HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor A Technical Bulletin for Histotechnology Vol. V, No. 4 - October, 1975

Four-Hour Processing Schedule for Mouse Tissue

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Our records show that in 1974 we necropsied, processed, embedded, sectioned and stained H&E slides on 98,847 adult and fetal mice. To handle this large volume of tissue it became necessary to develop a modified 4-hour processing schedule for the 10 Ultra tissue processors, used in the Histopathology Laboratory Branch.

The delay timer was modified to provide an overnight run of 450 Tissue-Tek® II Cassettes* (50 adult mice) and made ready for embedding in the morning when the histotechnologist reports for work. In addition, we scheduled a day run with 450 cassettes which are ready for embedding by 1:00 p.m. that same day. One to 7 organs are placed in each cassette and 1 to 10 cassettes per animal depending upon the experiment being conducted.

On the day schedule the various solutions are rotated and/or changed. The following solution changes are made just prior to starting the day processing schedule. (A) Replace the 70% and 80% alcohols (beakers 1 and 2) with fresh alcohols. (B) Discard the 95% and absolute alcohols (beakers 3 and 5). (C) Move 95% alcohol (beaker 4) to beaker station 3. Replace beaker station 4 with fresh 95% alcohol. The same rotational procedure is used with the absolute alcohol. Move beaker from stations 6 and 7 to beaker stations 5 and 6. Replace beaker 7 with fresh absolute alcohol. The same procedure can be followed for the xylenes. (D) The impregnating paraffins are replaced completely for both the day and overnight schedules.

The overnight run is loaded on the Ultra in the afternoon and the delayed timer used. For this run all alcohols and infiltrating media are replaced. To properly infiltrate mouse tissue, infiltration media must be kept fresh.

The processing temperature used is 35° C, paraffin baths are 57° C to 60° C. Animal tissue should not be exposed to temperatures over 60° C.

Four-Hour Processing Schedule

Beaker	Solutions	Time
1	70% ethyl alcohol	25 minutes
2	80% ethyl alcohol	10 minutes
3	95% ethyl alcohol	10 minutes
4	95% ethyl alcohol	10 minutes
5	100% ethyl alcohol	15 minutes
6	100% ethyl alcohol	20 minutes
7	100% ethyl alcohol	20 minutes
8	Xylene	10 minutes
9	Xylene	15 minutes
10	Xylene	15 minutes
11	Paraffin	20 minutes
12	Paraffin and Paraplast (2 parts) (1 part)	50 minutes

*Lab-Tek Products, Naperville, Illinois
Mention of products in this paper does not constitute an endorsement by NCTR, FDA, or EPA.

Automated Method for Deceration and Rehydration of Microscopic Slides

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Marine Research Laboratory
St. Petersburg, Florida 33701

Although there are automatic staining machines on the market today, it may be impractical to employ them in small clinical or research laboratories because of limited work loads. Fortunately, many of these laboratories have automatic tissue processors which can be used to perform a dual function.

Our laboratory uses a double decker Autotechnicon in the following manner: The lower level is set up for tissue processing while the upper level is used for decerating and rehydrating slides according to the following schedule.

1. Xylene, 5 minutes.
2. Xylene, 5 minutes.
3. Xylene, 5 minutes.
4. Absolute alcohol, 2 minutes.
5. 95% ethanol, 2 minutes. (See remarks)
6. 70% ethanol, 2 minutes. (See remarks)
7. 70% ethanol, 2 minutes.
8. Distilled water, 2 minutes.

Slides are transferred to coplin jars or staining dishes filled with distilled water or 70% ethanol, depending on which stain is to be used.

Remarks For Bouin's fixed material, insert a beaker of saturated sodium thiosulfate in 70% alcohol between steps 5 and 6. This solution removes the picric acid from the slides and insures better staining reactions. *Exposure time to this solution should be 5 minutes.*

If a mordant is required, as is the case with some special stains, that solution is inserted between steps 6 and 7. The exposure time is dictated by the stain used.

