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.

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In Defense of Cover Glasses

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The recent recommendation for the use of liquid substitutes for cover glasses (Buksa M: Liquid coverslipping media. Histo-Logic 11(4):167-168, 1981) compels me to present several observations about cover glasses that argue *against* the use of liquid substitutes. First made available commercially in 1840 by Chance Brothers of Birmingham, England,[1] cover slips should be regarded as more than just a "pane in the glass," as it were, that is used in a costly, time-consuming procedure and that drives people to look for a less costly and less tedious alternative. After all, there must be some valid reason why, ounce for ounce, cover glasses cost about 15 times more than micro slides.

Cover glasses are manufactured to meet well-defined optical and physical specifications (e.g., refractive index of 1.523 ± 0.005, dispersion value of 52.0, thickness, plane parallelism) that are related to the design of microscope objectives and that visibly influence image quality.[2,3,4] Indeed, cover glasses alter the path of light so substantially that lens designers consider them as the first lens in all microscope objectives! By extension, coverslipping can be regarded as the last step in an objective's assembly. Seen in this light, coverslipping deserves the best materials and methods.

Given a well-prepared cell spread or tissue section and a clean microscope adjusted to provide Kohler illumination, the correct thickness of cover glass and mounting medium will reveal crisp clear images. Too thick or too thin cover glass and mounting medium layers introduce spherical aberration that overall lowers image contrast, thereby producing images described variously as blurry, hazy, milky, watery, soft focus, out-of-focus, unfocusable, cloudy, flat, foggy, fuzzy, and washed-out. Edges and boundaries of cellular details become unsharp. Images are degraded in direct proportion to the degree of deviation of the actual thickness of cover glass and mounting medium from the intended thickness. The degree of image degradation can range from barely perceptible to ruinous.

Microscope objectives tolerate deviations from the design-specified thickness of cover glass and mounting medium as a function of their numerical aperture (NA). Numerical apertures range from about 0.04 to 0.95 for dry objectives, 1.0 to 1.3 for oil immersion ones, and are a measure of the light-gathering capacity of an objective — the higher the NA of an objective, the greater its resolving power. 10X objectives have NAs of 0.25 to 0.32 and are insensitive to cover glass thickness. Indeed, a micro preparation can be examined upside-down, with the slide serving as a thick cover glass, without noticeably degrading the image. On the other hand, 0.65 NA achromat objectives (i.e., those least corrected for optical aberrations) tolerate deviations only within ± 15 μm of 0.18 mm (American- and English-made microscopes) or 0.17 mm (European- and Japanese-made microscopes), and 0.95 NA apochromat objectives (i.e., those most highly corrected for optical aberrations) tolerate deviations only to within ± 3 μm![4] Indeed, the latter objectives would be impractical to use were it not for their having an adjustable correction collar that alters the spacing among the lens elements to compensate for cover glass and mounting medium thicknesses of 0.11 to 0.23 mm.

The thickness of cover glass recommended most often is the No. 1½. No. 1½ cover glasses range in thickness from 0.16 or 0.17 to 0.19 mm.[2,3] This range brackets the 0.17 and 0.18 engraved on some microscope objectives, numbers that are usually interpreted as indicating the thickness of cover glass only, no mounting medium, for which the objective should be used. No. 1½ cover glasses are correct only when there is little or no mounting medium between the cells or tissue and the cover glass. Examples include: (1) monolayer cell spreads that are heated on a hot plate (i.e., "cooked") to quickly evaporate the solvent of the mounting medium, thus leaving behind only a very thin layer of hardened mounting medium; (2) cells spread directly onto a cover glass (e.g., a blood film); (3) cells grown in culture on a cover glass; and (4) cells collected on a Nuclepore filter that is to be dissolved cell-side-down on a cover glass. These four examples are uncommon.

In routine practice, the No. 1 thickness cover glass is more correct. No. 1 cover glasses range from 0.13 to 0.17 mm in thickness. While thinner than usually recommended, No. 1 cover glasses allow for mounting medium, the consequences of which are curiously overlooked in most recommendations (even though mounting medium interacts with light as though it were glass). The thickness of mounting medium can often be substantial, exceeding by several times the most relaxed tolerance limits[4] (i.e., ± 15 μm for 0.65 NA achromats). Therefore, it is wise to use No. 1 thickness cover glasses and to apply as little mounting medium as will fully cover the specimen without subsequent retraction under the edge of the cover glass. The combined thickness of a No. 1 cover glass and mounting medium will more closely and more often equal the specified thicknesses of 0.17 or 0.18 mm "cover glass" for which objectives are corrected than a No. 1½ cover glass and mounting medium ever can.

The ruinous spherical aberration introduced by large deviations in cover glass and mounting medium thickness from the specified thickness, for 40X objectives especially, can be offset substantially by closing the aperture diaphragm of the substage condenser more than usual when establishing Kohler illumination in a microscope. The aperture diaphragm is ordinarily focused in the back focal plane of the objective and so determines the objective's working numerical aperture. Closing the aperture diaphragm, though never completely, reduces the objective's numerical aperture and lessens its sensitivity to cover glass thickness. In practice, simply close the aperture diaphragm until the best contrast is produced.

