

HISTO-LOGIC[®]

No reader should utilize materials and/or undertake procedures discussed in HISTO-LOGIC articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

Editor, Lee G. Luna, D. Lit., H.T. (ASCP)

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Ruas' H & E Stain for Stat Frozen Sections

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Technique: Cut frozen sections from fresh tissue as thin as possible; preferably not more than 8 microns. Pick sections up on albuminized slides. For next step, see staining procedure, step one.

Solutions:

Formal-Saline

Formaldehyde, concentrated	5.0 ml
Saline (isotonic)	45.0 ml

10% Aluminum Ammonium Sulfate

Aluminum ammonium sulfate	10.0 gm
Distilled water	100.0 ml

Hemalum (stock)

Solution 1: Dissolve 1 gm of hematoxylin in 20.0 ml of 95% ethyl alcohol with gentle heat. **Do not use open flame.**

Solution 2: Dissolve 0.8 gm of iodine crystals in 20.0 ml of 95% ethyl alcohol.

Solution 3: To 100 cc of the 10% aluminum ammonium sulfate, add 60 cc of distilled water.

Hemalum (working)

Quickly mix solution 1 and solution 2 in a large container. Immediately add solution 3 and mix well. Bring solution to a boil and boil for 1 minute. Remove solution from heat and stopper container. Allow solution to remain at room temperature for 24 hours. Do not agitate solution for this period. Filter and add sufficient distilled water to bring total volume to 200 ml. Store solution in a brown, well stoppered bottle. This solution is **unstable** and therefore should not be made in large quantities. For staining, filter 50.0 ml Hemalum working solution into coplin jar and add 1.0 ml acetic acid.

1% Eosin-Dichromate

Eosin Y	1.0 gm
Potassium dichromate	1.0 gm
Distilled water	100.0 ml

Mix well and filter before use.

Staining Procedure:

1. Place slides for 15 seconds in Formal-Saline solution (fixative).
2. Rinse slides in distilled water until clear.
3. Stain slides in hematoxylin for 30 seconds.
4. Dip slides in hot tap water.
5. Place slides in eosin and agitate for 4 seconds.
6. Remove excess eosin with distilled water, 2 changes, 5 dips each.
7. Rinse slides in ethyl alcohol, 80%, 95%, 100%; 3 changes each, 5 dips each.
8. Rinse in xylene, 3 changes, 5 dips each.
9. Coverslip slides with resinous media.

Results:

Similar to conventional H&E.

Quality Control:

1. Change the fixative weekly.
2. Change the distilled water daily.
3. Change the stains once a month. **Remove precipitate daily**, before staining slides.
4. Properly identify the slide.

Remarks:

This stain was developed with the cooperation of Max F. Lindquist, M.D., Chief Pathologist at Divine Redeemer Memorial Hospital, South St. Paul, Minnesota. We find this stain to be fast and the quality of the slide excellent.

References:

1. Jay, A.: Hemalum (Iodine Oxidase), Swedish Hospital, Minneapolis, Minnesota (personal communication).
2. Preece, A.: *A Manual for Histologic Technicians*, Little Brown and Co.

Important Notice to Histotechnologists

The Membership Committee of the National Society for Histotechnology recently mailed a large number of NSH informational letters and membership application forms to all histotechnologists certified by the American Society of Clinical Pathologists. Mailing was completed during July and August.

Purpose of this project was to inform histotechnologists of the existence of a professional organization which represents their interests, to introduce them to NSH activities and programs, and to offer them an opportunity to become NSH members.

Two items regarding this mailing have caused some concern and require explanation.

1. The last sentence appearing on the application form reads: "Your membership is renewable on Oct. 1 of each year". Many histotechnologists reading this were under the impression that if they joined NSH before October 1, they would be obligated to remit dues again in 1977. This is not correct. Persons responding to this letter who were **NOT PREVIOUS NSH MEMBERS** will not be required to pay dues again until October 1, 1978. If you join NSH now, your membership will be renewable on October 1, 1978.
2. Some NSH members have been confused as to whether they are to use the white (SL) or the yellow application forms for renewing their membership. In order to facilitate record keeping, we prefer members use the yellow form and pass the white application, along with the information letter, on to a non-member.

