HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor — A Technical Bulletin for Histotechnology — Vol. V, No. 1, January, 1975

Modification of Harris' Hematoxylin for Sections from Tissue Double Embedded with Nitrocellulose and Paraffin

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Harris' hematoxylin[1] was modified for staining 5 micra sections from tissues double embedded with nitrocellulose and paraffin. This modified solution is more concentrated, thus reducing the time element from 1½ hours to 10 minutes. The old Harris solution breaks down almost every month, whereas the solution described here remains stable for years. We have thus reduced our hematoxylin costs by 50%.

Solutions

Modified Harris' Hematoxylin

Hematoxylin crystals	5.0 gm
Alcohol, absolute	25.0 ml
Ammonium or potassium alum sulfate	25.0 gm
Distilled water	500.0 ml
Mercuric oxide (red)	2.5 gm

Prepare as in Harris' original: Dissolve the hematoxylin in the alcohol, the alum in the water by aid of heat. Remove from heat and slowly add the mercuric oxide. Reheat until solution becomes dark purple. Remove from flame immediately, and plunge the vessel into a basin of cold water until cool. Add 10 ml glacial acetic acid to the solution to increase precision of nuclear stain. Filter. Stain is ready for use as soon as it cools. Store at room temperature; filter each time before use.

1% Alcoholic Eosin (stock)

Eosin Y, water soluble	4.0 gm
Distilled water	80.0 ml
Dissolve and add alcohol, 95%	320.0 ml

Working Eosin Solution

1% alcoholic eosin (stock)	1 part
80% alcohol	1 part

Staining Procedure

1. Decerate and hydrate to distilled water.
2. Stain in hematoxylin solution for 10 minutes.
3. Rinse slides in tap water.
4. Dip slides 2 or 3 times in 0.25% concentrated (12N) HCl.
5. Rinse slides in tap water.
6. Blue in saturated aqueous sodium bicarbonate.
7. Wash slides in running tap water for 5 minutes.
8. Stain slides in eosin for 2 minutes.
9. Dehydrate in 3 changes of 95% alcohol, 2 changes of 100% alcohol, 2 minutes each.
10. Clear in xylene and mount coverslip with resinous media.

Result

A well differentiated stain with blue nuclei and bright rose background is obtained.

Reference

1. Manual of Histologic and Special Staining Techniques, 2nd ed., New York, Blakiston Division of McGraw-Hill, p. 26.

Preparation of this note was supported in part by grant RR00166 from the National Institutes of Health to the Regional Primate Research Center, University of Washington.

Additional Information on Problem in Stain Technology

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In the January, 1974 (Vol. IV, No. 1) issue of HISTO-LOGIC, the editorial dealing with the probable inconsistent staining reactions was of particular interest. As the editorial explained, dyes with the same Color Index Number can vary considerably in dye content, both from manufacturer to manufacturer, and at times from the same manufacturer. The pH of a dye solution is also dependent upon the dye content of a given dye.

I would like to share with my fellow histotechnologists a simple method that insures consistent staining reactions time after time. Since dyes are purchased in powder form and are dissolved in distilled water or, when necessary, ethyl alcohol, the final concentration is calculated on the basis of dye content and not gross weight. Thus, if a 2% solution is desired of a powder with a dye content of 80%, it is obvious that a solution containing 2 gm per 100 ml would produce a concentration of 1.6 and not 2%.

Calculation for the correct weight of dye to be added is a simple mathematical procedure. Let X be the weight value to be determined. Since X is going to be greater than 2, the following relationship holds:

$X:2::100:80 \qquad 80X=200 \qquad X=2.5$ gm/100 ml of solvent

Therefore, it takes 2.5 gm of 80% dye content per 100 ml of diluent to make a 2% solution.

Eliminate Wrinkles on Paraffin Section Slides

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To avoid the wrinkled sections that are the bane of histopathologists, add Kodak® Photo-flo 200 to the water bath.