The foregoing is but a minimum introduction to some important aspects of cover glasses; a thorough discussion would require many more pages. Undoubtedly, not all

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readers will have been convinced to continue to use cover glasses instead of liquid substitutes. To them in particular, and to all in general, it must be emphasized that the beneficial effects of cover glasses, as well as the detrimental effects of liquid substitutes, can be masked by poorly preserved specimens, pale staining, dirty microscopes, improperly adjusted microscopic illumination (e.g., the low contrast produced by spherical aberration is visually indistinguishable from that produced by glare when an aperture diaphragm is opened excessively or dirty lenses scatter light), or are observed only under a 10X objective. Under professionally controlled circumstances, the difference in the quality of images made possible by cover glasses and liquid substitutes is real, visible, and substantial. If good image quality is among one's goals for specimen preparation, then only properly selected and applied cover glasses can make it possible.

About the best that can be said for liquid substitutes for cover glasses is that they are inexpensive, fast-drying glues that are quick to use. In contrast to cover glasses and most mounting media, liquid substitutes: (1) produce greater volumes of potentially toxic vapors; (2) do not produce a coating of uniform thickness; (3) produce a wavy layer; (4) have uncontrollable thicknesses, often being thinner than is best, that will produce spherical aberration and hazy images; (5) have a low refractive index relative to that of cells and so produce a refractile appearance in cells; (6) can leave thick areas of cells uncovered; (7) may increase stain fading (the inclusion of an anti-oxidant does not ensure the stability of all dyes); (8) scratch easily; and (9) are not readily cleaned of immersion oil by xylene.

"If liquid substitutes for cover glasses are so unsuitable," it might be asked, "why have they been introduced to the microtechniques market?" Although only the manufacturers of these substitutes can answer this question, I suspect that someone saw a business opportunity in providing a product that would quickly and inexpensively cover a micro preparation with a thin protective film of rapid drying transparent medium. Without knowing the information in the cited references and others, a manufacturer might never stop to ask whether a cover glass has specific properties that a liquid substitute should have as well. Whatever their evolutionary path from concept to market, liquid coverslipping media cannot truly substitute for cover glasses; the laws of optics will not allow it. Using liquid substitutes for cover glasses means accepting limitations in the quality of one's work. So while much routine microscopic work can be done satisfactorily with less than optimal micro preparations, we should nonetheless strive to maintain the high standards of performance that are the hallmark of professionals and use cover glasses.

All things considered, liquid substitutes offer advantages only to the persons who must coverslip and to any administrator who does not have responsibility for quality of patient care or malpractice suits arising from less-than-accurate microscopic diagnoses. Conventional cover glasses benefit microscopists who must see highly resolved detail, especially pathologists with patients' health and lives at stake, by allowing them to see cells and tissues more clearly, and thereby increase the likelihood of their interpreting pathologic changes accurately. In the total picture, it is the needs of the microscopist and the patient that must be served. Liquid substitutes will not do, for there is more to a cover glass than meets the eye and the pocketbook.

References:

1. Bracegirdle, B.: *The History of Microtechnique*. Cornell University Press, Ithaca, 1978, p. 113.
2. ASTM Committee E-25 on Microscopy: Standard Specification for Cover Glasses and Glass Slides for Use in Microscopy - Designation E211-70, in *Annual Book of ASTM Standards*, American Society for Testing and Materials, Philadelphia, 1970.
3. General Services Administration: Interim Federal Specification - Cover Glass, Slide. NNN-C-001434A (GSA-FSS), GSA, Washington, D.C., Jan. 8, 1971.
4. Setterington, R.: The specification of a standard microscope cover glass. J. Roy. Micr. Soc., 73:69-76, 1953.
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Bis-Chloro-Methyl Ether (BCME): A Response

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Janet Maass' concern regarding the possible formation of bis-chloro-methyl ether (BCME), a potent carcinogen, as a result of formaldehyde being mixed with hydrochloric acid is understandable (Histo-Logic 10(4):152, 1980). Fortunately, however, published data indicate that BCME formation is highly unlikely under the conditions of use ordinarily encountered in safely operated histology laboratories.[1]

Whether BCME will result from exposure of formaldehyde to HCl depends on the concentrations of each in parts per million (ppm) in air, *not* in solution. When the concentrations of both chemicals are kept below their respective threshold limit values - time weighted average (TLVs-TWA), no detectable BCME is formed.[1] (TLV-TWA is "the time weighted average concentration for a normal 8-hour workday or 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect."[2]) The TLV-TWA for formaldehyde is 2 ppm; for hydrochloric acid (hydrogen chloride), 5 ppm. The TLV-TWA for BCME is 0.001 ppm or 1 part per billion (ppb). Such low values for all three chemicals place them in the highly toxic category. For comparison, the TLVs-TWA for xylene and ethyl alcohol are 100 ppm (upper limit of the highly toxic range) and 1,000 ppm (slightly toxic), respectively.[2]

Less than 0.5 ppb of BCME is formed from 20 ppm each of formaldehyde and HCl under a variety of conditions of humidity, temperature and UV radiation.[1] In other words, even when the gaseous concentrations of formaldehyde and HCl exceed their TLV-TWA by factors of 10 and 4, respectively, the amount of BCME produced within 12 to 24 hours is within safe limits.[1] Concentrations of formaldehyde and HCl this high are unlikely to occur, for the noxious vapors would alert the laboratory worker to take corrective action long before reaching such olfactorily intolerable levels.