The different timing schedules are determined by notching the timing discs according to procedure used. It only takes a few minutes to reposition the beakers and set the timing disc. Slides which are being stained in a variety of stains are identified by using the stain abbreviations or number coded. A single level tissue processor can be used for deceration and rehydration if one maintains an extra set of beakers filled with the proper solutions. These beakers replace the processing beakers and the properly set timing disc is used.

Decalcifying Solution for Hard or Soft Tissue[1]

Livia M. Molnar
Department of Orthodontics
University of Washington
Seattle, Washington 98195

Decalcification is thorough and rapid with this solution (1-7 days depending on the size of the hard or soft tissues) and there is no need to change the solution every day. Solution can

be kept in a dark bottle on a shelf for six months in good standing.

Decalcifying Solution

Distilled water	1010.0 ml
Formic acid	90.0 ml
HCl	80.0 ml
Sodium citrate	10.0 gm

Decalcifying Procedure

1. Fix tissue specimens in any type fixative. (Formalin preferred)
2. Transfer tissue to decalcifying solution.
3. Let stay for 1-7 days (depending on size of specimen).
4. Check with x-ray every 3rd day (again, depending on size of specimen).

Preparation of this note was supported in part by grant RR00166 from the National Institutes of Health.

[1]We wish to acknowledge Dr. K. Bankuthy (deceased) who developed this solution and shared it with our laboratory

Additional Hints

Brenda Cuevas and Jose Torres
Gorgas Hospital Laboratory
Balboa Heights, Canal Zone

We have read with great interest the hints which appeared in the AFIP Letter,* Vol. 133, No. 9, December 1974, paragraph four. We would like to share our procedure with readers of HISTO-LOGIC. The following solution is used in this procedure:

Glycerin-Alcohol Solution

Alcohol, 70%	90.0 ml
Glycerin	10.0 ml

Softening Procedure

1. Trim (rough cut) the paraffin block until the tissue is exposed.
2. Place paraffin block in a petri dish containing glycerin-alcohol solution and let stand for 1 hour.
3. Remove paraffin block from solution and remount on the microtome.
4. Chill block surface with a piece of ice and section.

*The AFIP Letter referred to contained the following information: Dried tissue, thyroid colloid, hemorrhage, eye lens, bone, etc., can be sectioned intact if some water is applied to the tissue in the following manner: Rough cut block until all tissue is exposed. Dip a piece of cotton into the flotation bath and squeeze out excess water. Apply water-soaked cotton to the surface of the block for a few seconds. Chill paraffin block surface with a piece of ice and section.

ALSO

In HISTO-LOGIC, Vol. 4, No. 2, April 1974, Shirley Orlando requested ideas pertaining to the difficulty of obtaining sections from specimens containing large amounts of fat; lipomas for example. We have developed the following procedure and find it most useful in dealing with this type of problem.

Solutions

10% Alcoholic Formalin

Formaldehyde, concentrated (37-40%)	10.0 ml
Alcohol, 80%	90.0 ml

Acetone-Chloroform

Acetone	100.0 ml
Chloroform	100.0 ml

Procedure

1. Tissue specimen should not exceed 2 mm in thickness.
2. Fix tissue in 10% alcoholic formalin solution for a minimum of 6 hours and preferably 24 hours.
3. Place tissue in acetone-chloroform solution for 2 hours in a 60° C oven.
4. Remove tissue from this solution and process in the usual manner.

Modified H&E Stain for Demonstrating Reed-Sternberg Cells

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This modified hematoxylin and eosin staining procedure has been used to good advantage in the demonstration of acidophilic nuclear material present in Reed-Sternberg cells.

Fixation Any well fixed tissue (10% buffered neutral formalin preferred).

Microtomy Cut sections at 3-4 microns

Solutions

Mayer's Hematoxylin

Hematoxylin crystals	1.0 gm
Distilled water	1000.0 ml
Sodium iodate	0.2 gm
Ammonium or potassium alum	50.0 gm
Citric acid	1.0 gm
Chloral hydrate	50.0 gm

Dissolve the hematoxylin in the distilled water, add the remaining chemicals in the order given, making sure each is completely dissolved before the next one is added. Use a magnetic stirrer. The final color of the stain is reddish-violet. Stain keeps well for months.