Contact Ken Urban, 1018 Diana Street, Iowa City, Iowa 52240 for: Questions or problems regarding membership; Change of name and/or address for members; Requests for membership application forms.

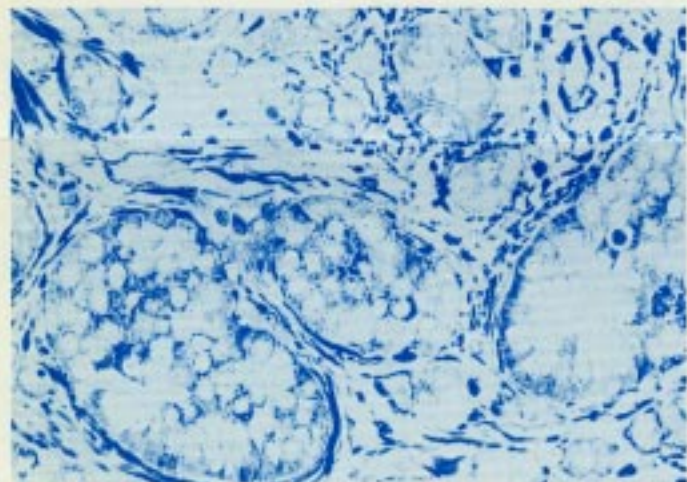
HISTO-HINTS

Sevier and Munger Silver Staining and Cryomethacrylate

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So far, we have been successful in staining for axons in sural nerve preparations, with and without myelin counterstains (though the plastic seems to subdue the Luxol Fast Blue) and carcinoids in GI tumors (see illus.), with the Sevier-Munger silver procedure.

The plastic leaves the material with very little distortion. The cytological features are clear. Anatomically, the tissue seems well preserved.



One micron section of carcinoid in GI tract. Cryomethacrylate section stained with the Sevier-Munger silver staining reaction.

JONES' STAIN

The Jones' stain for basement membranes has also been very well accomplished on cryomethacrylate sections.

FAN BRAIN TUMOR STAIN FOR PARAFFIN SECTIONS

The Fan stain reported by Miquel, Calvo and Rubenstein in *J. Neuropath. Exp. Neurol.*, 27:517, 1968, works well on paraffin sections if the staining time is extended to 5 minutes. The stain is a powerful method for discovery of astrocytes.

Gum Tragacanth — An Aid to Frozen Sectioning

Cutting frozen sections of certain intractable materials may be simplified by surrounding the material before freezing with gum tragacanth. The method is essential for muscle. It can make frozen sectioning of fixed and unfixed brain tissue much easier.

Simply surround the tissue with tragacanth, freeze, then trim straight edges close to the tissue and cut.

One reservation: If liquid nitrogen is used to freeze the tissue too long, an exposure to the nitrogen will cause the tragacanth to crack.

Gum Tragacanth Solution

Gum tragacanth	15.0 gm
Glycerine	6.0 ml
Distilled water	150.0 ml
Phenol (carbolic acid)	10 crystals

Dissolve the phenol and glycerine in water. Add the tragacanth. Use a tongue blade or other sturdy stirrer and stir. Let solution sit overnight. Stir 2 or 3 times the next day. The gum tragacanth solution should be ready for use by the second day. It will keep for many weeks. Mix another batch a few days before you wish to replace the old one.

Additional Information on Cleaning Tissue Cassettes

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In HISTO-LOGIC® Vol. VI, No. 3, 1976, Herbert Stevens presented his method for cleaning tissue cassettes with hot, soapy water. Many articles written on this subject have mentioned the expense of using xylene for cleaning purposes.

In the May 1976 edition of M.A.S.H., I presented a method of recycling xylol and other reagents used in a histology laboratory. The information presented below is provided as an additional method for cleaning tissue cassettes.

It is a fact that used xylene must be discarded in a safe manner. However, this waste xylene can be used for tissue cassette cleaning purposes. Warm xylene tends to clean faster than cold.* Metal tissue cassettes are placed in a metal pot containing used xylene obtained from the tissue processor. The container can be placed on top of a warming oven or on top of the paraffin bath of the Tissue-Tek® II Tissue Embedding Center. This will provide enough heat to warm the used xylene and assist in melting off the paraffin. The cassettes can then be dipped in used absolute alcohol to remove xylene residue. The cassette cleaning xylene can be changed as often as the solutions are changed on the tissue processor. Embedding molds can also be cleaned by this method.

