HISTO-LOGIC®

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GOLDEN FORCEPS AWARD WINNER

We are pleased to announce that Edna Prophet, B.A., H.T. (ASCP) has been selected as the recipient of the Golden Forceps Award for 1976. Her paper, "Technique for Processing Eye Specimens," was selected from articles submitted to HISTO-LOGIC* during the past year. Criteria for selection are clarity, originality, and scientific contribution. The Golden Forceps Award will be presented at the Symposium/Convention of the National Society for Histotechnology to be held in Minneapolis, Minnesota, October 8-15, 1976. Reprints of her article, which appeared in HISTO-LOGIC in January, 1976, are available from Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 N. Aurora Road, Naperville, Illinois 60540.

Data for Handling Toxic Chemicals Used for Fixation in Histopathology Laboratories

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If each individual operation in the preparation of tissues for microscopic examination were to be ranked according to its importance, *fixation* would certainly head the list. The selection of proper fixatives and adequate exposure of tissue to that fixative can result in slides of optimum clarity, differentiation, and unaltered cell morphology. Conversely, the choice of improper or chemically deficient fixatives can result in over- or under-fixation; shrunken or swollen cells; clumping of chromatin; halo effects (cytoplasm separating from nucleus); vacuolation; lysing of red blood cells.

The choice of a proper fixative depends on the working knowledge of the histotechnologist and his adherence to the use of that fixative which will yield the most satisfactory results. Assuming that his choice is a good one, many failures often occur simply because the material used is chemically deficient.

Most ready-made fixatives, as well as many chemicals used to prepare fixatives, have a definite shelf-life. The histotechnologist must have knowledge of the storage condition, stability and possible toxicological effect of these chemicals. Many histotechnologists simply do not have adequate working knowledge for safe, efficient laboratory operations. The information contained herein is intended to assist you in maintaining better control of chemicals commonly used for compounding fixatives.

The term "stable" as it applies to solutions, is rather difficult to define. Generally, fixative solutions have a shelf life of 1 to 2 years, but dry chemicals have a much longer shelf life. If properly stored and handled, many chemicals are stable for several years. When we speak of several years, remember that some chemicals are sensitive to moisture and will lose some of their stability if containers are opened and closed repeatedly.

Some simple rules to follow for handling chemicals in Histotechnology:

1. All dangerous, toxic, explosive, or flammable solutions and dry chemicals must be identified with appropriate labels.
2. Date chemicals as soon as they are received into your storage or receiving area.
3. Attention should be placed on safe storage, temperature control, ventilation, and proper lighting.
4. Be sure to pay particular attention to the lot number printed on the label of all chemicals. These lot numbers are important. The manufacturers can readily supply you with the ages and contents of chemicals you find suspect.
5. When preparing fixatives, the histotechnologist should always use ventilation hoods, as many chemicals used in fixatives are toxic and dangerous. The use of gloves when handling or mixing solutions should be adhered to.
6. Finally, good procedural technique suggests not using chemicals which have been on hand for several years.

The following chemicals are the most widely used for fixatives in histotechnology. A brief explanation of shelf-life and toxic effects of each chemical has been included.

37-40% Formaldehyde
Formaldehyde is stable for several years under proper storage conditions. That is, the containers should be kept closed and not opened until needed and should not be exposed to excess heat or cold. A cloudy precipitate of paraformaldehyde may form as the result of low temperature exposure.

Although 10-15% methanol is added to prevent the polymerization of paraformaldehyde, it has been reported that a temperature of only 65° F (18° C) has been sufficient to initiate polymer precipitation. When the solution is evaporated, some formaldehyde gas escapes, but most of it converts to paraformaldehyde.

Toxic effects are mainly those of irritation. If swallowed, it causes violent vomiting and diarrhea which can lead to collapse. In short, it is a strong irritant and has high toxicity if ingested or inhaled. Formaldehyde may cause skin dermatitis if gloves are not used when handling formalin-laden organs or the direct fixative.

Neutral Buffered Formalin
This solution has a shelf-life of approximately 2 years. This means that the solution remains stable for that period of time under proper storage and handling conditions.

