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. 3 - July, 1981

Carbol-Xylene (A Useful Tool)

An Editorial

Carbol*-xylene was a very useful "tool" in the histopathology laboratory some years ago, primarily due to its softening effect on tissue sections embedded in celloidin. Sections obtained from celloidin-embedded specimens had a tendency to harden when exposed to xylene, resulting in section curling. This often produced wrinkles and, of course, distortion of tissue section. Carbol-xylene was used extensively during the celloidin era to soften these sections and allow them to uncurl and lie flat against the slide prior to the coverslip mounting. The strong emphasis on the use of paraffin over the last fifteen years has relegated carbol-xylene to the disuse status. However, this solution can still be very useful on occasion when one is experiencing detachment of sections from the slide, causing section "foldover." This is a very useful solution for tissues such as bone, chitin, or sections consisting of fibrous components, all of which have a tendency to dislodge from the glass slide.

Solution:

Carbol-Xylene

Phenol (carbolic acid)	25.0 gm
Xylene	75.0 ml

The procedure is simple to use by employing the following directions:

1. A camel's hair brush is dipped into the solution of carbol-xylene.
2. The brush is then applied to the folded portion of the specimen. (The application of carbol-xylene softens the section and allows easy unfolding by a gentle touch with the brush.)
3. Remove excess carbol-xylene from section. (If carbol-xylene is left on the section, tissue will fade.)
4. Mount coverslip with a resinous media.

*Phenol or carbolic acid

Liver Trichrome Stain

William Dotson
The North Carolina Memorial Hospital
Chapel Hill, North Carolina 27514

The "liver trichrome" procedure is one of a battery of stains we do on all our liver biopsies. The pathologists seem to like it better than the Masson Trichrome, in this case, for its sharper color contrast. It is being presented here in the hope others will find it beneficial.

Fixation:
10% buffered formalin or Helly's solution

Microtomy:
Cut paraffin sections at 2 microns or desired thickness.

Solutions:

Alcoholic Picric Acid (Stock)

Picric acid	7.0 gm
Ethanol, 95%	100.0 ml

Alcoholic Picric Acid (Working)

Alcoholic Picric acid (stock)	2 parts
Ethanol, 95%	1 part

5% Aniline Blue

Anlline blue	5.0 gm
Acetic acid	2.5 ml
Distilled water	100.0 ml

1% Ponceau de Xylidine (Ponceau 2R)

Ponceau de xylidine (Harleco)	1.0 gm
Acetic acid	1.0 ml
Distilled water	100.0 ml

1% Acetic Acid

Acetic acid	1.0 ml
Distilled water	100.0 ml

1% Phosphomolybdic Acid

Phosphomolybdic acid	1.0 gm
Distilled water	100.0 ml

Staining Procedure:

1. Deparaffinize slides and hydrate to distilled water.
2. Stain slides in Harris' hematoxylin in 85°C for 5 minutes.
3. Dip slide once in 95% ethanol.
4. Place slides in working alcoholic picric acid for 10 minutes.
5. Wash slides in running tap water for 10 minutes.
6. Stain slides in ponceau de xylidine for 5 minutes.
7. Treat slides with 1% phosphomolybdic acid for 5 minutes (use solution only once).
8. Stain slides in 5% aniline blue for 2 minutes.
9. Rinse slides quickly in tap water to remove excess aniline blue.
10. Treat slides with 1% phosphomolybdic acid for 5 minutes.
11. Place slides in 1% acetic acid for 5 minutes.
12. Dehydrate, clear and mount coverslip with resinous mounting media.

Results:
Connective tissue and collagen — blue
Muscle and cytoplasm — red
Nuclei — purple

Making a Desk-Top Out of a Drawer

Jacob Lundy & Robert Moore
U.S. Army Biomedical Laboratory
Aberdeen Proving Ground, Maryland 21010

It is frequently advantageous to be able to provide an extra working or writing surface at a counter or desk. Some desks have such a facility at one or both ends in the form of a pull-out top.

It is not difficult to make such a utility out of any drawer

under a counter top, especially in the modular-type cabinet systems now popular, because in these cabinets the drawer-wells are considerably larger than the drawers.

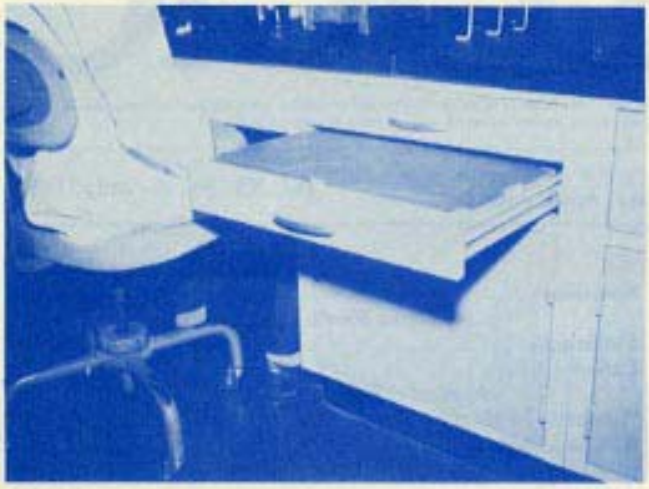

FIGURE 1.