While some data conclusively demonstrate that BCME is formed from gaseous formaldehyde and HCl, other data suggest that acidic solution (pH unspecified) which contains chloride ion and formaldehyde should be considered a potential source of BCME.[1] Whether B-5 fixative (sodium acetate-mercuric chloride-formalin: 9 parts distilled water, 1 part 40% formaldehyde, 6% $HgCl_2$ and 1.25% $NaC_2H_3O_2$ [anhydrous], pH 5.8 to 6[3]) is sufficiently acidic to produce BCME is uncertain, though it seems highly unlikely.

Knowing the TLVs-TWA of formaldehyde, hydrogen chloride and other toxic vapors are useless when we do not know the actual concentrations of vapors to which we are exposed. In the absence of such information, a useful rule-of-thumb is that the concentration of any odorous toxic chemical is excessive when we can smell it. The minimum concentration at which the odor quality of a compound under study can be described is its recognition odor threshold concentration (ROTC). A chemical's ROTC is usually substantially

National Society for Histotechnology Symposium/Convention
Boston, Massachusetts
September 13-17, 1982

The Eighth Annual Symposium/Convention of the National Society for Histotechnology will be conducted at the Sheraton Hotel in Boston. The enclosed program is complete with hotel reservation card and registration form.

HOTEL RESERVATIONS: We request you make hotel accommodations early, as all rooms blocked for NSH will be released one month prior to meeting date. **MAIL HOTEL RESERVATION DIRECTLY TO:** Sheraton, Prudential Center, Boston, Massachusetts 02199; 617/236-2000.

SYMPOSIUM REGISTRATION: Registration form 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. For workshops stipulating a LIMITED ATTENDANCE, please indicate 1st and 2nd choice. Your first choice will be honored as long as openings remain. Workshop quotas will be adhered to. LATE REGISTRATION CHARGE of $10 is applicable to registrations received after September 7th. Attendees registering upon arrival to the meeting will be assessed the $10 late fee. CANADIAN AND FOREIGN attendees should remit fees in U.S. currency. Checks and money orders are payable to: National Society for Histotechnology. **MAIL SYMPOSIUM REGISTRATION AND FEES DIRECTLY TO:** NSH, P.O. Box 36, LANHAM, MARYLAND 20706. For registration assistance, please call Roberta Mosedale, NSH Office, 301/552-9678.

REIMBURSEMENT POLICY: NSH will reimburse registration fees upon receipt of cancellation prior to September 7th. Fees not refundable after this date. After arrival to the meeting, NO REFUNDS will be made for unattended workshops, sessions or banquet. Banquet tickets may be purchased at the registration desk, but are not refundable. Monetary difference cannot be refunded when changing workshop attendance after arrival to the meeting.

NSH/Thomas Edison Program Schedule

COURSE REVIEWS: Students interested in review sessions should register for individual scheduled workshops below. Workshop registration is free to students formally enrolled in Thomas Edison College. STUDENT MUST SEND PROOF OF ENROLLMENT WITH REGISTRATION FORM.

HUMAN MICROSCOPIC ANATOMY
(Freida Carson, Ph.D., & John Ryan, HTL [ASCP])
MONDAY, WORKSHOP NO. 2 - 8:30 AM - 4:30 PM

Human Microscopic Anatomy workshop/review will be continued Tuesday morning for those planning to take this examination during the convention week. Review will allow informal discussion with faculty member and an opportunity for examinee to identify and review weak areas. Examinees are encouraged to attend both sessions.

INTRODUCTORY HISTOTECHNOLOGY/HISTOCHEMISTRY
(Richard Schroeder, M.A. & Jules Elias, M.A.)
TUESDAY, WORKSHOP NO. 13 - 8:30 AM - 4:30 PM

EXAMINATION SCHEDULE: WEDNESDAY, THURSDAY & FRIDAY — 7-9 AM

NOTE: Registrants planning to take Thomas Edison examination(s) during convention week must *pre-register* by sending NSH your name and address with examination title(s) you will be taking. This information may be included with your convention registration or submitted separately, but is more important for administrative purposes. Examination fees may be paid at time of administration; however, advanced payment is preferred and should be mailed to: NSH, P.O. Box 36, Lanham, Maryland 20706.

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National Society For Histotechnology Symposium/Convention
September 13-17, 1982

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WORKSHOPS

MONDAY, SEPTEMBER 13, 1982

No. 1 — **8:30 AM - 4:30 PM**
IMMUNOPEROXIDASE - THEORY, METHODS & CLINICAL APPLICATION
Elaine Kelsher, HT (ASCP)
LIMIT 30
This workshop will begin with an introductory lecture on basic immunology, immunohistochemistry and the various immunoperoxidase methods. Each registrant will also participate in an actual staining procedure. The session will conclude with a kodachrome presentation of stained sections. Detailed handouts will be provided.

No. 2 — **8:30 AM - 4:30 PM**
HUMAN MICROSCOPIC ANATOMY (Intermediate to Advanced)
Frenda Carson, Ph.D., & John Ryan, M.B.A., HTL (ASCP)
Workshop will emphasize basic tissues and their organization into the different organs. Visual identification, function and some special staining correlation will be included. Participants will be given a self assessment test, and handout material will be in a workbook format to be filled in during the workshop. Workshop is recommended for Thomas Edison students.
There will be a special review on Tuesday morning for students taking the Thomas Edison examination. (Not to be taken in lieu of the workshop.)