Although the formaldehyde used in fixatives is more dilute, the same precautions should apply.

Picric Acid Solution
Picric acid has a stability of approximately 2 years and contains about 1.2% picric acid at saturation. In addition to causing allergic as well as irritative dermatitis, it is regarded as a highly toxic poison if ingested.

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In the dry form it is considered an explosive and, for this reason, should not be allowed to dry excessively.

Acetic Acid
Acetic acid is also very stable when properly handled and stored. The same general precautions apply to acetic acid as to all acids. It is not only a strong irritant but also is highly toxic if ingested and is a *moderate fire hazard* when exposed to heat or flame. Since acetic acid can solidify at about 61° F (17° C), it should not be exposed to cool temperatures.

Potassium Dichromate
This item is also very stable because it is neither hygroscopic (absorbs moisture) nor deliquescent (dissolves by absorption of moisture). It is highly toxic by ingestion or inhalation and, being an oxidizing agent, is a dangerous fire risk when placed in contact with organic materials.

Sodium Phosphate, Dibasic, Anhydrous
This chemical is anhydrous and, being hygroscopic, will absorb moisture on exposure to air. It also may have a tendency to "cake up" and should be kept in tightly closed containers. This chemical has a low toxicity.

Calcium Chloride, Anhydrous
This item is not only hygroscopic, but also deliquescent and is extremely sensitive to moisture. It should be kept in tightly closed containers. When dissolved in water it liberates heat. It has a low toxicity.

Mercuric Chloride
The main precaution with mercuric chloride is its toxicity. It is highly toxic, and may prove fatal if ingested, inhaled or absorbed through the skin. Hands should be washed before eating or smoking. It is even slightly volatile at ordinary temperature and the *dust should not be inhaled.*

Sodium Sulfate, Anhydrous
This chemical also can absorb moisture and should be kept in tightly closed containers. The details on its toxicity are unknown but are suspected to be low.

Sodium Phosphate, Monobasic, Monohydrate
This item is slightly deliquescent and may have a tendency to "cake up" if affected by humidity. For this reason, it should be kept in tightly closed containers. The exact toxicity is unknown but is suspected to be low.

Ammonium Bromide
This chemical has a tendency to be hygroscopic and can slowly become yellowish in air due to decomposition. For this reason, the containers should be kept tightly closed. It is moderately toxic by ingestion and inhalation.

Sodium Acetate, Trihydrate
Sodium acetate is efflorescent. That is, it can become powdery through loss of water, or crystalline on exposure to the air. It may also have a tendency to "cake up" and should be kept in tightly closed containers. It has a low toxicity.

Sodium Chloride, Anhydrous
This chemical is found as colorless, transparent crystals or white powder, and is somewhat hygroscopic. It is stable in tightly closed containers. It possesses low toxicity except when heated to a high temperature, and the vapors emitted are irritating to the eyes.

References:

1. *The Merck Index*, Eighth Edition, published by Merck & Co., Inc., N.J.
2. *The Condensed Chemical Dictionary*, revised by Gessner G. Hawley, eighth edition, published by Van Nostrand Reinhold Co., New York, N.Y.
3. *Dangerous Properties of Industrial Materials*, N. Irving Sax, third edition, published by Van Nostrand Reinhold Co., New York, N.Y.
4. Chemical Safety Data Sheets from the Manufacturing Chemists Association, Washington, D.C.

Improved Design of New Cassette Makes Tissue Identification Easier

Don Hammer, Histology Manager
Epply Institute for Research in Cancer
University of Nebraska Medical Center
Omaha, Nebraska 68104

If you have ever used Tissue-Tek® II embedding cassettes* in the past, you may have encountered some problems with plastic warping and with the plastic writing surface. After some testing of the relatively new cassettes produced through a new manufacturing process, I am convinced the problems have been eliminated. The testing resulted in processing of tissue cassettes with no warping of the plastic. This eliminated the problem of the cassette lid popping open.

In addition, the writing surface has been improved considerably. It is now an excellent surface which enables the histotechnologist to label the cassettes very clearly. I have tried a variety of pencils and pens, and have found that an ordinary No. 2 pencil or the A. W. Faber Black Magic No. 850 pencil is best suited for use on the Tissue-Tek® II cassette writing surface.