Eosin-Phloxine B – 1% Eosin (stock)

Eosin Y, water soluble	1.0 gm
Distilled water	100.0 ml

1% Phloxine B (stock)

Phloxine B	1.0 gm
Distilled water	100.0 ml

Eosin-Phloxine B Solution (working)

1% Eosin (stock)	100.0 ml
1% Phloxine B (stock)	10.0 ml
Alcohol, 95%	780.0 ml
Glacial acetic acid	5.0 ml

Make up working solution as needed. Working solution should be changed at least once a week.

Staining Procedure

1. Decerate and hydrate to water in the usual manner.
2. If sections are Zenker-fixed, remove the mercuric chloride crystals with iodine and clear with sodium thiosulfate (hypo) in the usual manner.
3. Stain in Mayer's hematoxylin for 15 minutes.
4. Wash in running tap water for 20 minutes.
5. Counterstain with eosin-phloxine B for 8 minutes. For even staining, dip slides several times before allowing them to sit in the eosin for the prescribed time.
6. Dehydrate in 95% and absolute alcohols, 2 changes each. Pass slides through these solutions as rapidly as possible, making sure all water is removed.
7. Clear in xylene, 3 changes of 2 minutes each.
8. Mount coverglass in resinous media.

Results Nuclei – blue
Reed-Sternberg cell acidophilic nuclear material – bright red
Cytoplasm – various shades of pink

National Society for Histotechnology Symposium/Convention

The Second Annual Symposium/Convention of the National Society for Histotechnology will be conducted at the Sheraton and Holiday Inn, Silver Spring, Maryland, October 6-10, 1975.

Registration application is attached. We request filing your registration by September 15th, as the scientific sessions are limited to 600 registrants. Late applications will be accepted provided quota has not been filled. 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 are hotel reservation cards for the Sheraton and Holiday Inn. Please complete reservation card of choice and mail with registration to: Registrar, P.O. Box 36, Lanham, Maryland 20801, for recording purposes. As our workshop room rentals are determined by the number of registrants staying in the hotel, this will enable the NSH to have better negotiating power for prices. Reservation card will then be submitted to respective hotel who will send confirmation directly to you.

SHERATON RESERVATION CARD MUST BE USED as their Reservatron machine will be closed for this meeting. RESERVATION WILL NOT BE ACCEPTED IF TOLL FREE RESERVATRON IS USED. You may call Sheraton directly for late reservations.

Listed below are the titles for workshops, scientific sessions, and seminars being conducted:

Workshops

WORKSHOP NO. 1 **MONDAY, OCTOBER 6, 1975**

9:00 A.M. – 4:30 P.M. (Sheraton)

MANAGEMENT WORKSHOP

Dr. James Young

Topics for discussion during this workshop include, Communications in Management, Perception and the Behavioral Sciences, Listening as a Factor in Communications, How Can I Be a Better Manager, and a Workshop Summary. Learn to be a better manager by discussing and employing the latest behavioral sciences techniques with your peers. Each workshop member will be provided the opportunity to actively participate in the management process.

WORKSHOP NO. 2 **MONDAY, OCTOBER 6, 1975**

9:00 A.M. – 4:00 P.M. (Holiday Inn)

INTRODUCTION TO TISSUE IDENTIFICATION

Mrs. Edna Prophet and Mr. Charles Collard

The purpose of this workshop is to acquaint the participant with basic tissue structures. The value of this knowledge in microtomy and staining technology will be illustrated with the use of photomicrographs.

WORKSHOP NO. 3 **MONDAY, OCTOBER 6, 1975**

9:00 A.M. – 4:00 P.M. (Sheraton)

MICROTOMY

Mr. H. Keith Russell

The problems relative to poor versus good microtomy will be emphasized. The various steps in cutting, which may be responsible for variable results, will be discussed, including the effects on tissue sectioning of the following: Fixation, processing, embedding, microtomy, and tissue orientation. In-depth knife sharpening methods will be included. Participants are encouraged to bring "problem" blocks and microtome blades, as you will be sharpening blades, cutting tissue, staining slides, and taking home the finished product.