No. 3 — **8:30 AM - 4:30 PM**
HISTOTECHS LOOK AT THE ENDOCRINE SYSTEM (Basic to Intermediate)
Elizabeth Sasek, HTL (ASCP) & Elizabeth Carrillo, HT (ASCP)
This workshop will consist of lectures on the gross and microscopic anatomy and physiology of the organs of the Endocrine System, and stains used to demonstrate the tissue elements. Participants will perform selected special stains including one immunoperoxidase technique, and will be able to recognize properly stained slides. Glands covered in depth will be the pituitary, thyroid, pancreas and adrenals.

No. 4 — **8:30 AM - 4:30 PM**
PHOTOGRAPHY IN THE LABORATORY
(Robert Kershner)
Workshop will include lecture and demonstrations in the areas of both photomicrography and gross specimen photography. Information will cover all phases of instrumentation, types of film, technique and trouble shooting routine problems.

PHOTOMICROGRAPHY	GROSS SPECIMEN PHOTOGRAPHY
a. The microscope	a. The camera
b. The specimen	b. Lighting
c. The camera	c. Film and Processing
d. Film and processing	d. Projection
e. Projection	

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

No. 5 — **8:30 AM - 12 Noon**
SPECIAL STAINS FOR DEMONSTRATING MICRO-ORGANISMS
Charles Charaklas, B.A., HTL (ASCP)
LIMIT 40
Workshop will present an overview of the various types of micro-organisms and the recommended staining methods used for their identification. Participants will have the opportunity to perform a rapid Grocott methenamine-silver nitrate (GMS) method for fungi and Pneumocystis carinii, a modified Brown-Brenn method for Gram-positive and Gram-negative bacteria and Truant's fluorescent method for acid fast bacteria.

No. 6 — **8:30 AM - 12 Noon**
PLASTIC SECTIONS - AN OFTEN NEEDED SUPPLEMENT TO PARAFFIN
(Patricia Newman, B.A., HT (ASCP))
LIMIT 25
Participants, through audio visual aids and hands-on experience, will become familiar with all phases of making plastic sections for use with the light microscope. Workshop will also include several case studies demonstrating how plastic, in conjunction with paraffin sections, is invaluable in making a complete diagnosis.

No. 7 — **8:30 AM - 12 Noon**
TECHNIQUES FOR ARGENTAFFIN AND ARGYROPHIL CELLS
(Barbara Hewitt, HT (ASCP))
LIMIT 60
The lecture will include the identification of both argentaffin and argyrophil cells on 2x2 kodachrome slides using the following staining methods:
1. Schmorl's Method for Reducing Substances
2. A Modified Pascal's Argyrophil Method
3. Fontana-Masson Method for Argentaffin
These three methods are the stains the participants will perform in the wet workshop.

No. 8 — **8:30 AM - 12 Noon**
INSTRUCTIONAL TECHNIQUES USED IN TEACHING (Basic)
Jules Elias, M.A., & Janet Moore, HTL (ASCP)
This workshop is designed for teleist conferences. Workshop to include small group instruction, writing goals for a curriculum, effective communication, writing behavioral objective, preparing activity lists for instruction and making simple visual aids. Recommended for individuals interested in using teleist.

No. 9 — **8:30 AM - 12 Noon**
HOW TO PLAN AN EXPERIMENT, WRITE A SCIENTIFIC PAPER AND PRESENT DATA AT A SCIENTIFIC MEETING
Charles Culling, FIMLSC, M.R.C., PATH.
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. 10 — **1 - 4:30 PM**
MICROSCOPIC ANATOMY, PART I - BASIC
(Roy Kerton, M.D., & Hvery Albarelli, HT (ASCP))
LIMIT 100
An introduction to cell types, their variations, modifications, patterns, arrangements. Brief mention will be made of function and delineation of cell types by special stains. Method used for teaching will consist of study of a set of microscopic slides and projection of kodachromes with lecture. Slide sets and handouts will be kept by registrants.

No. 11 — **1 - 4:30 PM**
TECHNICAL AND DIAGNOSTIC ASPECTS OF THE LEDER'S STAIN
(R. Peter Wittmann, M.D., & Barbara Tarsio, HT (ASCP))
LIMIT 50
The Leder's Stain is a histochemical, enzyme selective stain used for the demonstration of neutrophilic myeloid cells, tissue mast cells, and Chediak-Higashi inclusions. The primary significance of this enzyme stain is that it can be performed on formalin fixed paraffin embedded tissue. This workshop will give participants the opportunity to perform the more intricate steps in the Leder's stain. Information on the diagnostic significance of the stain will also be given.

No. 12 — **1 - 4:30 PM**
MUSCLE BIOPSY WORK-UP — HOW AND WHY?
(Louis Savas, B.S., HTL (ASCP))
LIMIT 75
An in-depth look at the muscle biopsy, including normal histology of muscle, how biopsies are taken, the role of the Histotechnician in biopsy preparation, enzyme histochemistry and a discussion of muscle/nerve disease.

No. 13 — **1 - 4:30 PM**
NOMINAL GROUPING PROCESS APPROACH TO PROBLEM SOLVING
(Joyce Moore, HTL (ASCP))
Nominal grouping process allows a group to examine its own needs and interests and develop programs or solutions based on these findings. This workshop is designed to provide the student with a tool for identifying elements of a problem situation, identifying elements of the solution and establish priorities. The student will be taken through the steps in the Nominal Group Process. Most problems in a laboratory are created by the entire staff. Nominal grouping is an approach shared by all staff in creating a solution to the problems.