It is also much easier to write additional descriptive information on the edge of the redesigned cassette when and if needed. This writing can be smeared somewhat by rubbing, but smearing seldom occurs in normal usage.

*Lab-Tek Products, Naperville, Illinois

A Modified Auramine O-Rhodamine B Method for Better Demonstration of Acid-Fast Organisms with Fluorescent Microscopy

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Swedish Medical Center
Englewood, Colorado 80110

Fluorescent microscopy and fluorchrome acid-fast staining have provided a means for investigators of rapid screening of smears and sections (in particular where only a small number of bacilli are present). Although existing literature states *M. Tuberculosis* and *M. Leprae* are demonstrated by the Auramine-Rhodamine method,[1] it has been our experience that Lepra fluorchrome is very limited if evident at all by Truant's method.[2]

Since staining modes are probably similar to the traditional, conventional carbol fuchsin methods;[3] a modification such as Fite developed,[4] utilizing non-alcoholic solutions and "mild" sulfuric acid decolorization differentiation, was employed to

Tissue-Talk

Workshops

Workshop	Title	Instructor	Day	Date	Time	Location
1.	Introduction to Stain Mechanisms Seminar	Jack B. Wenger	Monday	Oct. 11	9:30 AM - 4:00 PM	Star of the North Hall Room
2.	Management Seminar	Dr. James N. Young	Monday	Oct. 11	9:30 AM - 4:00 PM	Mart, Room 202
3.	Fluorescent Antibody Techniques	C. F. A. Culling	Monday	Oct. 11	9:30 AM - 4:00 PM	Mart, Room 201
4.	Special Stain Workshop	Erwin Haas	Monday	Oct. 11	*9:00 AM - 12:00 noon *1:30 PM - 4:30 PM	Mart, Rooms 204 & 206
5.	Tissue Identification	Lee G. Luna Charles West	Tuesday	Oct. 12	9:30 AM - 4:00 PM	Star of the North Hall Room
6.	Innovative Histotechnology Workshop	H. Keith Russell Patsy Willard	Tuesday	Oct. 12	9:30 AM - 4:00 PM	Mart, Rooms 203 & 205
7.	Cryostat Technology	Frank Avallone	Tuesday	Oct. 12	9:30 AM - 4:00 PM	Mart, Rooms 204 & 206
8.	Electron Microscopy	Alice M. Harju	Tuesday	Oct. 12	*1:00 PM - 4:00 PM	Mart, Room 201
9.	Medical Photography	Robert Chu	Monday	Oct. 11	*9:30 AM - 12:00 noon	Mart, Room 205
10.	Tissue Culture	Mary Schmitt Pat Wirth Pin King	Monday	Oct. 11	*1:00 PM - 4:00 PM	Mart, Room 205
11.	Cytology	Carol Kanneberg Jo Ann Samuelson	Tuesday	Oct. 12	*9:30 AM - 12:00 noon	Mart, Room 202
12.	Enzyme Histochemistry	Andy Valls Michael Bentley	Tuesday	Oct. 12	*1:00 PM - 4:00 PM	Mart, Room 202
13.	Chromosome	Karen Kurvink	Tuesday	Oct. 12	*9:30 AM - 12:00 noon	Mart, Room 201

*(Half-day Workshops)

1. Introduction to Stain Mechanisms Seminar

Jack B. Wenger

This seminar will consist of lectures on staining theory, suggesting applications to some of the most often required special stains. Staining procedures will be approached by way of the following concepts: Salt-protein unions; isoelectric points; mordant mechanisms; the dual nature of silver and as time permits, reactions for minerals. Prerequisites: Some knowledge of special stains and solution formulation. It would be very helpful if the registrant would review some elementary material concerning the nature of electrolytic (ionic) solutions.

2. Management Seminar

Dr. James N. Young

A promotion or the preparation for advancement means more responsibility associated with management of additional people and resources. Learn and practice with your associates how you can become a better manager by employing the latest behavioral sciences management techniques. *Management* is the *key to your success;* prepare now to accept the challenge. We live in a world of rapid change necessitating new approaches to the age old problem of managing people and resources. The future is now — are you prepared to be an effective manager and to accept the challenge? Learn and practice with your associates the latest behavioral sciences management techniques.