WORKSHOP NO. 4 **TUESDAY, OCTOBER 7, 1975**

9:00 A.M. – 4:00 P.M. (Holiday Inn)

SPECIAL STAIN SEMINAR

Mr. Jack Wenger

This seminar on stain technology will deal with: (1) A review of staining theory including reactions of silver and other methods. (2) Preferred stains for demonstrating specific entities. (3) Permissible variables and innovative stain technology. (4) Common problems and solutions. (5) A question and answer period.

WORKSHOP NO. 5 **TUESDAY, OCTOBER 7, 1975**

9:00 A.M. – 4:00 P.M. (Sheraton)

CRYOSTAT TECHNOLOGY

Mr. Frank Avallone

This workshop is designed to 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.

WORKSHOP NO. 6 **TUESDAY, OCTOBER 7, 1975**

9:00 A.M. – 12:00 NOON (Sheraton)

FLUORESCENT ANTIBODY TECHNIQUES

Mr. C. F. A. Culling

(No abstract received by date of printing.)

WORKSHOP NO. 7 **TUESDAY, OCTOBER 7, 1975**

9:00 A.M. – 12:00 NOON (Sheraton)

MANAGEMENT IN HISTOLOGY LABORATORY

Ms. Elizabeth Mayle

Histotechnology laboratories in certain areas can be treated like any other organized laboratory setting. In many areas there are peculiarities and differences that need to be defined, investigated and improved upon, so that we may have more professionalism and efficiency.

This workshop will define particular difficult areas for both the one-technologist laboratory and the multi-technologist laboratory, whether it be research oriented or patient care oriented. It will give specific suggestions and guidelines to the histotechnologist so that these difficulties can be alleviated.

WORKSHOP NO. 8 **TUESDAY, OCTOBER 7, 1975**

1:00 P.M. – 4:00 P.M. (Sheraton)

PROCEDURES FOR DETERMINATION OF DRUGS IN BLOOD AND URINE

Dr. Maurice Goldbaum

(No abstract received by date of printing.)

WORKSHOP NO. 9 **TUESDAY, OCTOBER 7, 1975**

8:30 A.M. – 11:30 A.M. (20 Histotechnologists)
1:30 P.M. – 4:30 P.M. (20 Histotechnologists)

SPECIAL STAIN WORKSHOP (Sheraton)
Mr. Erwin Haas

This 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 a day, from 8:30 to 11:30 A.M. for twenty Histotechnologists, and from 1:30 to 4:30 P.M. for twenty Histotechnologists. If necessary, the workshop will be conducted on Monday and Tuesday to accommodate 80 Histotechnologists. A question and answer period and slide presentation will follow the practical exercise for Histotechnologists wishing a more in-depth discussion of the special stains performed during the practical exercise.

WORKSHOP NO. 10 **TUESDAY, OCTOBER 7, 1975**

6:30 P.M. – 7:30 P.M. (Sheraton)

SEMINAR ON FORMATION OF STATE HISTOTECHNOLOGY SOCIETIES
Mr. Donald Hammer

Seminar will present methods, hints and procedures helpful in organizing a State Histotechnology Society. Individuals interested in obtaining information on forming a Society in their state should avail themselves of this opportunity.