A flat, rigid board, about 1 cm thick and as wide as the drawer, is obtained to rest upon the right and left sides of the drawer (Figure 1). A board at least three-quarters as deep (front-to-back) as the drawer provides an adequate working surface for many purposes, including use as a stand for a dual-viewing microscope. On the underside of the board, two small rubber feet are fastened (by screws or tacks) in a vertical line near the right and left sides, as guides (Figure 2). The rubber guides are positioned at a distance from the edges of the board to hardly touch the sides of the drawer with the board in place, thus assuring a free back-and-forth movement without sideway. The distance the board-top can be pushed back, and the area of inner drawer-space exposed, depends on the location of the guides at the rear of the board; that is, the top can be pushed back until the rear guides strike the back side of the drawer.

To determine the proper location of the 2 gear guides, the board is laid on the right and left sides of the drawer with its back edge coinciding with the edge of the back side of the drawer. If, with the board in this position, access to the drawer is adequate, the rear guides are attached against the rear edge on the underside of the board. If, however, a greater opening is desired, the rear guides are secured on the board at a distance from the rear edge equal to the additional millimeters of opening desired.

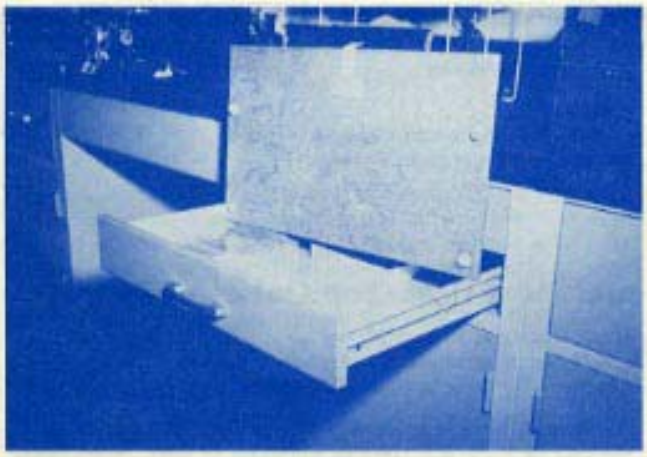

FIGURE 2.

A handy pull for manipulating the drawer-top can be made from a length of tape. Contact paper may provide a smoother surface and a more attractive appearance. In a cabinet where the drawer-well is too small to allow the entrance of a board atop the drawer sides, the board must be removed for reaching and looking into, and for closing, the drawer.

Note: The authors are thankful to Mr. Ray Clawson for the photography.

Muscle Quenching with Liquid Nitrogen and Talcum Powder

Leonard Noble and Venkata Challa, M.D.
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Winston-Salem, North Carolina 27103

To achieve enzyme localization and to eliminate ice-crystal artifacts in muscle biopsies, the use of liquid nitrogen as a coolant is prescribed. Isopentane and various fluorocarbons can be cooled down by liquid nitrogen to a temperature which will adequately quench muscle biopsies. However, the use of these agents in freezing techniques has now become outdated due mainly to storage and health hazards.

We feel that the method of choice is to use liquid nitrogen alone. However, to accomplish this, something must be done to prevent the gaseous layer which forms around tissue when thrust into liquid nitrogen. This layer of gas acts as an insulator and does not allow for artifact-free freezing. For this reason, a coating of talcum powder is put on the tissue prior to freezing to eliminate the gaseous layer. As the powder falls away from the specimen, the gas layer is eliminated. The biopsy itself is anchored in place with a talcum powder-OCT paste which we have devised to take the place of the more often used gum tragacanth.

Moline and Glenner[1] reported the talcum powder coating principle for rapid quenching of tissue specimens in 1964. Their experiments clearly showed that adequate quenching of tissue is possible using liquid nitrogen alone and not in combination with hydrocarbons or fluorocarbons.

When adapted to muscle biopsies routinely, this technique is safe and relatively inexpensive. Freezing can actually take place in an area adjacent to the biopsy room, thus avoiding any contact with saline. After quenching, the specimen remains in the liquid nitrogen until it is transported to the laboratory.

Materials:
Talcum powder
Liquid nitrogen
OCT embedding compound*
Cryostat chucks
Cork discs (2.4 cm in circumference x 0.4 cm in thickness)†
Dewar flask - 1 liter capacity

Solutions:
Talcum powder-OCT paste
To make, mix small amount of OCT embedding compound with talcum powder until a paste-like consistency is obtained. Discard any leftover paste after freezing specimen.

Method:
For best results, the biopsy should be 6 to 7 mm long and no more than 5 mm in cross section. If the dimensions are any longer, subsequent trimming may be necessary.

1. Cover one side of a cork disc with a generous amount of the talcum powder-OCT paste. Position the biopsy in the paste in such a way as to obtain a cross section of the muscle fibers when sectioning.
2. Coat the entire surface of the specimen as well as the paste and the cork disc with the talcum powder.

National Society for Histotechnology Symposium/Convention
November 16-20, 1981 Salt Lake City, Utah

The Seventh Annual Symposium/Convention of the National Society for Histotechnology will be conducted at the Little America Hotel, Salt Lake City, Utah. The enclosed program is complete with hotel reservation card and registration form. The convention will utilize 425 sleeping rooms in the Little America, with overflow accommodations in the Tri-Arc Hotel, 801/521-7373 (which will also accommodate several workshops), and the Hilton Inn, 801/532-3344. Both hotels are within a half block of the Little America and are in walking distance. All room reservations will be processed through the Little America. When this hotel is filled, reservations will be forwarded to other hotels for accommodations. PLEASE MAKE YOUR RESERVATIONS EARLY SINCE ALL ROOMS BLOCKED FOR NSH WILL BE RELEASED ONE MONTH PRIOR TO MEETING DATE.

Mail Hotel Reservation Directly to: Little America, 500 South Main, Salt Lake City, Utah 84101; 801/363-6781.