3. Fluorescent Antibody Techniques

C. F. A. Culling

This workshop will be divided into four sections, aimed at equipping the student to understand the underlying theory necessary for the practice and comprehension of fluorescent antibody technique.

Section 1, Elementary Microscopy: how a lens works, what is light, the component parts of the microscope.

Section 2, The Fluorescent Microscope: what and why is fluorescence, component parts of the fluorescent microscope, the various types of filters and how they are used in FAT (transmission v interference), transmission versus epi fluorescence.

Section 3, Modern Concepts of Immunity: what are antibodies, what are the various types of Ig, how are they formed, what they do, what are 'H', 'B', and 'T' cells.

Section 4, "Putting it all Together": the theory and practice of fluorescent antibody techniques, with a brief description of the use of peroxidase labelled antibody techniques.

4. Special Stain Workshop*

Erwin Haas

This half-day workshop will cover the following special stains: Verhoeff's Elastic; Gordon Sweet Reticulum; Masson Trichrome; Benhold's Congo Red; McCallum-Goodpasture's; Ziehl Neelsen. The workshop will be given twice on Monday; the morning workshop will be repeated in the afternoon. A question and answer period and slide presentation will follow the practical exercise for an in-depth discussion of the special stains performed during the workshop.

5. Tissue Identification

Lee G. Luna and Charles West

The purpose of this workshop is to acquaint the participant with basic tissue structures. By the end of the workshop each registrant will have acquired sufficient knowledge of the various tissues to enable them to perform most special stains and then check various tissue structures for proper staining.

6. Innovative Histotechnology Workshop

H. Keith Russell and Patsy Willard

All participants will actively engage in the following histological functions: Microtomy, knife sharpening, hematoxylin and eosin staining, microscopy, and a plastic microtomy technique session which will be conducted by a Sorvall representative. Short lectures on fixation and processing of tissues will be given. The workshop will end with a group discussion. This workshop is innovative because it covers many aspects of practical histological techniques, allows participants to work at their own pace, provides interaction between faculty, histotechnologist and exhibitor, and provides a chance for participants to use the latest equipment.

7. Cryostat Technology

Frank Avallone

Workshop will include information on basic and advanced techniques in the field of cryotomy. Lectures will be presented on different aspects of cryotomy and include such topics as general and specialized techniques in the field of histochemistry and immunofluorescence, routine staining procedures, and a short history of cryotomy. There will be a problem session and a limited number of cryostats for class use. Registrants are encouraged to discuss their problems, whether they deal with knives, tissues, or some other aspect of cryostat technology during the workshop meeting.

8. Electron Microscopy*

Alice M. Harju

The purpose of this workshop is to give basic information regarding proper handling of tissues which will be processed for electronmicroscopy. Discussion will include: (1) SAFETY while working with the toxic solutions involved. (2) TECHNIQUE with special emphasis upon the "time" factor involved in initial fixation. Procedures will be demonstrated and references will be given as handouts.

9. Medical Photography*

Robert Chu

Medical photography of general information will be presented by NIKON, covering a photographic refresher, specialized lenses, applications and techniques.

10. Tissue Culture*

Mary Schmitt, Pat Wirth and Pin King

Workshop will present Basic Tissue Culture — overview of techniques in staining of tissue cultured cells. Definitions of terms, media preparations and procedures, stain preparations and procedures will be part of the workshop.

11. Cytology*

Carol Kanneberg and Jo Ann Samuelson

Cyto-preparatory Techniques: Techniques will be discussed for preparation of all cytologic material. This will include aspiration cytology, bronchial brushing technique, and the cytocentrifuge.

12. Enzyme Histochemistry*

Andy Valls and Michael Bentley

Workshop will include lectures on history and general chemistry on phosphatase reaction, both lite and electron microscopy. Specific methodologies will be presented. A slide presentation will provide the suggested application for the techniques and reveal possible sources of artifacts.

