HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor A Technical Bulletin for Histotechnology Vol. V, No. 2, April, 1975

Erratum:

The following correction should be made concerning the Verfuerth-Luna article entitled "COUNTERSTAINS FOR HEMATOXYLINS," which appeared in Histo-Logic, Vol. V, No. 1, page 61, January, 1975. Under 1% Phloxine-B (stock), Phloxine B should read 1.0 gm; not 10.0 gm.

Useful Stains for a General Hospital Laboratory

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I thought readers of Histo-Logic might be interested in a list of stains we have found useful in routine histopathology work in a general hospital, and which we have available at all times. All three of our histology technicians are experienced with these stains. By limiting our special stain requests to those on this list, the technicians know what the pathologist expects and what reagents to keep on hand. Most of the stains have been on our list for the past three years; some have recently been added. This list may be of interest to other laboratories, especially to those whose special staining procedures are getting established. For purposes of quality control, we keep special tissues on hand for positive controls of these reactions.

1. Rapid polychrome stain for frozen sections, such as Paragon stain
2. Hematoxylin and eosin (for paraffin and frozen sections)
3. Masson Trichrome (method of Judge)
4. Phosphotungstic acid hematoxylin (method of Judge)
5. Wilder reticulum
6. Bodian silver
7. Fontana silver
8. Melanin bleach
9. Congo red for amyloid
10. Crystal violet for amyloid
11. Fat stain – ORO
12. Periodic acid Schiff (PAS), with and without diastase.
13. Gomori methenamine silver
14. Ziehl-Neelsen acid fast stain
15. Iron stain, Mallory
16. Giemsa (for bacteria)
17. Humberstone (for bacteria)
18. Brown-Brenn (for bacteria)
19. Brown-Hopps (method of Brown and Hopps, for bacteria)
20. Goodpasture's bacteria stain
21. Alcian blue
22. Mucicarmine (method of Preece)
23. Luxol Fast blue stain
24. Verhoeff's elastic stain
25. Movat pentachrome (method of Russell)
26. Schaeffer and Fulton stain for spores (method of Lillie)
27. Warthin-Starry stain for spirochetes

Bibliography:

1. Unless otherwise stated, the methods are those published in: 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, 1968. Other sources are:
2. Judge, M.S.: Quality control in special histologic staining. Amer. J. Med. Tech., *36*:2, 49-63, Feb. 1970.
3. Brown, R.C., and Hopps, H.C.: Staining of Bacteria in Tissue Sections: A Reliable Gram Stain Method. Amer. J. Clin. Path., *60*:2, 234-240, 1973.
4. Preece, A.: A Manual for Histologic Technicians, 2nd ed., Boston, Little, Brown and Company, 1965.
5. Russell, H.K. Jr.: A Modification of Movat's Pentachrome Stain. A.M.A. Arch. Path., *94*:187-191, Aug. 1972.
6. Lillie, R.D.: Histopathologic Technic and Practical Histochemistry, 3rd ed., New York, McGraw-Hill Book Co., 1965.

Luna's Method for Processing and Sectioning Eye Lenses

An Editorial

The following procedure has been developed for processing human eye lenses when the need for sectioning arises.

Fixation: 10% buffered neutral formalin

After fixation, for not less than 16 hours, place lens in tissue cassette and wash specimen in running water for 30 minutes.

1. Keep specimen in 80% alcohol until ready to place on automatic tissue processor.
2. Alcohol, 95%; 1 hour.
3. Alcohol, 95%; 1 hour.
4. Alcohol, 95%; 1 hour.
5. Alcohol, absolute; 1 hour.
6. Alcohol, absolute; 1 hour.
7. Alcohol, absolute; 1 hour.
8. Chloroform; 1 hour.
9. Chloroform; 1 hour.
10. Paraffin; 1½ hours.
11. Paraffin; 1½ hours.
12. Paraffin, under vacuum; ½ hour.
13. Embed.