Scientific Sessions

(All Sessions Held At The Sheraton)

WEDNESDAY, OCTOBER 8, 1975:

AN ALTERNATE APPROACH TO STAINING ACID FAST ORGANISMS
Mr. Wayne Kampa

THE ROLE OF HISTOPATHOLOGY IN THE MEDICO-LEGAL INVESTIGATION
Dr. Richard Froede

QUALITY CONTROL IN THE HISTOPATHOLOGY LABORATORY
Ms. Elaine Boyd

CATECHOLAMINE HISTOFLUORESCENCE
Mr. Robert Welsh

TELLTALE TISSUE TRACES
Ms. Barbara Campbell

IMMUNOFLUORESCENT VISUALIZATION OF GASTRO-INTESTINAL ENDOCRINE CELLS, USING PARAFFIN EMBEDDED BIOPSIES
Mr. Robert Escoffery

HISTO- AND CYTO-TECHNOLOGY IN VETERINARY MEDICINE
Mr. Thomas Palmer

WEDNESDAY EVENING:

7:00 – 9:00 P.M. NATIONAL SOCIETY FOR HISTOTECHNOLOGY BUSINESS MEETING, EVERYONE WELCOME!

THURSDAY, OCTOBER 9, 1975:

HISTOTECHNOLOGY IN AN INDUSTRIAL PATHOBIOLOGY OPERATION – A UNIQUE APPROACH
Dr. Gary Johnson

EXPERIENCES WITH AMYLOID STAINS AND RECOMMENDATIONS FOR IMPROVING THEIR USE AND INTERPRETATION
Dr. Robert Mowry

THE RUSSELL/MOVAT STAIN, ITS VALUE TO THE PATHOLOGIST
Dr. Daniel Connor

THE AMA AND THE ACCREDITATION PROCESS
Dr. John Beckley

MUSCLE ENZYME HISTOCHEMISTRY
Ms. Alice Harris

CONTROL OF AND TROUBLESHOOTING FOR SPECIAL STAINING PROBLEMS
Mr. John Koski

SILVER METHODS FOR RETICULIN AND CARBOHYDRATES ARE SPECIFIC, AREN'T THEY?
Mr. C. F. A. Culling

THURSDAY EVENING:

6:30 – 7:30 P.M. Complimentary cocktails sponsored by LAB-TEK PRODUCTS, Naperville, Illinois

7:30 – 9:30 P.M. National Symposium Banquet

Banquet Speaker: Ralph C. Kuhli, MPH, Director, Department of Allied Medical Professions & Services, American Medical Association, Chicago, Illinois.

Subject Title: "NATIONAL COOPERATION FOR ALLIED MEDICAL EDUCATION"

FRIDAY, OCTOBER 10, 1975:

SUCCESSFUL LOBBYING – HOW TO CONVINCE YOUR CONGRESSMAN, HOSPITAL, PATHOLOGIST, TO DO WHAT YOU WANT
Mr. Keith Russell

ALDEHYDE FUCHSIN STAINING OF ELASTIN AND MUCINS
Ms. Beatrice Macdonald

A PERIODIC ACID-METHENAMINE SILVER-CHROMOTROPE 2R METHOD FOR STAINING BASEMENT MEMBRANE
Mr. Wayne Kampa

PREPARATION OF OSSEOUS TISSUE EMPLOYING THE WET CELLOIDIN TECHNIQUE
Mr. Gerald Armstrong

PROBLEMS OF PSEUDO-MINERALIZED BRAIN
Mr. Celester Carter

HISTOCHEMISTRY
Dr. Frank Johnson

HISTOLOGICAL TECHNIQUES USEFUL IN PARASITE IDENTIFICATION
Mr. Ronald Neafie

AN EVALUATION OF THE PROBLEMS IN LYMPH NOD PREPARATION
Ms. Elaine Boyd

CLOSING REMARKS
Mr. Lee G. Luna

Day of above lectures subject to change pending finalization of program.

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SCIENTIFIC SESSIONS_____ $35.00 (Wed, Thur, Fri)

BANQUET_____ $13.00 (Thursday Evening)

CHECK OR MONEY ORDER **MUST ACCOMPANY** REGISTRATION APPLICATION.