Symposium registration application may be photocopied if more than one individual from the same activity desires to attend. To avoid delays and unnecessary complications, registrations AWAITING FUND APPROVAL will be accepted and held in abeyance until final commitment is received. Please include a note to this effect on your registration form.

To avoid a LATE REGISTRATION CHARGE, be sure your registration is received prior to NOVEMBER 10th. Late registrations and "walk-ins" at the meeting will be assessed a $10 LATE FEE.

REIMBURSEMENT of registration fees will be made upon receipt of cancellation notification prior to November 10th. NO REFUNDS WILL BE MADE AFTER THIS DATE. Refunds for unattended workshops, sessions or banquet ticket, WILL NOT be made after arrival to the meeting. Refund will not be made when changing workshop attendance after arrival to the meeting.

For clarification or assistance, please call Roberta Mosedale, NSH Office, 301/552-9678. MAIL registration and check to: NSH, P.O. BOX 36, LANHAM, MARYLAND 20706.

NSH/Thomas Edison Program Schedule

COURSE REVIEWS: Students interested in review sessions should enroll in one of the workshops scheduled Monday. Workshop registration is free to students formally enrolled in Thomas Edison College. STUDENT MUST SEND PROOF OF ENROLLMENT WITH REGISTRATION FORM.

Tuesday review sessions are only for those planning to take examinations during the convention week, and will allow informal discussion with faculty members, and an opportunity for the examinee to identify and review weak areas. Examinees are encouraged to attend both sessions.

INTRODUCTORY HISTOTECHNOLOGY/HISTOCHEMISTRY
(Richard Schroeder) HT (ASCP)
Monday - see Workshop #3
Tuesday review, Nov. 17: Powell Room 9 AM - Noon

HUMAN MICROSCOPIC ANATOMY
(Freida Carson, Ph.D.)
Monday - see Workshop #4
Tuesday review, Nov. 17: Hayden Room 9 AM - Noon

CURRENT CONCEPTS IN DIAGNOSTIC HISTOPATHOLOGY
(Jules Elias, M.A.)
Monday - see Workshop #5
Tuesday review, Nov. 17: Escalante Room 9 AM - Noon

EXAMINATION SCHEDULE:
Wednesday, Thursday & Friday, 7-9 AM, BRIDGER ROOM, 2nd floor

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Reservations must be received 2 weeks prior to the opening date of the meeting. Reservations received after 2 week cut off will be on a space available basis only. First night's deposit or payment guaranteed by credit card required.

If a room at the rate requested is unavailable, one at the nearest available rate will be reserved.

All rooms subject to applicable city tax.

NATIONAL SOCIETY OF HISTOTECHNOLOGY
November 16 - 20, 1981 ROOM RESERVATION REQUEST

NAME _____

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CITY _____ STATE/ZIP _____

GROUP NAME _____

I will be arriving _____ AM PM on _____ DAY DATE

I will depart _____ AM PM on _____ DAY DATE

Credit Card Guar. # _____ Exp. Date _____ Type of Card _____

ACCOMMODATIONS

Please reserve the following:		Tower
SINGLE OCCUPANCY	$50	$65
DOUBLE OCCUPANCY (2 persons, 1 bed)	$56	$71
TWIN OCCUPANCY (2 persons, 2 beds)	$56	$71

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Employer: _____

Address: _____
(street)

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Work Telephone No. ( ) _____
area code

Prefer Home_____ Work_____ address for mail.

This is your first attendance to an NSH Symposium/Convention. Yes_____

Are you an NSH Member? Yes_____ No_____

Please check functions you desire to attend and complete Registration.

DO NOT USE THIS SPACE

LATE REGISTRATION FEE OF $10 required after November 10, 1981.
CANADIAN registrants please remit fees in U.S. currency.
Make check/money order payable to: National Society for Histotechnology
MAIL complete form & fees to: NSH
P. O. Box 36
Lanham, Maryland 20706

Workshop Program
Monday, Nov. 16

All Day	Morning	Afternoon
#1___	#7___	#10___
#2___	#8___	#11___
#3___	#9___	repeat PM___
#4___		
#5	8-11 PM	#12___
#6___		

Workshop Program
Tuesday, Nov. 17

All Day	Morning	Afternoon
#13___	#17___	#21___
#14___	#18___	#22___
#15___	#19___	#23___
#16___	#20___	#24___
	#20 repeat	PM___

Wednesday, Thursday & Friday

Scientific Sessions_____
Thursday Banquet_____

Wednesday, Open Seminar on Parliamentary Procedure, 7-9 PM: no charge_____

Workshop Registration

	Member Fees	Non-Member Fees
#1	___$40	___$45
#2	___$40	___$45
#3	___$40	___$45
#4	___$40	___$45
#5	___$40	___$45
#6	___$40	___$45
#7	___$20	___$25
#8	___$20	___$25
#9	___$20	___$25
#10	___$20	___$25
#11	___$20	___$25
#12	___$20	___$25
#13	___$40	___$45
#14	___$40	___$45
#15	___$40	___$45
#16	___$40	___$45
#17	___$20	___$25
#18	___$20	___$25
#19	___$20	___$25
#20	___$20	___$25
#21	___$20	___$25
#22	___$20	___$25
#23	___$20	___$25
#24	___$20	___$25
Scientific Sessions	___$50	___$60
Banquet	___$18	___$18
$10 LATE FEE (after Nov. 10th)	____	____
Total Fee: $	_____	$_____

Workshops

Monday, November 16, 1981

No. 1: Introduction to Stain Mechanisms
Jack Wenger, HT(ASCP) **8:30 AM - 4:30 PM**
Workshop is presented in lecture form with the following subjects discussed in depth: Introduction to electrolytes; salt-protein formations; iso-electric points and how to use them; mordant dyeing; practical applications and various facets of silver reactions, including mechanism of argyrophil and argenaffin reactions, principles of bacterial staining and mineral reactions. Advanced level workshop.