For disposal of other reagents, the Laboratory Disposal Manual of the Manufacturing Chemists Association 1970 is suggested, and in the Matheson Coleman & Bell Chemical Reference Manual, Vol. II, page 339, June 1976, hydrocarbons, alcohols, ketones and esters are discussed.

Any liquid including acetone, benzene, cellosolve, 1,4-dioxane, ethanol, methyl alcohol, toluene and xylene, etc., can be atomized into an incinerator. If your hospital or place of employment has its own incinerator, they can dispose of your waste materials. However, combustion may have to be improved by mixing in a more flammable solvent. While storing these flammables before incineration, they should be kept in safety cans and should be stored in a non-flammable locker. Used paraffin can also be incinerated rather than placed in a compactor.

The toxicity of fumes found in an histology laboratory can be measured. The insurance company covering your place of employment can arrange for someone to come to your lab and measure the parts per million of those toxic fumes. If a problem exists, a solution for the poor ventilation can be suggested.

*Editor's Note: Since xylene has a very low flash point, exercise caution and do not use open flame to warm.

Tissue-Talk



The Use of Specimen Bags In Toxicology Studies

Hedwig Baxter
Pathology Department
Ayerst Research Laboratories

Our laboratory has used 3,500 to 4,000 Specimen Bags* for storage of formalin fixed animal tissues from drug safety evaluation studies for the past year and a half. These bags have been found extremely useful in our work. There are many advantages obtained from the use of these bags. For example:

The extra wide, special double lock lip closes quickly and easily, does not allow fluid leakage and reopening is simple. There is never the problem associated with heat sealing or rebagging due to insufficient plastic remaining, such as may be the case with homemade or other types of manufactured bags.

A bag holder can be utilized to hold the bags in an open and upright position for easier insertion of tissue specimens.

In our laboratory, all animal tissues are initially placed in glass jars containing the fixative. This enables prompt fixation. Following fixation, the specimens are trimmed and placed in proper size bags with enough fixative fluid to cover tissues completely. The bags are sealed and properly identified. They are then placed in permanent storage. Bags may be stored in boxes, card file cabinets, or hung with a storage hook on a wire stretched across the ceiling of a storage room.

The 3" x 6" bags hold a complete set of tissues from a rat necropsy and the 7" x 8" bags hold a complete set of tissues from a monkey or dog necropsy.

Specimen bags reduce the storage space for wet tissue by one fourth of that required for glass jars. Several bag sizes are available to eliminate waste of space. This is important since storage space has become increasingly limited.

The bags require far less fixative solution. Also, we have experienced less solution evaporation over a period of time. In addition, the overall weight of wet tissues and bag is considerably less than that experienced with the use of glass jars.

Transparent, semi-transparent and semi-rigid type bags are available, depending upon the type and strength desired. It has been found that the Series V semi-transparent, semi-rigid bags are excellent for storage of wet tissue containing sharp bone spicules since they will not puncture the plastic.

We have been very pleased with the ease and neatness of wet tissue storage since initiating the use of specimen bags in our laboratory. We highly recommend them for use in toxicology studies.

EDITORIAL COMMENT: I am familiar with the use of these specimen bags and would recommend them highly for all types of tissue storage. This system has been used extensively at my place of employment for many years.

* Tissue-Tek®II Specimen Bags are marketed by Lab-Tek Products, Division Miles Laboratories.

A Rapid Mallory's PTAH Method for Fibrin

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The following modification of Mallory's phosphotungstic acid hematoxylin is a rapid procedure which gives consistent results. It is especially useful for demonstrating fibrin since an eosin solution is employed which stains the erythrocytes red and differentiates them from the blue fibrin.

Fixation:
10% buffered neutral formalin

Microtomy:
Cut paraffin sections at 6 microns.