MAKE PAYABLE TO: NATIONAL SOCIETY FOR HISTOTECHNOLOGY

MAIL REGISTRATION TO: REGISTRAR, P.O. BOX 36, LANHAM, MARYLAND 20801

WORKSHOPS

No. 1 ___	$20.00	(Monday)
No. 2 ___	$20.00	(Monday)
No. 3 ___	$20.00	(Monday)
No. 4 ___	$20.00	(Tuesday)
No. 5 ___	$20.00	(Tuesday)
No. 6 ___	$20.00	(Tuesday) ½ day
No. 7 ___	$20.00	(Tuesday) ½ day
No. 8 ___	$20.00	(Tuesday) ½ day
No. 9 ___	$20.00	(Tuesday AM _____) (Tuesday PM _____)
No. 10 ___	No Fee	(Tuesday Evening)

NATIONAL SOCIETY FOR HISTOTECHNOLOGY
SYMPOSIUM/CONVENTION
OCTOBER 6-10, 1975

SHERATON – SILVER SPRING
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Reservations must be received not later than two weeks prior to arrival date, and ROOMS WILL BE HELD ONLY UNTIL 6 P.M. on DATE OF ARRIVAL, unless guaranteed in writing. CHECKOUT TIME IS 1 P.M.

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NATIONAL SOCIETY FOR HISTOTECHNOLOGY
SYMPOSIUM/CONVENTION
OCTOBER 6-10, 1975

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6 P.M. reservations will be held until 6 o'clock, then cancelled.
Guaranteed reservations will be held all night and guest will be billed for full amount of room if he fails to show up.
Prepaid reservations will be held all night.

ARRIVAL DATE _____ TIME _____ A.M. _____ P.M.

DEPARTURE DATE _____ TIME _____ A.M. _____ P.M.

SINGLE ____ $18.00 DOUBLE ____ $22.00 (All rates plus 7% Maryland Tax.)

*Holiday Inn approximately three blocks from Sheraton, meeting headquarters.

MAIL RESERVATION TO: REGISTRAR, P.O. BOX 36, LANHAM, MARYLAND 20801

Russell's Fixative Solution

An Editorial

The substitution of zinc chloride for mercuric chloride in Zenker's fluid.

Distilled water	1000.0 ml
Zinc chloride	50.0 gm
Potassium dichromate	25.0 gm
Sodium sulfate	10.0 gm
Acetic acid, glacial	50.0 ml

This modified Zenker fixative utilizes zinc chloride in place of mercuric chloride. The use of mercuric chloride in fixative solutions should be discontinued due to the potential toxic effects of this chemical to the environment. The staining results produced by this fixative are similar to those produced by Zenker fixative containing mercuric chloride. Cadmium chloride may also be substituted for zinc chloride. Zinc and cadmium chloride are removed from sections by the usual dehydrating procedures; that is, no extra steps are required to remove crystals of the salts as are required after fixation with Zenker's solution containing mercuric chloride.

It is interesting to note that this procedure was developed in 1941 because of the high cost of mercuric chloride – not the environmental problem posed by mercuric chloride.

Reference

Russell, W. O.: J. Tech. Meth. Bull., Int. Assoc. Med. Mus., *21*:47-49, 1941.

Mounting and Storing Frozen Tissue Specimens

Marie I. Doman
Department of Oral Biology
University of Washington
Seattle, Washington 98195

The following method is used in our laboratory for mounting and storing tissue specimens destined for cryotomy. The method has proved most useful and convenient.

Method

1. Collected tissue specimens are oriented in O.C.T.* embedding media on a 1/8 inch slice of cork and plunged into liquid nitrogen.
2. The frozen specimen can now be stored in the freezing compartment of the refrigerator in an air tight container or mounted on the cryostat metal object holder.
3. If the tissue requires sectioning all the way through the block, there is no risk of damaging the knife edge on the metal object holder.
4. The specimen cut surface can be recovered with O.C.T., the cork removed from the object holder and the mounted specimen stored in a covered jar. If additional sections are required at a later date, the specimens will still be in their original orientation when the cork is remounted on the object holder.

This is done by first placing a drop of water on the object holder, then placing the specimen on the water for attachment to the object holder. The specimen can now be sectioned or stored while it remains attached to the cryostat object holder.

We find the following advantage to this method: It is a fast, handy method for orienting and storing large numbers of tissue specimens destined for cryostat technology.

We wish to thank Dr. James A. Claggett for his encouragement in writing this article.