No. 2: The Use of Histochemistry in Diagnostic Pathology: An Important Role of Histotechnology in Patient Care
Hugh A. McAllister, M.D., COL, USA) **8:30 AM - 4:30 PM**
The primary objective of this workshop is to discuss the utilization of special histochemical stains in establishing diagnoses. This role of the histotechnologist in patient care will be illustrated with actual case presentations, as appropriate. Emphasis will be placed on the practical approach to diagnosis of various disease categories.

No. 3: Introductory Histotechnology/Histochemistry
(Richard Schroeder, M.A.) **8:30 AM - 4:30 PM**
Workshop is designed as an introduction and refresher for the discipline of histotechnology. Concepts of fixation, tissue preparation, sectioning and staining will be presented. Staining procedures utilized as routine in histopathology laboratories; i.e., carbohydrates, lipids, proteins, minerals, bacteria, are discussed. The histochemistry program provides the participant with a more in-depth understanding of routine and sophisticated procedure mechanisms. Cryostat and cryogenic techniques are discussed.

No. 4: Human Microscopic Anatomy
(Freida Carson, Ph.D.) **8:30 AM - 4:30 PM**
Workshop will emphasize basic tissues and their organization into the different organs. Visual identification and function will be included. Participants will be given a post-test, and handout material will be in a work book format to be filled in during the workshop. Workshop is recommended for Thomas Edison students.

No. 5: Current Concepts in Diagnostic Histopathology
Dales Elias, M.A.) **8:30 AM - 4:30 PM**
A unique course which encompasses the recommended procedures for handling surgical specimens in the histopathology laboratory. The intimate details of resection as they apply to the handling of small biopsy specimens as well as the rules for grossing tissue from the major human organ systems are included. The course is geared to the experienced histotechnologist working in a clinical environment. It is advisable that the participant purchase a copy of "Basic Techniques in Diagnostic Histopathology" by A. Kennedy, Churchill Livingstone, New York, 1977.

No. 6: Immunoperoxidase Techniques Utilized in Both Research and Clinical Settings
(Diane Miller, HT(ASCP) & Kay Jenkins, HTL(ASCP)) **8:30 AM - 4:30 PM**
Limit: 40
Workshop will include demonstrations of herpes, toxoplasmosis, viruses, etc. Monoclonal antibodies: Both indirect method and direct methods that are used in a research and clinical setting. Detailed information will be provided in lecture form and handouts.

No. 7: Principles of Enzyme Histochemistry
(Richard W. Dapson, Ph.D.) **8:30 AM - 12 Noon**
Workshop will present an organized treatment of a seemingly bewildering array of procedures. Proper fixation is critical to immobilizing the enzyme in a functioning state. Following that, there are essentially five ways in which a colored product can be produced to mark the site of endogenous enzymes: Direct synthesis, simultaneous coupling, post-incubation coupling, formation and demonstration of metallic salts, and reduction reactions. Emphasis will be on theoretical aspects so that participants can understand and correct problems experienced with these tests in the laboratory.

No. 8: Time Management
(Renwick Nicholls, B.A.) **8:30 AM - 12 Noon**
Limit: 30
Developing effective time management is a very important skill that allows you to extend yourself and stretch your abilities. In today's laboratory, time management is a vital skill. At this workshop you will develop forceful plans that will help you learn how to work smarter, not harder, and increase your productivity.

No. 9: Water Soluble Embedding Media for Light Microscopy
(Jane Woodruff & Diane Barics, HTL(ASCP)) 8:30 AM - 12 Noon
Limit: 25 Repeated 1:00 - 4:30 PM

Recently there has been an increased interest in the use of plastic embedding for many histopathologic techniques. One of the most versatile and widely used plastics is the water soluble JB-4 Embedding Kit™. This workshop will focus on the advantages and disadvantages of using the JB-4 embedding plastic as compared with the more traditional embedding medium, paraffin. In the course of the workshop, the participants will experience the relative ease of embedding, sectioning and staining with the JB-4 Embedding Kit™.

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No. 10: Practical Immunopathology: Fluorescence Microscopy (& PAP's)
(Charles Culling, Prof.) 1 - 4:30 PM

This will be a lecture discussion workshop and will cover basic immunology, routine and epi-fluorescence and the peroxidase, anti-peroxidase (PAP) techniques, their theory and application in practical immunopathology.

No. 11: Laboratory Calculations
(Dale Largent, HT, MT(ASCP)) 1 - 4:30 PM

Workshop is designed to give a basic knowledge of preparation of molar and normal solutions and the correct calculations for weighing and diluting solutions. Prior knowledge of units of measurement and atomic weights may be beneficial to participants. Bring your calculators.

Workshop 12: Instructional Techniques Used in Teaching 8-11 PM
(Janet Mazza, HTL (ASCP), Jules Elias, M.A., & Prof. Charles Culling)

Tele-net conferences explained by Dr. Elmer Konneman, Editor of Laboratory Medicine; small group instruction on student attitude towards returning to school, writing goals for a curriculum, effective communication, writing behavioral objectives, preparation of learner activity list, teacher activity list and instructional material list, evaluation techniques, adjusting instruction to learner readiness and making simple visual aids. Workshop is recommended for individuals that have a degree *or* currently enrolled in school or contemplating returning to school.