TUESDAY, SEPTEMBER 14, 1982

No. 14 — **8:30 AM - 4:30 PM**
INTRODUCTORY HISTOTECHNOLOGY HISTOCHEMISTRY (Basic)
(Richard Schroeder, M.A., & Jules Elias, M.A.)
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. Workshop is recommended for Thomas Edison students.

No. 15 — **8:30 AM - 4:30 PM**
THE USE OF HISTOCHEMISTRY IN DIAGNOSTIC PATHOLOGY: AN IMPORTANT ROLE OF THE HISTOTECHNOLOGIST IN PATIENT CARE
(Hugh McAllister, M.D., COL, USA)
The primary object of this workshop is to discuss the utilization of special histochemical stains in establishing diagnoses. The 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. 16 — **8:30 AM - 4:30 PM**
THE HISTOLOGY AND HISTOTECHNOLOGY OF THE ENDOCRINE PANCREAS
(Robert Escoffery, B.S., HT (ASCP) & Carl Emilson, HT (ASCP))
LIMIT 35
Workshop is aimed to present current knowledge and concepts of the content and structure of the Endocrine Pancreas, (Islets of Langerhans), with particular reference to the changes that occur in both Zollinger-Ellison and Verner-Morrison syndromes. Pertinent methodologies will be presented and photographs and histological sections will be reviewed. Participants will be able to do their own immunoperoxidase.

No. 17 — **8:30 AM - 4:30 PM**
SEVEN KEYS TO SUCCESS IN MANAGEMENT FOR HEALTH CARE PROFESSIONALS
(Jack Broderick & Phyllis Russell, B.A.)
LIMIT 50
1. **Performance** based on strength and reality.
2. Managerial styles that work.
3. **Time** is your best management resource.
4. **Negotiation** from a position of strength and leadership.
5. A win-win **communications** system.
6. You as a "**change agent**" in the 80's.
7. Your **career development** bandwagon.

You gain insight into the necessity of developing skills and techniques for you and the people that work with you. You should improve your ability to create a work environment that really works, that increases worker response and department productivity. Job stress is reduced to manageable levels, better results are achieved, and employee motivation and job attitude are enhanced.

No. 18 — **8:30 AM - 12 Noon**
MICROSCOPIC ANATOMY, PART II - ADVANCED
(Roy Kerton, M.D., & Hvery Albarelli, HT (ASCP))
LIMIT 100
A continuation of the analysis of cells. Emphasis will be placed on the arrangement of cells into tissues. Patterns of arrangement and techniques for analyzing patterns will be discussed. Unifying principles for histologic identification will be presented. Lecture will be presented with kodachromes. Slide sets will be kept by registrants.

No. 19 — **8:30 AM - 12 Noon**
CRYOTOMY TECHNOLOGY
(Frank Avallone, B.A.)
This workshop is designed to teach all those individuals, technicians, technologists, and researchers both experienced and non-experienced who wish to become more competent in the field of cryotomy. The program contents will include a multitude of subjects related to all phases of the art. A brief history of frozen sectioning, the technique and instrumentation from its inception to present day. The main contents of the workshop will include such areas as detailed information on sectioning, orientation of specimen, cutting and section evaluation. The problems encountered the reasons for and the solutions to. Anti-roll plate technology, adjustment, problems, solutions and repairs. Cryostat knives, types, sharpening, angle adjustments. The cryostat, purchasing, cleaning and maintenance. The problems associated with, the remedies and Cryostat evaluation. Staining methods for routine surgicals and other related tissues. Histochemical reactions, immunofluorescence and immunoperoxidase techniques. Kidney and muscle biopsy technology will be discussed at length. Methods for handling of specimens shipping, freezing, sectioning and staining. Frozen section artifacts, mounting medias, coverslipping, slide readings and transport media will be discussed, in addition to numerous tricks of the trade. A cryostat will be available for demonstration and instruction for those who desire additional help.

No. 20 — **8:30 AM - 12 Noon**
PRINCIPLES AND PRACTICE OF TISSUE PROCESSING
(Paul Mirabile, B.S., HT (ASCP))
This workshop will provide a more thorough understanding of tissue processing by considering the various stages of processing at a molecular level. Principally for histotechnologists who do not have a background in basic physics and chemistry. Part I will consist of a review of basic physical/chemical principles which are involved in tissue processing, such as molecular motion, diffusion, density, viscosity, miscibility, convection, etc. Part II will consider specific features of automated tissue processors (vacuum, temperature, agitation, etc.) in terms of their effects on the physical/chemical processes previously discussed. Part III will offer participants an opportunity to examine several different models of automated tissue processing equipment, and to evaluate their respective features.

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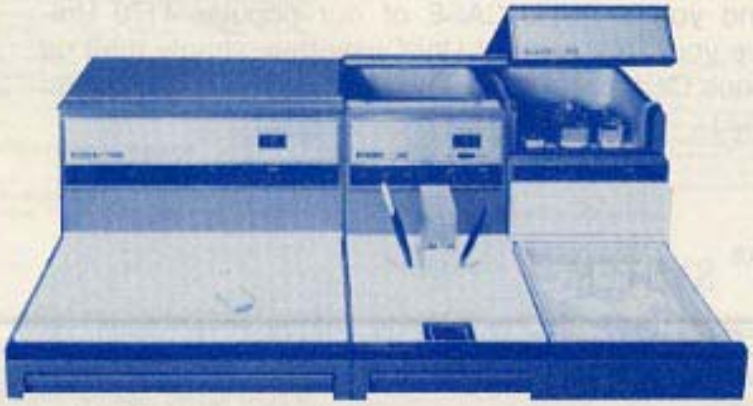