Technique:

1. Prepare a solution of Bacto-Agar (0.25%) in distilled water, with a few crystals of Thymol.
2. Heat the solution to dissolve all of the agar, and pour 45 ml into a 2750 ml water bath maintained at 54° C.
3. Add 10-15 drops of Kodak® Photo-flo 200 solution.
4. Float the paraffin sections in the water bath and attach sections to clean slides.
5. Dry the slides in a slide dryer, or oven. The temperature should be adjusted to the melting point (or just slightly less) for the paraffin used.
6. Cool at room temperature and stain.

Glycerinized egg albumin (albumen) has long been used as an adhesive for histologic sections. A disadvantage of egg albumin is that in the process of staining, much of the stain adheres to the slides, requiring extra time and work to clean the stained slides. We have found that plain agar is a successful and stain-free adhesive.

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Luna-Verfuerth Stat. H&E Staining Procedure[1]

An Editorial

The staining procedure presented below for stat. frozen sections produces excellent staining results. Staining time is approximately 2½ minutes.

Fixation: Formol-methanol alcohol
Cryotomy: Cut frozen sections at 8 micra.
Solutions:

Formol-Methanol

10% buffered neutral formalin	80.0 ml
Methanol, absolute	20.0 ml

Bullard's Hematoxylin

Hematoxylin crystals	8.0 gm
Alcohol, 80%	144.0 ml
Glacial acetic acid	16.0 ml

Dissolve by gentle heat and add mixture of:

Ammonium or potassium alum	20.0 gm
Distilled water	250.0 ml

Heat to boiling and then remove from flame and add slowly:

Mercuric oxide	8.0 gm

Cool quickly, filter and add mixture of:

Alcohol, 95%	275.0 ml
Glycerin	330.0 ml
Glacial acetic acid	18.0 ml
Ammonium or potassium alum	40.0 gm

Eosin Y-Phloxine B Solution

1.0% Eosin Y (stock)

Eosin Y, water soluble	1.0 gm
Distilled water	100.0 ml

1.0% Phloxine B (stock)

Phloxine B	1.0 gm
Distilled water	100.0 ml

Working Solution

1.0% Eosin (stock)	100.0 ml
1.0% Phloxine (stock)	10.0 ml
Alcohol, 95%	780.0 ml
Glacial acetic acid	4.0 ml

1.0% Acid Alcohol

Alcohol, 70%	100.0 ml
Hydrochloric acid, concentrated	1.0 ml

1.0% Lithium Carbonate

Lithium carbonate	1.0 gm
Distilled water	100.0 ml

Staining Procedure:

1. Cut frozen sections and pick up on acid clean slides. Keep sections frozen until ready to stain. This will inhibit autolysis.
2. Fix in formol-methanol; 30 seconds.
3. Tap water (change frequently); 5 dips.
4. Bullard's hematoxylin; 1 minute.
5. Running tap water (change frequently); 5 dips.
6. 1% lithium carbonate; 1 dip.
7. Tap water (change frequently); 5 dips.
8. Alcohol, 80%; 3 dips.
9. Eosin phloxine; 30 seconds.
10. Alcohol, 95%; 3 dips.
11. Alcohol, 100%; 3 dips.
12. Alcohol, 100%; 3 dips.
13. Xylene; 3 dips.
14. Xylene; 3 dips.
15. Mount coverglass with resinous media.

Results:

Nuclei – blue   Cytoplasm – pink to red

Reference:

1. Luna, L. G. and Verfuerth, R. J.: Histopathology Laboratories Division, Armed Forces Institute of Pathology, Washington, D.C. 20306.

How to Make a Countertop Dispenser

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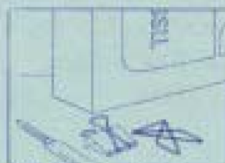
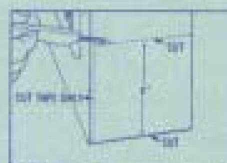
An ingenious method of converting a Tissue-Tek® II Cassette shipping carton into a handy Tissue-Tek® II Cassette dispenser or organizer has been developed. Illustrated steps for making them for your own laboratory are shown below.

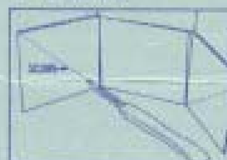


1. Tools needed for converting a Tissue-Tek processing cassette case into a dispenser pack for shelf or countertop use.



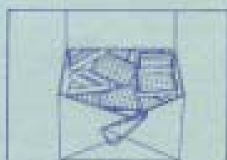
2. The first cut is made 4 inches in and parallel to top edge of carton. Next cut just the taped edge as illustrated.