Tuesday, November 17, 1981

No. 13: Tissue Identification
(Lee G. Luna, HT(ASCP) & Edna Prophet, B. S., HT(ASCP)) 8:30 AM - 4:30 PM

Primary objective of this workshop is to give each participant a basic knowledge of the microscopic structures of some of the commonly processed organs in the histopathology laboratory. It is anticipated that each histotechnologist will be sufficiently motivated to do further study on his/her own to gain in-depth knowledge of histology. The knowledge gained can then be applied to determining properly stained slides. In addition to learning the morphology, participants will be taught how to recognize proper staining qualities of numerous special stains.

No. 14: Photography in the Laboratory
(Robert Kershaw) 8:30 AM - 4:30 PM

Workshop will include lecture and demonstrations in the areas of both photomicrography and gross specimen photography. The information will cover all phases of instrumentation, types of film, technique and trouble shooting routine problems.

Photomicrography:
a. The microscope
b. The specimen
c. The camera
d. Film and processing
e. Projection

Gross Specimen Photography:
a. The camera
b. Lighting
c. Film and processing
d. Projection

Slide presentation covering both areas of photography will be presented. Question and answer session will cover specific problems presented by attendees.

No. 15: Moving Management for a Safer Workplace
(Diane Barics, HTL(ASCP), Walter Scott, Ph.D., & Jane Woodruff) 8:30 AM - 4:30 PM

Presentation will demonstrate methods for the technologists in advising management in safer workplace needs. The handling and usage of plastics and related compounds will be discussed. Employee environmental problems will be identified. Methods and resources for gaining management's cooperation in providing a healthy climate will be practiced.

No. 16: If the Student Hasn't Learned, the Instructor Hasn't Taught
(Elaine Boyd, HT(ASCP)) 8:30 AM - 4:30 PM
Limit: 40

Objective of this workshop is to assist instructors in becoming proficient in techniques and methods that make for successful, productive teaching. It is designed to provide orientation and skill development in preparation and presentation of the instructional materials to students through instructional strategies. The workshop is centered around the skills each instructor needs to productively teach, and evaluate the student's progress in the classroom or laboratory. It includes concepts of teacher evaluation by the student. Modules which have been established to achieve the purpose of this workshop are:
A. Basic concepts of teaching with emphasis on how student learns.
B. Examining variety of teaching methods.
C. Developing basic skills for integrating theory and practice.
D. Evaluation techniques designed to measure teacher effectiveness and student achievement in course objectives.

No. 17: How to Plan an Experiment, Write a Scientific Paper and Present Data at a Scientific Meeting
(Charles Culling, Prof.) 8:30 AM - 12 Noon
Limit: 24

This will be a hands-on workshop where participants will actually write a paper for publication. One participant will be selected and placed on the Scientific Session Program for Friday, to present their paper written during this workshop.

No. 18: Basic Chemistry of Staining
(Ada T. Feldman, M.S., HT(ASCP)) 8:30 AM - 12 Noon

Primary objective of this workshop is to define chemical terminology pertinent to staining procedures. Examples of subjects to be covered are: Bond types, oxidation, reduction, pH and pH signatures, isoelectric point, the major macromolecules-carbohydrates, lipids, proteins), acids, bases, acidophilia, basophilia, metachromasia, mordanting, argyrophilia, and argentaffin reaction. Examples will be drawn from general clinical histology. All materials will be introduced at a level which assumes the participant has no previous knowledge of chemistry.

No. 19: Motivational Dynamics for Supervisors
(Resavich Nicholls, B.A.) 8:30 AM - 12 Noon
Limit: 30

Would you like to get the most out of your staff and reduce technical and people problems? Would you like to cut down on turnover? At this workshop we will be discussing self-actualization motivation, including detailed planning and setting up of a motivational system tied to performance-oriented management by objectives. We will also be discussing the relationship between supervisor and staff, the methods of handling different types of people, and the hiring and firing of staff, as well as the games people play and how to stop them. In summary, we will be discussing the ways of motivating staff members through the use of a motivational values system.

No. 20: Cytochemistry of Leukemias — Enzyme and Special Stains Used for the Diagnosis and Differentiation of Leukemia
(Michael Johnson, B.S., HT(ASCP) & Pauline Keegan, HT(ASCP)) 8:30 AM - 12 Noon
Limit:25 Repeated 1:00 - 4:30 PM

Workshop is designed to provide the student with hands-on experience performing enzyme histochemical stains including myeloperoxidase, non-specific esterase and chloroacetate esterase as well as a discussion of their use along with PAS and Sudan Black LAP and acid phosphatase for diagnosing leukemias. Hausbrots and Leidschrume slides will be used to supplement practical and didactic presentations. Each student will be able to return to their laboratory with several stained slides.

No. 21: Cryotomy & Cryostat Technology
(Edward Bareda, HT(ASCP)) 1 - 4:30 PM

Workshop provides description of the microtome and cryostat, contact techniques for embedding specimens, knife and angles of adjustment for the knife. Operation will be placing the knife in the microtome, cutting angle, temperature of the cryostat and application of compound to the tissue, desired thickness of section, placing section on slide. Discussion of various staining procedures for frozen sections. Factors affecting tissue sectioning will be covered, which include the cryostat temperature, allowing a perfect cut of the sections whether warm or cold. The practical application will then be given. Tissue-Tek II Cryostat will be used in the workshop.