13. Chromosome*

Karen Kurvink

Procedures for human tissue culturing chromosome harvesting, slide preparation, chromosome banding and karyotyping will be emphasized. Additional techniques for obtaining chromosomes from skin, amniotic fluid and bone marrow will be available.

***Half-day workshops**

Scientific Sessions

(Star of the North Hall Room)

WEDNESDAY, OCTOBER 13, 1976

Session	Speaker
Dermatological Specimens: When a Little Can Mean a Lot	*Carolyn A. Durkowski*
The Use of Histologic Technique for Determination of Tissue Response to Thyroid Allografts	*Marlyce George*
The Uses and Abuses of Histochemistry	*C. F. A. Culling*
Barbs in the Pee Patch or Techniques of a Renal Biopsy	*Deborah Hillhouse*
I Wonder If (The Unemplanted Embryo Studies and the Alizarin Red S Stain for Animal Skeleton)	*E. Louise Russell*
Workload Recording and Quality Control in the Histotechnology Laboratory	*Erna E. Austin*
Histologic Study of Degeneration: (A) Routine Staining (B) Histochemical Staining (C) Special Procedures	*Virginia Havener*
Basic Chemistry	*Vera Hinkelman*

THURSDAY, OCTOBER 14, 1976

Session	Speaker
The Histotechnologist's Role in Muscle Biopsy Procedure	*Eugene W. Nash*
Formaldehyde as a Fixative for Light and Electron Microscopy	*Freida L. Carson*
Pathology of the Human Placenta	*Dr. Ralph A. Franciosi*
College of American Pathologists Inspection	*Norma Carmody*
Improved Method for Demonstrating Spirochetes	*Jack B. Wenger*
Fixation With Microwave Ovens	*Dan Maneval*
Diagnostic Special Stains for Surgical Pathology	*Erwin Haas*

FRIDAY, OCTOBER 15, 1976

Session	Speaker
Can You Specifically Demonstrate Connective Tissue Elements?	*C. F. A. Culling*
A Combination Stain for Acid Fast Bacilli and Fungi	*Jerry Meade*
Kidney Biopsies	*Frank Avallone*
Potpourri Histologically Speaking	*Lee G. Luna*
Acid Fast Contaminants	*Freida Carson*

(DAY AND TIME OF ABOVE LECTURES SUBJECT TO CHANGE ON FINAL PROGRAM)

Evening Sessions

WEDNESDAY, OCTOBER 13, 1976	**Symposium Banquet**
THURSDAY, OCTOBER 14, 1976	**NSH Membership Meeting**

National Society for Histotechnology
Symposium/Convention

The Second Annual Symposium/Convention of the National Society for Histotechnology will be conducted at the Radisson Downtown Hotel, Minneapolis, Minnesota, October 11-15, 1976.

Registration application is attached. Please submit your registration by September 15. Registration forms may be photocopied if more than one individual from the same activity wishes to attend. Mail registration and check to: **Registrar, P.O. Box 36, Lanham, Maryland 20801.**

Also attached is a Radisson Hotel reservation card. Please complete and mail directly to the hotel.

The NSH/Thomas Edison College exam program will be conducted prior to the Symposium on SUNDAY, OCTOBER 10th. Exams will be held in the Radisson Mart Rooms, 3rd floor. Reviews for each exam will be presented Sunday A.M., and the examination given in the P.M. All three examinations will be completed on Sunday: Introductory Histotechnology, Histochemistry and College Chemistry.

All examinees must be pre-registered before taking the exam. If you would like to take the exam and have not registered, submit $5.00 for study guide to Roberta Mosedale, P.O. Box 36, Lanham, Maryland 20801.

NATIONAL SOCIETY FOR HISTOTECHNOLOGY
SYMPOSIUM/CONVENTION
OCTOBER 11-15, 1976

radisson hotel downtown

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PLEASE MAKE THE FOLLOWING RESERVATIONS:

	Room	Rate
____	Single Room	$25.00
____	Double Room (one double bed)	$28.00
____	Twin Room (two twin beds)	$30.00
____	Suites (one bedroom)	$60.00 to $90.00
____	Extra Bed	$ 7.00

If requested rate is not available, the nearest rate will apply.