3. Score the panel diagonally as illustrated. DO NOT cut through. Then cut the second panel.

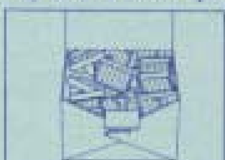


4. Score the second panel as in illustration.

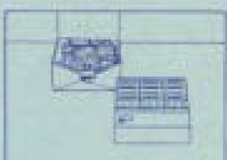
5. Fold left and right flaps inward along scored lines. Lower inner flap to meet other flaps.



6. Secure the folded flaps with a paper clip.



7. Secure the folded flaps with a paper clamp as an alternate method.



8. Cassette carton is now ready to be placed on shelf or bench counter to serve as dispenser.

A Special Technique in Processing Small Specimens

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One of the most frequent problems encountered in processing small biopsies and body fluids for cell blocks, is the lack of sufficient material and the difficulty in finding minute specimens for embedding after they have been processed.

We have been using a very simple procedure in our laboratory which makes finding such specimens much easier. When a specimen is received in the laboratory, it is immediately fixed in 50 ml of 50% ethyl alcohol to which 3 to 5 ml of 0.4% eosin has been added. The specimen is allowed to stand for approximately one-half hour before placing on lens paper and then in the tissue processor. The specimen stained by the eosin will retain the dye during processing, making it much easier to find for embedding.

Counterstains for Hematoxylins

Richard Verfuerth[1] and Lee G. Luna
An Editorial

A study of various eosin counterstains proved that most produce similar staining results. The basic difference was in the shade of cytoplasmic color produced. It was determined that the following counterstains provided the most significant staining results. These counterstains are highly recommended for routine use.

Alcoholic Eosin

1% Alcoholic Eosin (stock)

Eosin Y, water soluble	1.0 gm
Distilled water	20.0 ml
Dissolve and add:	
Alcohol, 95%	80.0 ml

Eosin Solution (working)

Eosin solution (stock)	1 part
Alcohol, 80%	3 parts

Just before use, add 0.5 ml of glacial acetic acid to each 100 ml of stain.

Staining Procedure:

1. Decerate and hydrate to water in the usual manner.
2. Proceed with desired hematoxylin stain (Mayer's preferred).
3. After 15 minute water wash, bring slides to 80% alcohol for 2 minutes.
4. Stain in working eosin solution for 2 minutes.
5. Dehydrate in 95% and absolute alcohols, two changes of 2 minutes each.
6. Clear in xylene, 2 changes of 2 minutes each.
7. Mount with resinous media.

Results:

This counterstain provides very little tinctorial differentiation of the various acidophilic structures. The cytoplasm generally stains a light yellowish-pink.

Eosin-Phloxine-B

1% Eosin (stock)

Eosin Y, water soluble	1.0 gm
Distilled water	100.0 ml

1% Phloxine-B (stock)

Phloxine B	10.0 gm
Distilled water	100.0 ml

Eosin-Phloxine-B Solution (working)

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

Staining Procedure:

1. Decerate and hydrate to water in the usual manner.
2. Proceed with desired hematoxylin stain (Mayer's preferred).
3. After 15 minute water wash, bring slides to 80% alcohol for 2 minutes.
4. Stain in eosin-phloxine-B working solution for 2 minutes.
5. Dehydrate in 95% and absolute alcohols, two changes of 2 minutes each.
6. Clear in xylene, two changes of 2 minutes each.
7. Mount with resinous media.

Results:

The combination of eosin and phloxine B produces a cytoplasmic stain which tinctorially differentiates many acidophilic structures. For example, muscle stains slightly darker than collagen, inclusion bodies are demonstrated better than by the use of eosin alone. An excellent counterstain.