Microtomy:

1. Rough cut paraffin block carefully and slowly until the entire width of the lens is exposed.
2. Soak block with a piece of cotton which has been saturated with room temperature water. The block should be soaked for several minutes.
3. Chill the surface of the block and knife with an ice cube.
4. Section specimen with a slow even turn of the microtome wheel.
5. Float sections on albuminized slides and dry on slide warmer overnight or in a 60° C oven for 45 minutes.
6. Stain as desired.

Remarks:

Do not use paraffins which contain plastic polymers since the sections will not spread out adequately on the flotation bath. The success of the procedure is dependent on two factors: (1) A sharp knife, and (2) adequate soaking of the specimen. The specimen has not been soaked adequately if it shatters during sectioning.

Reference:

Luna, L.G.: Histopathology Laboratories Division, AFIP, Washington, D.C. 20306.

Luna-Parker Method for Rhabdomyoma and Nemaline Rods

An Editorial

Fixation: 10% buffered neutral formalin preferred. Zenker's or Bouin's fixative may be used.

Microtomy: Cut paraffin sections at 6 microns.

Solutions:

5% Ferric Ammonium Sulfate

Ferric ammonium sulfate	5.0 gm
Hydrochloric acid, concentrated	2.0 ml
Distilled water	100.0 ml

1% Phosphotungstic Acid

Phosphotungstic acid	1.0 gm
Distilled water	100.0 ml

1% Acid Fuchsin (Stock)

Acid fuchs 1% Acid Fuchsin (stock)	1.0 gm
Distilled water	100.0 ml

1% Oxalic Acid (stock)

Oxalic acid	1.0 gm
Distilled water	100.0 ml

Acid Fuchsin (working)

1% acid fuchsin (stock)	1.0 ml
1% oxalic acid (stock)	1.0 ml
Distilled water	98.0 ml

20% Alcohol (stock)

Alcohol, absolute	20.0 ml
Distilled water	100.0 ml

2% Picric Acid (stock)

Picric acid	2.0 gm
Distilled water	100.0 ml

1% Fast Green (stock)

Fast green FCF	1.0 gm
Distilled water	100.0 ml

Counterstain and Differentiating Solution

20% alcohol (stock)	50.0 ml
2% Picric acid (stock)	20.0 ml
1% Fast green (stock)	1.0 ml

Staining Procedure:

1. Decerate and hydrate slides to distilled water.
2. Place slides in ferric ammonium sulfate solution for 30 minutes.
3. Rinse slides in 3 changes of distilled water (pour on and off).
4. Place slides in phosphotungstic acid solution for 15 minutes.
5. Wash slides for 3 minutes in running tap water, then rinse in distilled water.
6. Place slides in acid fuchsin staining solution for 1 hour in a 55-60° C oven.
7. Remove slides from oven and cool for 15 minutes.
8. Place slides in counterstain and differentiation solution for 4-10 minutes. Agitate once every minute.
9. Dehydrate slides in absolute alcohol and clear in xylene, 3 changes each.
10. Mount coverglass with resinous media.

Remarks:

Step 8 is critical since it is possible to overdifferentiate the rod by overexposure to the differentiation solution. Slides may be restained in the acid fuchsin working solution if overdifferentiation occurs.

Mallory's phosphotungstic acid hematoxylin (PTAH) should be performed in conjunction with this procedure when rhabdomyoma and nemaline rod demonstration is desired.

Results:

Rhabdomyoma and Nemaline Rods (Z band material)	bright red
Erythrocytes	red
Background	light green

Reference:

Luna, L. G., & Parker, A. J.: Armed Forces Institute of Pathology, Washington, D.C. 20306. (To be published.)

Modification of Verhoeff's Elastic Stain for Sections from Tissue Double Embedded with Nitrocellulose and Paraffin

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Verhoeff's elastic stain was modified for staining 5 micra sections from tissues double embedded with nitrocellulose and paraffin. The modified solution is more concentrated, thus reducing the time element from 30 minutes to 5 minutes. The modified stain is stable for one month, whereas the original Verhoeff's solution should be made up the same day it is used. The breakdown point of the original stain is 30-40 slides; the modified stain is stable for 200-300 slides. The modification saves not only time, but valuable chemical elements.