All rooms held until 6 p.m. unless the room is guaranteed, or a deposit has been received.

(PRINT) ORGANIZATION Natl. Society of Histotechnologists-October 8-15, 1976

(PRINT) NAME ________ PHONE (____) ________

ADDRESS ________ CITY ________ STATE ________ ZIP ________

ARRIVAL DATE ________ ☐ A.M. ☐ P.M. DEPARTURE DATE ________

NATIONAL SOCIETY FOR HISTOTECHNOLOGY
SYMPOSIUM/CONVENTION
OCTOBER 11-15, 1976

DO NOT USE THIS SPACE

NAME ________ (Last, First, Initial)

HOME ADDRESS ________ (Street, City, State, Zip)

EMPLOYER ________

ADDRESS ________ (Street, City, State, Zip)

Please check functions you desire to attend:

Scientific Sessions____$40.00 (Wed - Fri)

Banquet____$13.00 (Wednesday Evening)

WORKSHOPS

Monday

No. 1 ____ $20
No. 2 ____ $20
No. 3 ____ $20
No. 4 ____ $20 (1/2 day AM)
____ $20 (1/2 day PM)
No. 9 ____ $20 (1/2 day AM)
No. 10 ____ $20 (1/2 day PM)

Tuesday

No. 5 ____ $20
No. 6 ____ $20
No. 7 ____ $20
No. 8 ____ $20 (1/2 day PM)
No. 11 ____ $20 (1/2 day AM)
No. 12 ____ $20 (1/2 day PM)
No. 13 ____ $20 (1/2 day AM)

(Please read program carefully. Do not check an all day and half day workshop on the same day.)

Check or Money Order **MUST ACCOMPANY** Registration!
Payable to: National Society for Histotechnology
Mail Registration to: Registrar, P.O. Box 36, Lanham, Maryland 20801
PLEASE NOTE: Reimbursement of registration fees will be made upon receipt of cancellation notification prior to October 1st. **NO REFUNDS WILL BE MADE AFTER THIS DATE.**

Visit our booth at the Convention to see . . .

Four LAB-TEK® products that add efficiency through added convenience

Tissue-Tek® II Cold Plate
Tissue-Tek II Cold Plate is designed to keep paraffin blocks chilled without ice cubes or containers of chipped ice. After blocks are embedded at Tissue-Tek II Tissue Embedding Center, they can be placed on the Cold Plate and kept at the microtome ready for sectioning. Each Cold Plate holds 24 Tissue-Tek II Process/Embedding Cassettes. Tissue-Tek II Cold Plate contains a special formulation that freezes overnight when placed in a conventional freezer. Stack trays for greater cold retention. The styrofoam base supplied with each package will further prolong cooling.
No. 4650 — Tissue-Tek II Cold Plate — packages of six with one insulator base.

To modernize embedding procedures New Tissue-Tek® II Forceps Warmer
New Tissue-Tek II Forceps Warmer No. 4655 eliminates the need for flaming tissue forceps. An electrically operated unit, it provides three wells to hold up to three pairs of forceps in paraffin at a temperature suitable for tissue embedding. When the forceps are put into the paraffin, the temperature of the tips is elevated to approximately 85° C. Paraffin does not stick to the tips of the forceps during tissue orientation. Insulating material for gripping surfaces for three pairs of forceps is provided. Operating instructions with directions for applying forceps insulation are supplied with each Tissue-Tek II Forceps Warmer.
For safe and efficient forceps warming in your laboratory, use Tissue-Tek II Forceps Warmer.

Space Saving — Tissue-Tek® II Slide Staining Sets
Tissue-Tek II Slide Staining Set (No. 4451) provides a compact, efficient system for histologic or cytologic slide staining. Each staining set consists of 12 chemically inert plastic staining dishes with lids and a metal carrier with drain tray/cover. The dishes have been designed to minimize space requirement and solution carry over. The lids can be stored conveniently in the rear of the tray during staining.
Each dish in the set easily accommodates a Tissue-Tek II Slide Staining Holder (No. 4465) carrying 25 slides. To increase the flexibility of the set, double slide holders carrying 50 slides are available (No. 4466).
For convenience and economy without the expense of instrumentation use Tissue-Tek II Slide Staining Sets and Holders.