No. 22: Knife Sharpening with Application to Microtomy
(Ernestine Sims, HTL(ASCP)) 1 - 4:30 PM
Limit: 35

Understanding the basics of the sharpening of microtome knives and the application of sharp knives to the art of microtomy will be the theme of this workshop. There will be emphasis on safe handling and use of knives while sharpening and performing microtomy. Guidelines to be used for self evaluation of the sharpness of knives will be reviewed along with troubleshooting cutting problems that may or may not be the results of poorly sharpened knives. An "on hands" demonstration for proper use of the Tissue Tek, Shandon Mark V, and Hacker Knife Sharpeners will be presented during this program. Representatives from the companies that manufacture or distribute the sharpeners will be assisting with the "on hands" presentation.

No. 23: Staining Techniques for the Demonstration of Legionella Pneumophila
(Patricia Greer, A.H. MT, HTL(ASCP) & Aida Van Gelder, HT(ASCP)) 1 - 4:30 PM
Limit: 25

One difficulty in diagnosing Legionnaires' disease is the inability of the usual tissue gram stains to demonstrate the organism in paraffin embedded tissue sections. However, *Legionella pneumophila* is readily demonstrated by using the Dieterle silver impregnation procedure in paraffin embedded sections and the Gimenez stain or the Brown-Hoppe procedure in frozen sections or tissue scrapings of formalin fixed tissue. Workshop participants will perform these procedures on appropriate specimens for the demonstration of the Legionnaires' disease bacterium.

No. 24: Proceedings on the Third Basic Science Workshop in Histology
(Antonio Villanueva, M.A. & Jules Elias, M.A.) 1 - 4:30 PM

Following topics will be covered in this workshop:

1. **Typing of Specific Lymphocytes in Skin Biopsies from Patients with Lymphoproliferative Disorders:** *Heikn Heinzerling, Ph.D., & Linda Selak, HT (ASCP)*

Many dermatological diseases exhibit a lymphocyte or histiocyte infiltrate in the dermis of the skin. Characterization of the lymphocytes infiltrating the skin in various diseases, such as mycosis fungoides and many dermatitises, has been the object of numerous investigations and may enable better diagnosis and understanding of the etiology of these diseases. Recently Ortho Pharmaceutical, New England Nuclear, and Becton Dickinson companies have produced monoclonal antibodies that bind to specific cell surface antigens and permit identification of T-cell subtypes, B-cells, and monocytes. Our laboratory is investigating the typing of lymphocytes in frozen sections of skin biopsies. Methods will be discussed which (1) fix skin sections without denaturing specific glycoprotein antigens, and (2) amplify the detection of monoclonal antibody binding to cell surfaces, using immunofluorescence, horseradish peroxidase complexes, and antibody-bound microspheres. Immunological methods of cell identification may add new dimensions to histopathology by permitting a better understanding of disease etiology.

2. **Recent Advances in Dental Implants:** *John A. Hess, D.D.S., M. Phil.*

Many different materials have been used over the centuries for the replacement of bone and in the stabilization of fractures. Nearly 100 years ago, the first joint implant was placed. In more recent times, there has been an increased interest in the utilization of implant materials for the replacement of teeth. This interest reflects the development of materials with greater biocompatibility, an improvement in design and in physical properties. There are several types of implants used in dentistry, including endosteal, subperiosteal and transosteal. There are a number of different materials used in the construction of implants, including metals, ceramics, medical polymers and carbon with variations and combinations of each. A review of dental implant types and materials will be presented along with some of the technical problems that have been encountered.

3. **Optimal Condition for Feulgen Hydrolysis:** *Vincent Della Speranza, B.S., HT (ASCP)*

The cytophotometric determination of the DNA content of individual cell nuclei has proven to be quite useful in diagnosing biopsies of the thyroid gland. The classical Feulgen procedure employs a one normal hydrochloric acid solution at a 60° C temperature as a means of disrupting the purine-desoxyribose bond. The aldehydes produced during hydrolysis are derived from the deoxyribose and will chemically link to leuko-schiff, restoring its characteristic magenta color. The duration of hydrolysis is often determined by the type of fixative used and is usually quite narrow for aldehyde containing fixatives. More recent studies have clearly demonstrated that if the hydrolysis reaction is done at room temperature using a more concentrated acid solution, the maximum amount of aldehyde groups that develop extends over a wider range of times. We reinvestigated the role that temperature plays in the removal of purine bases and the subsequent development of stainable aldehydes in formalin fixed tissue.

4. **Argentaffin and Argyrophilic Reactions:** *Sharon Lear, HTL (ASCP)*

This presentation deals with the origin and distribution of the Kultschetsky cell and the variety of associated staining reactions employed in verifying this presence. Included is a kodachrome demonstration of the differences between argentaffin and argyrophilic reactions using the following histochemical techniques: Fontana-Masson, Diazo coupling, Schmorl's, Sevier-Munger, Bodian, Bielschowsky's and Gremilius. The advantages and disadvantages of each methodology will also be considered.

5. **Epithelial Cells: Morphologic Characteristics in Health and Disease:** *Richard Schroeder, M.A.*

Neoplasia is manifested through cellular aberrations. In order to more fully understand these changes, it is imperative to have insight of the cell in health, inflammation and preneoplastic states. Morphologic characteristics observed in these states will be discussed along with disciplines used in their elucidation.