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4170

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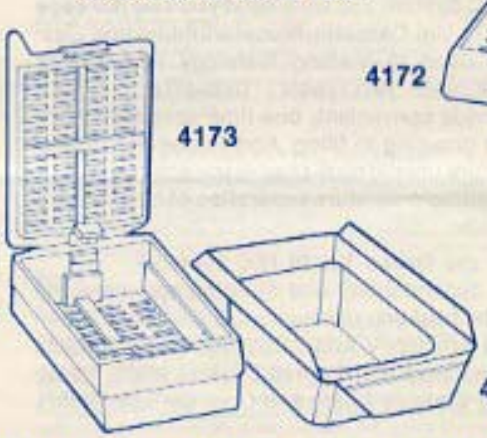
4172



4173

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No. 21 — 8:30 AM - 12 Noon
REPEATED 1:00 - 4:30 PM

WATER SOLUBLE EMBEDDING MEDIA FOR LIGHT MICROSCOPY

(Jane Woodruff, Product Specialist)

LIMIT 25

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 for histology is glycol methacrylate. This workshop will focus on the advantages and disadvantages of using water soluble 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 plastics for light microscopy.

No. 22 — 1 - 4:30 PM

CONNECTIVE TISSUES - FAMILY TREE AND STAINING CHARACTERISTICS

(Lee O. Luna, HT (ASCP))

Purpose of this course is to familiarize participants with the entire family tree of the connective tissue system. This will include the development from Mesoderm to Mesenchyme, primordia, connective tissue proper and supporting connective tissues. Included will be the forms of connective tissue; i.e., fibroblast, fibrogia, collagen, elastin, reticulin, osseous, ligaments, cartilage, mucous, adipose, tendons and blood which is a form of specialized connective tissue. The function of connective tissue will also be included. This will be followed by an in-depth discussion of the preferred fixatives, processing method and staining procedures for the demonstration of all connective tissues. Course will be conducted with the aid of charts and photomics.

No. 23 — 1 - 4:30 PM

EXAMINATION DEVELOPMENT: CERTIFICATION/CLASSROOM

(Mary Luna, Ph.D.)

LIMIT 50

The procedures for writing and evaluating multiple choice items for certification (BOR) and classroom use is the focus of this workshop. The following topics will be covered: (1) Writing item stems and responses; (2) evaluating item stems and responses; (3) taxonomy levels in developing examination items; (4) statistical analysis of examinations; (5) examinee reporting mechanisms including score profiles and item descriptors. Participants should plan to bring five multiple choice test questions they have developed and a histology reference book. Participants will work together to develop and critique examination items as well as learn basic theories of examination construction utilized by the Board of Registry.

No. 24 — 1 - 4:30 PM

DESIGN FOR HEALTH

(Norman V. Steere, P.E., & Diane Burica, HTL (ASCP))

This half-day workshop will address the issue of laboratory design as it relates to the Histology Laboratory. A special area of concern is ventilation, and we will look at methods of controlling fumes which are inherent to Histology, i.e., formaldehyde and xylene.

No. 25 — 1 - 4:30 PM

PROCEEDINGS ON THE FOURTH BASIC SCIENCE WORKSHOP IN HISTOLOGY

(A. R. Villanueva, Ph.D. & Jules Elias, M.A.)

Workshop will include the following lecture topics:
- Bone Marrow Cells - Identification, Morphology and Pathology (Rocco Lotarocco, M.D.)
- Progress in Staining Decalcified Bone and Teeth (John Rees, D.D.S.)
- Immunohistochemistry - Plastic Embedding and Staining (Sharon Van de Velde, B.S.)
- Metabolic Bone Disease in Gastrointestinal Disorders (Dhanwade Rao, M.D.)
- Immunohistochemical Identification of Cells in Contact Dermatitis (Jules Elias, M.A.)
- Undecalcified Bone Stains - Review and Comments of Certain Stains (Antonio Villanueva, Ph.D.)
- Discussion, Questions and Answers

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On application below, please indicate first and second choice on limited workshops.

Check to ensure registration is for ONE, all day workshop, OR an AM and PM combination.

LATE REGISTRATION FEE OF $10 required after September 7, 1982.
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Make check/money order payable to: National Society for Histotechnology
MAIL complete form & fees to: NSH, P.O. Box 36, Lanham, Maryland 20706

MONDAY

		Member Fee	Non-Member Fee
All Day	#1	____$40	____$45
All Day	#2	____$40	____$45
All Day	#3	____$40	____$45
All Day	#4	____$40	____$45
AM	#5	____$20	____$25
AM	#6	____$20	____$25
AM	#7	____$20	____$25
AM	#8	____$20	____$25
AM	#9	____$20	____$25
PM	#10	____$20	____$25
PM	#11	____$20	____$25
PM	#12	____$20	____$25
PM	#13	____$20	____$25
Totals:			

TUESDAY

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All Day	#15	____$40	____$45
All Day	#16	____$40	____$45
All Day	#17	____$40	____$45
AM	#18	____$20	____$25
AM	#19	____$20	____$25
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PM	#22	____$20	____$25
PM	#23	____$20	____$25
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PM	#25	____$20	____$25

WEDNESDAY-THURSDAY-FRIDAY

	Member Fee	Non-Member Fee
Scientific Sessions (Sessions & fee include Wed. thru Fri.)	____$50	____$60