Tissue-Tek® II Tissue Processor
Tissue-Tek II Tissue Processor (No. 4640) can carry the whole load or act as an economical backup in an overloaded laboratory.
Table-top size fits under exhaust hoods. Smooth-surfaced deck is easy to clean.
Timing dial regulates entire cycle. The delayed timing capability offers dependable processing over weekends and up to 50 hours when needed.
Two stainless steel perforated tissue baskets are in constant motion for thorough tissue penetration. Each holds up to 50 cassettes. Insulated stainless steel paraffin pots have separate thermostats.
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overcome this problem, as well as excessive extraction of dye from fluorchromed tuberculosis bacilli.

Fixation: 10% buffered neutral formalin

Microtomy: Cut sections at 5 microns.

Solutions:

Xylene-Peanut Oil Solution

Peanut oil	1 part
Xylene	2 parts

29% Ferric Chloride

Ferric chloride	29.0 gm
Distilled water	100.0 ml

Add just before Weigert's hematoxylin solution.

Weigert's Iron Hematoxylin Solution
Solution A

Hematoxylin crystals	1.0 gm
Alcohol, 95%	100.0 ml

Solution B

29% ferric chloride	4.0 ml
Distilled water	100.0 ml
Hydrochloric acid, concentrated	1.0 ml

Auramine-Rhodamine Solution

Auramine O	1.5 gm
Rhodamine B	0.75 gm
Glycerin, USP	75.0 ml
Phenol (melted crystals)	10.0 ml
Distilled water	50.0 ml

1% Sulfuric Acid

Sulfuric acid, concentrated	1.0 ml
Distilled water	99.0 ml

Staining Procedure:

1. Decerate through two changes of xylene-peanut oil solution for 12 minutes each.
2. Drain slide, wipe off excess oil, blot carefully and thoroughly with bibulous paper.
3. Place in Weigert's iron hematoxylin solution for 10 minutes. (This step may be omitted, but it offers some background fluorescence inhibition. It also offers some gross visualization of the section but not a dark nuclear staining such as in Truant's method.)[5]
4. Wash slides in running tap water for 10 minutes.
5. Place slides in Auramine-Rhodamine solution at 60° C for 10 minutes.
6. Wash slides in running tap water for 2 minutes.
7. Decolorize slides individually with 1% sulfuric acid solution until section is translucent orange-pink (or clear).
8. Wash slides in running tap water for 5 minutes.
9. Rinse slides in distilled water.
10. Dehydrate slides in 95%, 100% alcohol and clear in xylene, two changes each.
11. Mount coverglass with Histoclad or other non-fluorescent media.

Examination:
Examine with suitable light source (the 360-420 millimicron range is suggested)[6] and filter.

Results:
Bacilli fluoresce — reddish yellow
Background tissue — green to flat black
Artifacts produce a — greenish yellow fluorescence

Remarks:
It is difficult to obtain suitable fluorchroming on decolorized material previously stained with carbol fuchsin, but material first stained with fluorchrome technique may be decolorized and stained with carbol fuchsin procedures.[7] There is also a possibility Norcardia filaments may be demonstrated by this method. This warrants further investigation.

References:

1. Thompson, S. W.: Selected Histochemical and Histopathological Methods, C. C. Thomas, Springfield, pp 951, 1038-1043, 1966.
2. Truant, J. P.: Henry Ford Hospital Medical Bulletin, *10*:287, 1962.
3. Thompson, S. W.: Selected Histochemical and Histopathological Methods, pp 1026-1029, 1966.
4. Fite, G. L., Cambre, P. J., and Turner, M. H.: Arch. Path., *43*:624-625, 1947.
5. Luna, L. G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill Book Co., Blakiston Division, p. 219, 1968.
6. Thompson, S. W.: Selected Histochemical and Histopathological Methods, C. C. Thomas, Springfield, pp 1036-1043, 1966.
7. Mansfield, R. E.: Am. J. Clin. Path., *53*:394-406, 1970.