6. **Identification and Enumeration of T and B Lymphocytes:** *Jules Elias, M.A.*

The morphological homogenous population of circulating lymphocytes can now be delineated into two separate groups by surface markers demonstrated by specific immune mechanisms (B cells) and by surface markers which have no immunological basis (T cells). The rationale for the study of human T and B cells is aimed at determining numerical deviations from "normal" T and B cell ratios in the circulation as an aid in the diagnosis of diseases that affect the lymphoid system. The techniques involved appear to be quite simple but accurate and reliable results can only be obtained by protocols which include steps which preclude false positive or negative readings. Equally as important as good techniques for obtaining interpretable results is an awareness of the rather complex immunology that is "surfacing" as newer methods are devised for identifying populations of T cells, cells without characteristic surface markers, etc. There now appears to be evidence that the clear dichotomy that initially appeared to characterize the human lymphocyte system is

Workshops, continued

not the true picture, but instead there exists a continuum of slightly different lymphocytes. The latter population of lymphocytes makes a significant contribution to a lymphocyte pool containing at one end of its spectrum T lymphocytes whose surface markers and functions are furthest removed from those of the B lymphocytes occupying the opposite extreme. Careful consideration of methodological pitfalls and the immunology of T and B cells should be incorporated in any interpretation of enumeration results of these cells in both health and disease.

7. Relationships Between Osteoid Widths and Types of Osteoblasts in Bone
(A. R. Villanueva, M.A.)

Four types of osteoblasts are normally present lining the intima of osteoid seams. They assume a variety of shapes which we have classified as types 1, 2, 3 and 4 cells. Type 1 cells resemble both mesothelial and fibroblast-like cells frequently present in a very recently formed osteoid. The osteoid formed here are mostly protruding, collagenic fibers. The nucleus is large, oval shaped and averages 9 micrometers in diameter and 15 micrometers in length, and contains numerous chromatin particles. Tetracycline staining osteoid seams adjacent to these cells are not usually evident. Type 2 cells are the classic, or typical and often designated as active osteoblasts. It assumes a variety of shapes, such as columnar or cuboidal, or pyriform. These osteoblasts can be identified easily by the presence of adjacent nuclear clear zone otherwise known as juxta-nuclear vacuoles or cytocentriums, a large, round, clear area at the center of the cell about the size of the nucleus (7um diameter and 10um length) which it adjoins. The width of the osteoid seam adjacent to these cells is approximately 17.4um. Type 3 cells are flattened cells and average about 15 to 20 micrometers in length. The nucleus is spherical or rectangular, centrally or distally located, and averages approximately 4 micrometers in diameter. 10 micrometers in length with an elongated, occasional wavy cytoplasm. The width of the osteoid seam adjacent to these cells is approximately 15.7um. Type 4 cells are transitional, stretched, reticulum-like cells with small elongated nuclei measuring approximately 2.5 micrometers in diameter and 10 micrometers in length and an extremely thin cytoplasm. The width of the osteoid adjacent to these cells is approximately 5 mm.

Scientific Sessions

Wednesday, November 18, 1981

A.M. Session

Malignant Lymphoma, Recent Advances	*Carl Kjeldsberg, M.D.*
Open Lung Biopsy	*Jerrold Abraham, M.D.*
Forensic Histopathology	*Wallace Graham, M.D.*
Lung Cancer — Histology Correlation with Response to Treatment	*Harmon Eyre, M.D.*

P.M. Session

Mortality Study of Histologic Technicians Certified Between 1948-1970	*Dan Grauman*
Routine and Special Stains for the Diagnosis of Liver Disease	*Randy Lee, M.D.*
Estrogen Receptor Analysis; An Immunofluorescent Method	*M. Elizabeth Hammond, M.D.*
Hormonal Evaluation of the Cytologic Sample	*Catherine Keebler, CT (ASCP) CFIAC*
7 - 9 P.M.: "Simplifying/Enjoying Parlimentary Procedures"	*Lois Cook, B.S., HT (ASCP)*

Session will have a brief introduction to the basics of parliamentary procedure. There will be mock sessions using different situations that illustrate how to accomplish varying results starting at the same place. Finally there will be parliamentary "gamesmanship."

Thursday, November 19, 1981

A.M. Session

Testing for Evidence of Rape	*T. Paulette Sutton, MT (ASCP)*
Histochemistry and Sports Medicine	*Peter Senzig, HT (ASCP)*
The Pathology of Tumors	*Robert Flinner, M.D.*

P.M. Session

Alcohol — The Chemical Drug We Drink	*Richard Schroeder, M.A.*
Bone Marrow Preparations and Interpretations	*Catherine Brunst, HTL (ASCP), Marilyn Irwin, HT (ASCP) and Kathy Davis, HTL (ASCP)*
Toxicology — Drug Testing	*Thomas Jennison, Ph.D.*
Immunoperoxidase (PAP) Techniques	*Joe Marty, M.S.*

Friday, November 20, 1981

A.M. Session:

The Interpretation and Application of Diagnostic Special Stains for Surgical Pathology	*Erwin Haas, HT (ASCP)*
Animals with a Total Artificial Heart: Blood and Tissue Interactions	*Donald Olsen, D.V.M.*
Toxic Shock Syndrome	*Allen Paris, M.D.*
Histologic Technique: Skin Sectioning for the Dermalpathologist	*Diane Miller, HT (ASCP)*
Lecture from Paper Written During Workshop on Tuesday	

3. Quickly immerse the cork, with tissue down, into the liquid nitrogen.
4. The frozen specimen is then left in the liquid nitrogen for at least one minute.
5. The cork with frozen specimen attached is then transferred to the cryostat.
6. Wait for a period of 15 to 30 seconds before attaching the cork to a cryostat chuck with OCT embedding compound.
7. Allow the temperature of the frozen specimen to warm to the temperature of the cryostat. Sectioning can now begin.

Reference:

1. Moline, S.W., and Glenner, G.G.: Ultrarapid Tissue Freezing in Liquid Nitrogen. J. Histochem. Cytochem., 12:777-783, 1964.

*OCT embedding compound is a product of LAB-TEK DIVISION, Miles Laboratories, Inc., 30W475 North Aurora Road, Naperville, IL 60566.