BANQUET ____$18

TOTAL REGISTRATION

Monday Workshops $_____
Tuesday Workshops _____
Scientific Sessions _____
Banquet _____
Late Fee $10 (required after Sept. 7, 1982) _____

TOTAL FEES: _____

SCIENTIFIC SESSIONS

WEDNESDAY, SEPTEMBER 15, 1982

HISTOTECHNOLOGY: THEN AND NOW	*Israel Diamond, M.D.*
HANDLING HAZARDOUS MATERIAL IN THE LABORATORY	*Guy Fragala, Ph.D.*
ESTROGEN RECEPTOR ASSAY IN BREAST CANCER	*Neptune Mizrahi*
HT/HTL PRACTICAL EXAMINATION CRITERIA	*William Kingsley, M.D.*
HISTOCHEMICAL COMPARISON OF SENILE PLAQUES IN MAN AND DOG	*Robert Pinzok, M.S.*

PROFESSOR C.F.A. CULLING LECTURE

IMMUNOHISTOLOGY - A NEW FRONTIER IN SURGICAL PATHOLOGY	*Clive Taylor, M.D.*
CHRONIC AND LATENT EFFECTS OF SODIUM ARSENITE IN THE AMERICAN OYSTER (Crassostrea Virginica)	*Carolyn Barszcz, B.S., HT (ASCP)*
WHAT EVERY HISTOTECHNOLOGIST SHOULD KNOW ABOUT MALIGNANT MELANOMA	*John Kaiser, M.D.*
SUDDEN DEATH IN HEART ATTACK	*Norman McLetchie, M.D., FRCPM, FCAP*
NSH MEMBERSHIP MEETING (Opening Meeting) 4:45 P.M.	

THURSDAY, SEPTEMBER 16, 1982

CAP WORKLOAD RECORDING SYSTEM	*Suzanne Carbone, HT (ASCP) & Thomas Webb*
THE USE OF HISTOPATHOLOGICAL TECHNIQUES IN THE DIAGNOSIS OF FISH DISEASES	*Arthur Hauck, M.S., FHI (AFS)*
PROBLEMS IN DIAGNOSTIC HISTOPATHOLOGY - CASE STUDIES	*Earl Kasdon, M.D.*
ROLE OF HISTOLOGY LABORATORY IN FORENSIC MEDICINE	*Luke Tedeschi, M.D.*
SLOW VIRAL INFECTIONS IN THE CENTRAL NERVOUS SYSTEM	*William Pendlebury, M.D.*
MARINE PATHOLOGY	*Paul Yevich, B.S.*
HISTOLOGY LABORATORY HAZARDS: FIRE AND FORMALDEHYDE	*Nicholas Hardin, M.D.*
MORTALITY STUDY OF HISTOLOGIC TECHNICIANS CERTIFIED BETWEEN 1948-1970 — FINAL RESULTS	*Dan Grauman, M.A.*

FRIDAY, SEPTEMBER 17, 1982

SKIN IS IN - SPECIAL DERMATOPATHOLOGY TECHNIQUES AND WHY EVERY HISTOTECH SHOULD KNOW THEM	*Florence Nygaard*
METHODOLOGY AND INTERPRETATION OF FINE NEEDLE ASPIRATION	*Rosa Enriquez, M.D.*
UPDATE ON THE EFFECTS OF IN UTERO EXPOSURE TO DIETHYLSTILBESTROL IN FEMALE AND MALE OFFSPRING	*Donald Antonioli, M.D.*
FROM SPILSBURY TO QUINCY: SCIENTIFIC DETECTION OF CRIME - THE DIAGNOSIS OF MURDER	*William Sturner, M.D.*
SPORTS MEDICINE	*Arthur Pappas, M.D.*
LECTURE from Paper Written During Workshop on Tuesday	

less than its TLV-TWA. For example, xylene's TLV-TWA is 100 ppm, but its ROTC is 0.47 ppm — about 200 times less than its TLV-TWA. Thus, while the concentration of xylene in laboratory air is usually less than 100 ppm and is ordinarily within "safe" levels, its presence is readily detected by smell at far less concentrations. Smelling even very low concentrations of xylene can cause headaches. In such instances, the concentration of xylene is unacceptable, even though within "safe" limits.

The vapor concentrations of formaldehyde and HCl, as well as other toxic chemicals, can be maintained below their TLVs-TWA by taking the following measures: (1) minimize their exposure to air by keeping all containers tightly capped or securely covered when the solutions are not in use; (2) use the solutions away from evaporation-promoting circumstances such as draughts from air conditioners, people traffic, proximity to doorways and sources of heat; and (3) use these chemicals only within the work space of a conventional, properly functioning fume hood.[4]

Self-contained partial enclosures with charcoal sorbents (e.g., Labconco's Fume Absorber,[5] Lerner Laboratories' Fume-Gard,[6] and Lipshaw's Fume-X-Peller[7]) cannot reduce the concentration of vapors as effectively as conventional fume hoods and are not recommended. Parenthetically, these units are misnamed, for it is not fumes they attempt to control, but vapors. Vapors are the gaseous forms of substances which are normally in the solid or liquid state (at room temperature and pressure). On the other hand, fumes are solid particles generated by condensation from the gaseous state, generally after volatilization from molten metals.[8] Fumes are not ordinarily produced in histology laboratories.