A Method for Cleaning Tissue Capsules

Herbert Stevens
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Dearborn, Michigan 48124

The method most often used for cleaning tissue capsules after embedding is to submerge them in one of the paraffin solvents for several hours. Generally they are exposed to several changes of the solvent to assure that all paraffin has been removed.

Our laboratory uses 80-120 tissue capsules a day. The cleaning of this number of capsules requires two gallons of xylene a week. This is quite an expensive method of cleaning tissue capsules. Secondly, xylene is a derivative of crude oil, making it difficult to obtain at times. Thirdly, xylene-cleaned capsules require washing in alcohol to remove the xylene film attached to the capsule. This prevents xylene contamination of the first solutions on the tissue processor. Because of the problems mentioned above, the following method was devised and has worked well in our laboratories.

1. After embedding, capsules are collected in a stainless steel or similar container.
2. Add a generous portion of edisonite or any other good surgical detergent cleaner.
3. Fill the container with tap water.
4. Place the container on a hot plate and bring to a boil. About five minutes of boiling is required to melt the paraffin which will rise to the surface.
5. A paper towel or scoop can be used to remove the excess paraffin and debris from the surface.
6. Remove the container from the hot plate and place under hot running tap water.
7. Flush well with running tap water.
8. Place capsules on paper towels to dry. If time is limited, capsules can be dried easily in a drying oven.

One note of caution! If you have been using xylene to clean your capsules over an extended period, it will be necessary to lengthen the boiling and flushing time. This is due to the excess build-up of xylene film.

Summary:

This method of cleaning tissue capsules has saved considerable time and expense, and results in much cleaner tissue capsules. Also, by reducing the cleaning time to less than an hour a day there is a reduction in the number of capsules which must be kept on hand.

HISTO-LOGIC®
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Neatest new idea in the HISTO LAB

Tissue-Tek® II Microtome Waste Collection System

- glare-free tray
- waste slot
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- disposable plastic bag

New Tissue-Tek II Microtome Waste Collection System 4657 keeps histologic work areas and floors neat and free from paraffin shavings. The plastic tray is placed beneath the microtome which holds it securely in place. As sections are trimmed and cut, any waste matter falls onto the tray and is funneled through the open chute directly into a disposable plastic bag. When the bag is filled, it is removed, sealed, and discarded. Replacement bags are easily attached. The unique new Microtome Waste Collection System makes cleanup a simple matter of wiping the tray with any of the usual laboratory cleaning solutions. Your Lab-Tek dealer will be glad to show you this new system, or call us directly.

NO MICROTOME SHOULD BE WITHOUT ONE.

Lab-Tek Products . . . setting the standards by which performance is judged.

Technique for Preserving Wet-mount Slides

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Gibbstown, New Jersey 08027

In cases where water-mounted material must be examined, such as crystal violet for amyloid or Paragon solution for frozen sections, some method must be used to "ring" the coverslip to prevent evaporation of water.

The following has been used by the author for preserving wet-mount slides for up to four weeks:

1. Wipe excess water from the slide around the tissue.
2. Place one or two drops of distilled water on the tissue specimen and coverslip.
3. Hold the slide on edge and allow the excess water to drain onto absorbent paper. The coverslip should be at the edge of the slide and in contact with the paper.
4. Center the coverslip and allow the excess to dry from the edges of the coverslip.
5. Using a wooden applicator stick which has been flattened approximately 1/2 inch by shaving, apply a sealant around the edges.

The sealant consists of CoverBond* diluted 1:5 with ethyl acetate. Ethyl acetate, which has the characteristic odor of nail polish remover, evaporates much more quickly than usual mounting media. Because the solvent and the resin are both immiscible with water, the aqueous preparation does not leak.

The sealant used is much thinner than regular mountant. Consequently, it flows faster and should be transferred in small quantities. Several applications may be necessary to ensure that the seal is complete. The extra time is worthwhile, however, when one considers that the slide will stay permanent for four weeks, and that the coverslip cannot be moved when the sealant has dried.

*Scientific Products, 8855 McGaw Road, Columbia, Maryland 21045

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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 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.