

Reference:
1. Culling, C.F.A.: *Handbook of Histopathological and Histochemical Techniques*, Third Edition, 1974.

New Fixative For Frozen Sections

Franklin J. Rude, M.D.
Debbie Schmidt
Michelle Maurer
Memorial Hospital
Nacogdoches, Texas 75961

Shrinkage of cytoplasm and unreliable nuclear staining were recurring problems with alcohol (100% isopropyl) fixation of frozen section slides. This was especially true on endometrial cases. Several texts recommended formalin, especially warm formalin, to be the preferred fixative. We found that 10% warm buffered formalin alone still allowed cytoplasmic shrinkage, although it did enhance the nuclear detail. Alcohol followed by formalin was no better than formalin alone.

Bouin's fixative, having acetic acid to prevent shrinkage, was tried with good success, but formalin was still needed to enhance chromatin detail. Formalin after the Bouin's completely washes out the picric acid in just a few seconds, unlike water or alcohol. We hope you will try this procedure in your laboratory and find it as helpful as we have.

Procedure:
1. Cut frozen sections at 6-8 microns.
2. Dip in Bouin's for 15-20 seconds without agitation.
3. Dip in Bouin's for 10-15 seconds with agitation.
4. Wash in either distilled water or tap water for a few seconds.
5. Dip in warm (56°C) formalin until yellow is gone.
6. Wash again in DI or tap water for a few seconds.
7. Proceed with your routine H&E for frozen sections.

HISTO-LOGIC®
A Technical Bulletin for Histotechnology

LAB-TEK DIVISION
Miles Laboratories, Inc.
30W475 North Aurora Rd.
Naperville, IL 60566
Address Correction Requested

Lab-Tek Division MILES

☐ NEW NAME ☐ NEW ADDRESS ☐ NEW SUBSCRIPTION

Please PRINT correct information below and return with old label (except new subscribers) to Lab-Tek. Mailing list updated quarterly. Allow 90 days for change to be made.

Name _____

Address _____

City, State _____ Zip _____

☐ Please take my name off the **Histo-Logic** mailing list. (Attach old label.)

BULK RATE
U.S. POSTAGE
PAID
PERMIT NO. 4954
Chicago, Illinois

Histotechnology Training — Help Us Meet the Need

The National Society for Histotechnology is interested in improving practical training in the field of histotechnology. It is recognized that the conventional seminar-type training is beneficial, but inadequate. We are convinced that "on-the-job refresher courses" will provide the necessary training to upgrade the quality of histotechnology worldwide.

In order for the Society to provide information to the many national and international requests received in this regard, we are soliciting your assistance. It is requested that any hospital, university, research activity, etc., willing to accept histotechnologists for refresher and/or advance training from a week to several months, notify NSH. It is most important that you provide the area of training you would be willing to conduct; i.e., electron microscopy, immunopathology, surgical histotechnology, eye, bone, special stains, medical photography, etc. Please include the name of the person to contact for correspondence purposes and complete mailing address of your institution.

Your assistance in this project is greatly appreciated, particularly since it will provide training information to anyone desiring to further their education in histotechnology. Send information to: Roberta Mosedale; National Society for Histotechnology; P.O. Box 36; Lanham, MD 20706.

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 60566
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 20706. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.