Solutions:

0.25% Eosin	
Eosin Y, water soluble	0.25 gm
Distilled water	5.0 ml
Ethyl alcohol, 80%	95 ml
Before use, add glacial acetic acid	0.5 ml

5% Periodic Acid	
Periodic acid	5.0 gm
Distilled water	100 ml

Phosphotungstic Acid Hematoxylin (PTAH)	
Hematoxylin	1.0 gm
Phosphotungstic acid	20.0 gm
Distilled water	1000 ml

Dissolve the solid ingredients in separate portions of the water. Use gentle heat for dissolving the hematoxylin. Combine solutions when cool. Ripening requires several weeks. However, the addition of 0.177 mg of potassium permanganate will cause the stain to ripen at once.

Staining Procedure:

1. Decerate and hydrate sections to 80% alcohol.
2. For fibrin staining only, place slides in eosin solution for 30 seconds.
3. Wash slides in distilled water for a few seconds.
4. Place slides in 5% periodic acid solution for 10 minutes.
5. Wash slides in tap water for 3 minutes and rinse in distilled water.
6. Place slides in PTAH solution for 30-90 minutes in a 60° C oven. Check slides for proper staining, without drying, after 30 minutes.
7. Dehydrate with 95% alcohol, absolute alcohol and clear in xylol.
8. Mount with resinous mounting media.

Results:

Nuclei — blue
Fibrin and platelets — blue
Erythrocytes — red
Muscle — blue, with cross striations well defined
Collagen — red to reddish brown

References:

1. Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed., McGraw-Hill, New York, 35-36, 199-200, 1968.
2. Mallory, F.B.: *Pathological Technique*, Hafner Publishing Co., 76-77, 1968.

An Editorial

Lillie's B-5 fixative has been found to be very useful for fixation of a variety of specimens, lymph nodes, bone marrow, etc., in recent years. The editor has received many inquiries requesting the source of information for compounding this fixative. This information is provided below. However, interested individuals are encouraged to read the related information in Dr. Lillie's book (see reference).

Lillie's (B-5) Fixative	
Distilled water	90.0 ml
Mercuric chloride	6.0 gm
Sodium acetate (anhydrous)	1.25 gm
Formaldehyde (concentrated)	10.0 ml
Add the concentrated formaldehyde just before use.	

NOTE: If the sodium salt available is trihydrate (NaCO₃·CH₃·3H₂O) use 2.074 gms.

Reference:

1. Lillie, R.D.: *Histopathological Technique and Practical Histochemistry*, 3rd ed., McGraw-Hill Book Co., New York, page 48, 1965.

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Technically Speaking

Robert A. Clark, Technical Services
Lab-Tek Products, Naperville, IL 60540

Do you drive your automobile 100,000 miles without an oil change? Do airlines continue to fly a plane without periodic servicing? Of course not! Yet, Technologists expect a Tissue Embedding Center to run 24 hours a day, 365 days, year after year. Like any other piece of laboratory equipment, the Tissue Embedding Center needs a little tender loving care to keep it running trouble free. It is a good idea to start a preventive maintenance program to help eliminate desperate calls to the dealer service agency for help and a loaner.

To maintain your unit and help keep it functioning, do the following on a routine basis:

1. Clean the outside of the unit by wiping occasionally with a xylene dampened cloth.
2. Remove all moisture from the Cold Plate and Cold Drawer.
3. Every six months remove the Foil Vent from behind the unit and wash with warm soapy water. Like all refrigeration systems, lint is

drawn into the Foil Vent, preventing adequate circulation.

4. Periodically clean the Mold Warming Caddy to remove excess paraffin.
5. Remove all paraffin from behind the Paraffin Dispenser Touch Plate.
6. Remove the Paraffin Pot from the unit every six months and examine the compressor area for paraffin leakage.
7. Note any slight changes in performance which might indicate trouble and check them out before they become serious.

The following parts, if kept on hand, will allow your hospital maintenance department to fix the unit immediately and at a reduced cost to you:

Heater Strip (Paraffin Pot) 40211016; Heater Strip (Orientation Platform) 40211018; Thermostat (Paraffin Pot) 40570022; Thermostat (Orientation Platform) 40570021; Solenoid 40542002; "O" Rings 50421016 - 50421015; and Starter Relay (Compressor) E2190740.

The Operating Manual will provide useful information for other on-the-spot servicing.

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 North Aurora Rd., Naperville, Illinois 60540.

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.