Aniline-Chromotrope 2R[2]

Aniline Chromotrope 2R Solution

Chromotrope 2R	1.0 gm
Alcohol, 80%	100.0 ml
Aniline	3.0 ml
Glacial acetic acid	1.0 ml

Staining Procedure:

1. Decerate and hydrate to water in the usual manner.
2. Proceed with desired hematoxylin stain (Mayer's preferred).
3. After 15 minute water wash, stain in aniline-chromotrope 2R solution for 10 minutes.
4. Wash in running tap water for 2 minutes.
5. Dehydrate in 95% and absolute alcohols, two changes of 2 minutes each.
6. Clear in xylene, two changes of 2 minutes each.
7. Mount with resinous mounting media.

Results:

This counterstain produces a salmon colored cytoplasm. It stains erythrocytes darker than other counterstains. Some granules, such as zymogen, eosinophiles, mast cell, etc., appear to be more distinctly stained. Excellent counterstain, particularly if one desires a counterstain which is easy on the eyes during extended use of the microscope.

Phloxine-B Safran

2% Picric Acid

Picric acid	2.0 gm
Distilled water	100.0 ml

1.0% Aqueous Phloxine B

Phloxine B	1.0 gm
Distilled water	100.0 ml

2.0% Alcoholic Safran

Safran du Gatinais[3]	2.0 gm
Alcohol, 100%	100.0 ml

Dissolve the safran in the alcohol by placing the solution in a 56 - 60° C oven. It is recommended the solution be retained in the oven at all times. The container must be well stoppered to prevent excessive evaporation.

Staining Procedure:

1. Decerate and hydrate to water in the usual manner.
2. Place in 2% aqueous picric acid for 5 minutes.
3. Wash in running tap water for 5 minutes.
4. Proceed with desired hematoxylin stain (Mayer's preferred).
5. After 15 minute water wash, stain in 1.0% aqueous phloxine B solution for 1 minute.
6. Wash in running tap water for 5 minutes.
7. Place slides in three changes of absolute alcohol for 2 minutes each.
8. Stain in 2.0% alcoholic safran for 2½ minutes.
9. Rinse with two changes of absolute alcohol.
10. Xylene, two changes of 2 minutes each.
11. Mount with resinous media.

Results: Cytoplasm - reddish pink or yellow.

NOTE: This is an excellent counterstain for routine staining since tinctorial differentiation results in two distinct colors. For example, collagen stains yellow, while muscle stains red. The value of this counterstain can only be appreciated by personal experience. The procedure is longer and more complicated than most counterstains, but the tinctorial differentiation of various tissue structures may justify its use.

1. Richard Verfuerth was formerly Chief of the Research Laboratory Branch, AFIP. Mr. Verfuerth is now Vice President and Director of Laboratories, American HistoLabs, Inc., Silver Spring, Maryland 20910.
2. Luna, L. G.: Histopathology Laboratories Division, AFIP, Washington, D.C. 20306.
3. Roboz Surgical Instrument Co., Washington, D.C. 20006.

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Recycling Xylene

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Introduction

With the onset of petroleum shortages, we must consider the future possibility of a xylene shortage. In a small laboratory with limited storage space and budget, it is not always possible to purchase large quantities of xylene. Because of these problems, a study was undertaken to investigate the possibility of reclaiming xylene. Not only has it helped alleviate the xylene storage problem, it also has resulted in saving money for the hospital. The following procedure for reclaiming xylene has been successful in our laboratory.

Materials Required

1. Silica gel, grade 12, 50 mesh. (Obtained from Fisher, at $8.55 for a five pound can.)
2. Filter paper, fine grade.

Method

1. During routine solution changes of the tissue processor and H&E staining dishes, the xylene is returned to its original containers and 100 gm of silica gel is added for every gallon of xylene. This is a saturated solution.
2. The xylene and silica gel mixture is allowed to stand for a week at room temperature. Occasional agitation may be helpful.
3. The mixture is then filtered through fine grade filter paper into a clean, dry container and capped tightly.
4. The reclaimed xylene is now suitable for re-use in the tissue processor or H&E staining dishes.

The use of reclaimed xylene in the tissue processor did not adversely affect tissue. It was as well processed as when using fresh xylene. The reclaimed xylene was also successfully used to decerate and clear slides before staining with H&E. The specific gravity of reclaimed xylene was the same as unused xylene; both read 0.858 at room temperature.

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.