†Pre-cut cork discs are available from Slee International, Inc., New York, NY 10011.

Replies to Inquiry

Editor's Note: The following replies were received in regard to why *plastic* conical tip centrifuge tubes produce more compact buttons than *glass* conical tip tubes. The original article, authored by Brenda Cuevas, appeared in **Histo-Logic**, Vol. X, No. 2, pg. 146, April 1980.

Priscilla Ann Gregory
Osteopathic General Hospital
1750 N.E. 167th Street
North Miami Beach, Florida 33162

In response to the article by Brenda Cuevas, following is our procedure for Cell Block Preparation.

Cell Block Preparation: Plasma - Thrombin Technique

1. Centrifuge the fluid sample at 1500 to 2000 rpm for 15 minutes.
2. Decant supernatant.
3. Add 3 to 5 drops of plasma (plasma with normal PT time).
4. Gently stir with wooden applicator stick to permit plasma to permeate the sediment.
5. Add 3-5 drops of thrombin (Ortho Brain Thromboplastin).
6. Gently stir with wooden applicator stick to allow thrombin to form a clot.
7. After clot forms, place in lens paper and place in embedding cassette.
8. Place in formalin for tissue processing.

Reference:

Pathology Annual, Part I, Vol. 12, University of Miami Medical Center, 1977.

George W. Chang
University of California
College of Natural Resources
Berkeley, California 94720

I was interested to read Ms. Brenda Cuevas' note about "Cell Blocks from Specimens of Body Fluids." I suspect her observation has something to do with the fact that water doesn't really wet the plastic tubes. Thus, there is less water remaining at the bottom of the tube after she pours off the supernatant. The smaller amount of water (fluid) remaining may be less likely to resuspend the compact button of cells.

Section Adhesive for Paraffin Sections

Alice Esposito
Methodist Hospital
Philadelphia, Pennsylvania 19148

The following procedure for adhesion of tissue sections to microscopic slides has been found to be very beneficial in our hands. Some of the benefits are: (1) reduced tissue section detachment from glass slide; (2) improved staining qualities; (3) reduced residue on microscopic slide; (4) less wrinkles evident on finished slide; and (5) saved time.

Procedure:

The tissue section ribbon which is obtained from microtomy is placed in the flotation bath containing the following solution. The water bath temperature should be at a minimum of 55 °C.

Adhesive Solution

Alcohol (ethyl or isopropyl)	100.0 ml
Distilled water	2400.0 ml
Elmer's Glue-all*	6.0 drops

Mix well.

Sections are picked up on glass slides and placed in a staining rack which is then placed in a slide dryer at 65 °C for 15 minutes. Sections are now ready for deparaffinization and staining. Note: Flotation bath and slide dryer temperatures are most important when using this section adhesive. The adhesive solution should be added to water bath just prior to use.

*Can be purchased at most drugstores and food stores.

Problems Related to Staining of Connective Tissue Embedded in Water-Soluble Plastics

Peter O. Gerrits
Department of Anatomy
University of Groningen
9713 EZ Groningen
The Netherlands

In our laboratory we routinely prepare embedding media on the basis of water-soluble methacrylates (JB_4, GMA, according to Ruddell,[1] 1967, and Sims,[2] 1974). The following problem is experienced.

Although most staining procedures we use in routine histology do not offer any significant problems (occasionally only slight modifications appear to be necessary), we are repeatedly confronted with unsatisfactory results regarding the staining of connective tissue components. Most investigators do not mention similar difficulties, while to our knowledge, an effective connective tissue staining procedure for plastic-embedded specimens has not been reported.

Perhaps someone can provide us with technical protocols or suggestions regarding this issue, in order to obtain sharp contrasts between nuclei and cytoplasm in relation to other tissues. I would gratefully appreciate any suggestions on this matter.

Editors Note: Please forward a copy of replies to the Editor, and to Mr. Gerrits at the address above.

References:

1. Ruddell, C.L.: Embedding Media for 1-2 Micron Sectioning. Hydroxethyl Methacrylate Combines with 2-Butoxyethanol. Stain Tech., 42: 253-255, 1967.
2. Sims B: A Simple Method of Preparing 1-2 Micron Sections of Large Tissue Blocks Using Glycol Methacrylate. J. Microsc. (Oxf.), 101:223-227, 1974.

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Can You Help?

Rob Bosma
Laboratorium voor de Volksgezondheid
Jelsumerstraat 6, afd. P.A.
8917 EN Leeuwarden
The Netherlands

I have a problem in determining and coloring spirochaetes. The methods used are Levaditi's method and the Warthin-Starry method. Who can help me to positively identify (1) Treponema Pallidum (syphilis) and (2) Leptospira Ictrohaemorrhagiae (or Weil's disease) in liver tissue or lympho-gland tissue (fixed in formalin). Should there be other techniques (i.e., fluorescence methods) for determining of spirochaetes, I would like to be informed.

Editor's Note: Please forward a copy of any replies to the Editor, and to Mr. Rob Bosma at the address above.

An Atlas of Tissue Artifacts

This is the only comprehensive book on tissue artifacts available to histopathology technicians and pathologists. It contains more than 400 photographs illustrating many common and uncommon artifacts produced in all facets of histotechnology. More important, it contains detailed written information on how these artifacts are *identified, produced, prevented and/or eliminated.* This book is the result of more than 20 years of studying tissue artifacts. It is authored by Samuel Wesley Thompson, D.V.M., M.S., and Lee G. Luna, HT (ASCP). The book is available from: Charles C. Thomas, 301-327 E. Lawrence Avenue, Springfield, Illinois 62717.

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.
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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.