Solutions:

Modified Verhoeff's

Dissolve 2 gm of hematoxylin in 22 ml of absolute alcohol in an open dish on a hot plate. Cool, filter and add 8 ml of 15% aqueous solution of ferric chloride and 8 cc of iodine solution (4 gm iodine, 8 gm potassium iodide dissolved in 100 ml distilled water).

2% Ferric Chloride

Ferric chloride	2.0 gm
Distilled water	100.0 ml

Van Gieson's Stain

Acid fuchsin, 1% aqueous solution	5.0 ml
Saturated aqueous picric acid	100.0 ml

5% Sodium Thiosulfate (Hypo)

Sodium thiosulfate	5.0 gm
Distilled water	100.0 ml

Staining Procedure:

1. Decerate and hydrate to distilled water.
2. Place slides in modified Verhoeff's solution for 5 minutes.
3. Rinse in distilled water.
4. Differentiate in 2% ferric chloride solution – only a few seconds. Check slides under the microscope and if differentiated too far, return to staining solution.
5. Place in 5% hypo solution for 1 minute.
6. Wash in tap water for 5 minutes.
7. Counterstain in Van Gieson's stain for 1 minute.
8. Differentiate in 95% alcohol, 2 dips (2 changes).
9. Absolute alcohol, 2 changes for 2 minutes each.
10. Xylene, 3 changes.
11. Mount coverslip with resinous media.

Results:

Elastic fibers	blue-black to black
Nuclei	blue to black
Collagen	red
Other tissue elements	yellow

Reference:

Mallory, F.B.: Pathological Technique, Philadelphia, W. B. Saunders Co., p. 170, 1942.

A Modification of the Klüver-Barrera Stain

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Introduction:

Although there are many modifications of the Klüver-Barrera stain such as Luxol Blue – P.A.S., Luxol Blue – H&E, etc., we have found this stain very useful in working with C.N.S. sections.

Sections cut at 8-10 microns take the most selective staining, and when dried in the oven at 37° C overnight, may be handled with ease. The Luxol Blue stains the myelin sheath a greenish-blue whereas the protargol stains axis cylinders of both myelinated and non-myelinated fibers dark brown. The neuronal perikarya and neurofibrillar networks are light blue. We find this modification time saving, reliable, and reproducible for both the technician and the pathologist.

Solutions:

0.1% Luxol Fast Blue

Luxol Fast Blue, MBS	0.1 gm
Alcohol, 95%	100.0 ml

Dissolve dyes in alcohol, add 0.5 ml of 10% aqueous glacial acetic acid to each 100 ml.

1% Protargol

Protargol	1.0 gm
Distilled water	100.0 ml

Sprinkle the protargol on the surface of the water and allow it to remain undisturbed until it dissolves. Add 5 grams of clean copper shot to this solution just before use.

Reducing Solution

Hydroquinone	1.0 gm
Sodium sulfite	5.0 gm
Distilled water	100.0 ml

We find a pinch of Kodalk* added to the reducing solution is helpful in that it brightens the stain.

1% Gold Chloride

Gold chloride	1.0 gm
Distilled water	100.0 ml

2% Oxalic Acid

Oxalic acid	2.0 gm
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 to 95% alcohol.
2. Place slides in 0.1% Luxol Fast Blue solution in 60° C oven overnight.
3. Rinse slides in 95% alcohol for 30 minutes.
4. Rinse slides in distilled water for 2 hours. Change distilled water several times.

NOTE: Sections will be uniformly blue at this stage.

5. Place slides in protargol solution for 24 hours in a 37° C oven.
6. Rinse slides in distilled water.
7. Place slides in reducing solution for 1-2 minutes or until white matter (blue) is differentiated from gray matter.
8. Rinse slides in 3 changes of distilled water.
9. Place slides in 1% gold chloride for 2-5 minutes. Sections may be light brown before they are placed in gold chloride. This brown color will disappear in the gold chloride, but may reappear if the section is left in this solution too long.
10. Rinse slides in 3 changes of distilled water.
11. Place slides in 2% oxalic acid, approximately 2-7 minutes. In this step the gray matter takes on a grayish-violet color and white matter remains blue.
12. Rinse slides in 3 changes of distilled water.
13. Place slides in 5% sodium thiosulfate for 3-5 minutes. This fixes the silver oxide.
14. Rinse slides in 3 changes of distilled water.
15. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, 2 changes, 2 minutes each.
16. Mount coverslip with resinous media.