MDA Scientific's Captair 2007C is also a self-contained partial enclosure designed to filter toxic vapors. It uses a catalytic-reaction molecular filter as a sorbent and is more effective than charcoal. Its filtration efficiency has been demonstrated by an independent testing laboratory to be nearly 100 percent.[9] The Captair unit appears to be a worthwhile alternative to the expense of installation and operation of a conventional fume hood.

In summary, formaldehyde and hydrochloric acid are highly unlikely to yield bis-chloro-methyl ether under ordinary conditions of use in a histology laboratory. B-5 fixative is an improbable source of BCME. The concentration of a chemical's vapors in air should be kept so low that its presence escapes detection by smell. Self-contained recirculating partial enclosures that use charcoal sorbents to remove toxic vapors from the air are not recommended. Toxic chemicals such as formaldehyde and hydrochloric acid should be used under a conventional fume hood to ensure personnel safety.

References:

1. Frankel, L.S., McCallum, K.S., and Collier, L.: Formation of Bis (chloromethyl) Ether from Formaldehyde and Hydrogen Chloride. Env. Science Technol. 8:356-359, 1974.
2. American Conference of Governmental Industrial Hygienists: Threshold Limit Values for Chemical Substances in Workroom Air Adopted by ACGIH for 1975. ACGIH, Cincinnati, 1978.
3. Lillie, R.D., and Fullmer, H.M.: *Histopathologic Technic and Practical Histochemistry*, 4th Edition. McGraw-Hill, New York, 1976.
4. Scientific Apparatus Makers Association: Standard for Laboratory Fume Hoods. SAMA, Washington, D.C., 1975; 1980 revision available end of 1980. (Free copy available by writing SAMA, 1101 16th Street, N.W., Washington, D.C., 20036, or by calling (202) 223-1360.)
5. Labconco Corporation, 8811 Prospect, Kansas City, Mo. 64132.
6. Lerner Laboratories, 17 James Street, New Haven, Conn. 06513.
7. Lipshaw Manufacturing Corporation, 7446 Central Avenue, Detroit, Mich. 48210.
8. American National Standards Institute: Fundamentals Governing the Design and Operation of Local Exhaust Systems - ANSI Z9.2-1979. ANSI, New York, 1979.
9. MDA Scientific, Inc., 800 Busse Highway, Park Ridge, Ill. 60068.

Decalcifying Fluid Explosion

Leigh Winsor
James Cook University
School of Biological Sciences
Queensland 4811, Australia

The following is a recent report by Pirie and Soretire[1] regarding an explosion of a winchester of nitric acid-formalin decalcifying fluid:

"... an amber winchester half full of a 10% nitric acid-formalin decalcifying fluid exploded spontaneously with such force as to cover a room approximately 22 feet by 26 feet (6.6m x 7.8m) with fragments of broken glass. The bottle, which had been used earlier that morning, was standing in the place it had occupied for several weeks. Neither the room nor the weather was hot and the bottle was not in direct sunlight ... nobody was in the room at the time of the incident ... it is suggested that a build-up of gaseous methyl nitrite, formed by the reaction of traces of nitrous acid with the methanol usually added to prevent formaldehyde polymerization, is a possible cause."

Comment:

This incident bears certain similarities to explosions of sealed containers of chromic acid and aqua regia reported in the literature.[2] Possible explanations for these explosions include oxidation of contaminants and autodecomposition catalyzed by impurities present in the reagents involved. Similar mechanisms may, in general, apply to the nitric acid-formalin explosion.

Numerous decalcifying fluid mixtures and techniques have been thoroughly investigated, evaluated and rationalized in two excellent studies by Clayden[3] and Brain.[4] They have clearly demonstrated that 5-10% simple aqueous solutions of nitric acid or formic acid are the best choice of decalcifying fluids for routine diagnostic purposes. More complex and exotic mixtures were found to be generally inferior to these two simple fluids, providing that the latter were used correctly. It has also been found that substances, including formalin, added to nitric acid or formic acid decalcifying fluids to prevent maceration, serve no purpose when employed on well fixed tissues.[4] Simultaneous fixation and decalcification of tissues saves the time required for separate fixation. However, this time-saving is more than offset by the inferior quality of the resulting tissue sections.[5]

Although more limited, my experiences with decalcifying fluids lead me to concur with the foregoing findings, and I would urge that laboratories which at present employ nitric acid or formic acid-formalin mixtures, replace them with the safe, single decalcifying fluids recommended. Should personal preferences and prejudices see the retention of the nitric acid-formalin type fluids, then safe use can be simply attained by preparing the mixture immediately before use, or by storing the decalcifying fluid in a vented container.

The report highlights the safety problem inherent in many of the classical brews which abound in Histotechnology. A trend towards the use of scientifically formulated, rationalized, simple and effective reagents would be welcome.

References:

1. Pirie, C.J., and Soretire, E.A.: Decalcifying Fluid Explosion. Chemistry in Britain, 15:11-12, 1979.
2. Winsor, L.: Potential Health Hazards and Safety in the Histology Laboratory: A Review. Austral. J. of Med. Technol., 9:149-166, 1978.
3. Clayden, E.C.: A Discussion on the Preparation of Bone Sections by the Paraffin Wax Method with Special Reference to the Control of Decalcification. J. of Med. Lab. Tech., 10:103-123, 1952.
4. Brain, E.B.: *The Preparation of Decalcified Sections*. Charles C. Thomas, Springfield, Ill., 1966.
5. Wallington, E.A.: *Histological Methods for Bone*. Butterworths, 1972.

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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.