*O.C.T. Compound, Lab-Tek Products, Naperville, Illinois

A Modified Argentaffin Silver Reaction

Karole Cecich
Holy Family Hospital
Des Plaines, Illinois 60016

The following silver nitrate procedure for argentaffin granules and melanin pigment is a modification which provides the following advantages:

1. The method can be performed faster than other argentaffin type procedures.
2. It is simpler to perform.
3. The method gives excellent results.

This method has been used for the demonstration of melanin pigment, argentaffin and chromaffin granules.

Fixation 10% buffered neutral formalin

Microtomy Cut sections at 10 micra.

Solutions

Ammoniacal Silver Solution

To 10.0 ml of a 10% aqueous solution of silver nitrate add 2.0 ml of a 10% aqueous solution of potassium hydroxide. Add ammonium hydroxide (drop by drop) until all silver nitrate granules are dissolved. To this solution add 5.0 ml of a 10% silver nitrate solution. Pour above solution into 55.0 ml of distilled water. *Filter solution before use.*

10% Silver Nitrate

Silver nitrate	10.0 gm
Distilled water	100.0 ml

10% Potassium Hydroxide

Potassium hydroxide	10.0 gm
Distilled water	100.0 ml

10% Formalin

Formaldehyde, concentrated (37-40%)	10.0 ml
Distilled water	100.0 ml

5% Sodium Thiosulfate (Hypo)

Sodium thiosulfate	5.0 gm
Distilled water	100.0 ml

Staining Procedure

1. Decerate and hydrate sections to distilled water.
2. Wash sections in running tap water for 5 minutes.
3. Rinse slides in 3 changes of distilled water.
4. Impregnate slides in ammoniacal silver solution for 1 hour in a 58° C oven. Control silver deposition microscopically. Remove staining dish from oven when granules appear light brown.
5. Rinse slides in 3 changes of distilled water.
6. Reduce slides in 10% formalin solution for one quick dip. This solution intensifies the reaction and turns the granules black.
7. Rinse slides in 3 changes of distilled water.
8. Place slides in 5% sodium thiosulfate for 5 minutes.
9. Rinse slides in 3 changes of distilled water.
10. Counterstain slides with eosin working solution of choice.
11. Dehydrate in 3 changes of 95% alcohol, 2 changes of 100% alcohol, 2 minutes each.
12. Clear in xylene, 3 changes, 2 minutes each.
13. Mount coverslip with resinous media.

Results Argentaffin granules – black
Background – pink

Remarks All glassware must be clean and free from anything which may contaminate the silver nitrate solution. Tissue has a tendency to fall off slides in ammoniacal silver solutions. A preventive measure to this problem is to use gelatin in the flotation bath and egg albumen adhesive on the glass slide.

A Technical Bulletin for Histotechnology Vol. V, No. 4

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HISTO-LOGIC

HISTO-LOGIC™ – Statement of Policy

There has been some misunderstanding by many people concerning who is eligible to receive HISTO-LOGIC. HISTO-LOGIC is available free of charge to any histotechnologist who wishes to receive it. You do not have to be a member of any organization or society, or fulfill any prerequisites. The only requirement is that you make a request to be put on HISTO-LOGIC mailing list and furnish your name and home address. Once you have been added to the mailing list, you will receive HISTO-LOGIC four times a year; January, April, July and October.

In addition, you will receive "NSH In Action." NSH In Action is the official newsletter for the National Society for Histotechnology. This newsletter contains news pertaining to all activities concerning the endeavors of the Society in the field of Histotechnology.

We ask that you encourage all histotechnologists to be placed on our mailing list. We also ask that you send all inquiries pertaining to HISTO-LOGIC to the Editor, P. O. Box 36, Lanham, Maryland 20801.

The editor feels that as Histotechnologists we are fortunate that Lab-Tek Products publishes HISTO-LOGIC and NSH In Action at no cost to the individual histotechnologist. I am sure most of you recognize this is a costly endeavor for Lab-Tek in both money and time.

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.

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