Remarks:

Exposure of slides to the protargol solution for more than 24 hours may cause some Luxol Fast Blue to fade.

*Kodalk is obtainable from: Eastman Kodak, 1669 Lake Avenue, Rochester, New York 14650.

Luna's Method for Softening Keratin

An Editorial

Neet® is a proprietary term for a hair-removing lotion which can be purchased at most cosmetic counters. This lotion has been used in our laboratories for softening keratin, particularly fingernails, in order to make sectioning easier and in some cases possible. Although Neet is used in this procedure, it is felt that any hair-removing lotion will perform satisfactorily if the following procedure is followed:

Softening Procedure:

1. Fix fingernail or keratin material in 10% buffered formalin for 6 hours.
2. Place specimen in Neet or other hair-removing solution until keratin is soft.
3. Wash specimen in running tap water for 30 minutes.
4. Dehydrate, clear and impregnate with paraffin or process as desired.

Remarks:

Fixation in 10% buffered formalin is necessary to prevent keratin from dissolving completely in Neet.

Reference:

Luna, L.G.: Histopathology Laboratories Division, AFIP, Washington, D.C. 20306.

Special Training Film Now Available

"The Theory and Application of Embedding Techniques in the Histopathology Laboratory"

A 17-minute color film on techniques developed and used in the Histopathology Laboratory at the University of Texas M. D. Anderson Hospital and Tumor Institute is available for showing without charge in your hospital. It demonstrates the key stages in the production of good quality diagnostic slides with emphasis on proper tissue embedding. The entire sequence of steps in well-organized tissue preparation is covered from processing, embedding, ribbon sectioning, and mounting sections on slides, through storage of tissue blocks. Six basic types of specimens are discussed – single pieces of tissue, multiple pieces of tissue, skin specimens, decalcified bone, lymph nodes, and cylindrical tissue specimens – and proper orientation of each type in paraffin is shown.

To have this film shown without charge in your hospital, write to Lab-Tek Products, the publisher of Histo-Logic. Because of the limited number of films available, we would appreciate your selection of three alternate dates a few weeks apart. The film is available for purchase for $100.00 from the Audio Visual Department, M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas.

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Editor's Corner

Quality Control for PTAH Staining

An Editorial

Mallory's phosphotungstic acid hematoxylin (PTAH) is very useful for staining a wide variety of tissue elements. However, its effective use is often hindered by variable staining results. Staining results are determined by the stage of oxidation of the solution when used. Control of PTAH staining can be achieved by the use of 6-micron sections of brain to test the tinctorial quality of the PTAH staining solution. The piece of brain should contain a fair number of neurons with dendritic processes. *Good staining PTAH solution will demonstrate the following tinctorial qualities:*

Neuronal cytoplasm	Salmon
Nuclei	Blue
Dendrites	Blue
Collagen	Salmon

It is imperative to produce a salmon colored neuronal cytoplasm and blue dendritic processes (Fig. 1). The entire section should appear blue (for the most part) microscopically. Conversely, a poor staining PTAH solution will produce the following tinctorial qualities:

Neuronal cytoplasm	Salmon
Nuclei	Blue
Dendrites	Light Gray to Salmon
Collagen	Salmon

The entire section will appear salmon colored (for the most part) microscopically (Fig. 2).

Figure 1: Notice that dendritic processes (arrows) are stained well and more detail can be seen in this section on which good working PTAH solution was used. PTAH x 250

Figure 2: Notice in this section that most of fine fibrillar detail seen in Figure 1 is not well defined. Also, the dendrites (arrows) are not staining. PTAH x 250