HISTO-LOGIC™

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Further Information on Eosin Preparation

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In Histo-Logic, Vol. II, No. 3, July, 1972, I read about a problem with eosin. We use eosin every day for routine H&E staining and never have any problem with it. It is true that eosin gets weaker by carrying solutions from previous staining dishes. To keep eosin the same strength, we add fresh eosin once a week. Our eosin is prepared in a special way and was preferred by the famous pathologist, Dr. Arthur Perdu Stough, Columbia University, Medical Center, New York, N.Y.

Eosin staining solution is prepared as follows:

1. Eosin Y (water and alcohol soluble).....10.0 gm
   Distilled water.....1000.0 cc
   Hydrochloric acid.....10.0 cc
   Mix and leave overnight.
2. Filter and wash in distilled water for 3 days, (plug funnel at the end of each day and start rinsing next morning – 3 days).
3. At the end of the third day, let water drain completely and dry precipitate on filter paper at room temperature.
4. Put dry eosin from filter paper into bottle with 1000.0 cc of 95% alcohol.
5. For working solution, dilute 1 part eosin in 3 parts 95% alcohol.

Additional Information on Histochemical Calcium Techniques

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In Histo-Logic, Vol. II, No. 2, April, 1972, Mrs. Willa Mikowski mentioned "difficulties encountered with traditional histochemical calcium methods." Our laboratory routinely uses the von Kossa silver nitrate technique and the Alizarin Red S method for demonstrating calcium, and we have no trouble with either one.

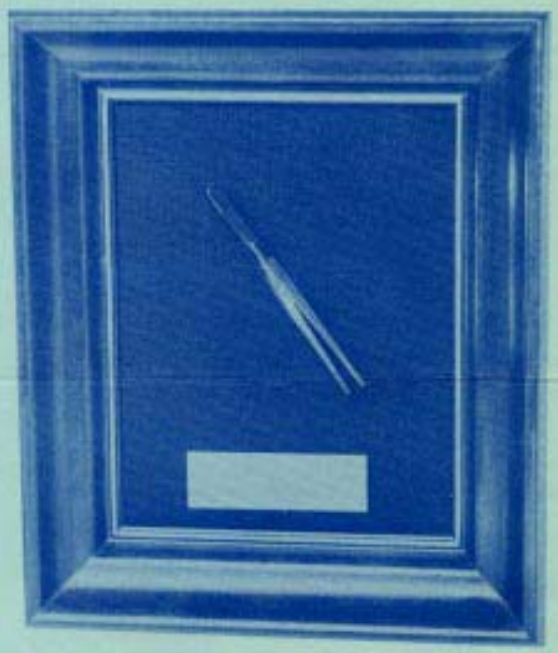
The von Kossa technique requires merely placing the decerated and hydrated slide in 5% silver nitrate solution in a chemically clean coplin jar (covered) outdoors for one hour. Sunshine is helpful, but even when the sky is overcast the silver nitrate causes calcium deposits in the sections to blacken.

With Alizarin Red S we find that if the 1% Alizarin Red S is brought to pH 6.5 by using a weak (.1%) sodium hydroxide solution (aqueous) instead of ammonium hydroxide the Alizarin Red remains stable for several months. Our present solution is over 3 months old and still working perfectly. Furthermore, the use of sodium instead of ammonium hydroxide to regulate the pH makes the stain much less likely to "bleed" when sections are differentiated in water and counterstained with 1% aqueous Light Green. We let slides stand in the Alizarin Red for at least 1 hour.

Let's Get Our Facts Straight

An Editorial

The method most often used for determining the sharpness of a microtome knife is tactile sensation. Although commonly used, this method is not recommended due to the hazard involved with handling a sharpened microtome knife. Exhibit extreme care if this procedure is followed. The thumb is gently placed on the bevel of the knife and stroked gently at right angle to the knife edge, producing a so-called "bite". Many histotechnologists believe that the greater the "bite" the sharper the knife edge. This is not true. A good "bite" only indicates the knife has a serrated (saw tooth) edge. Serrations produce the "bite". A good knife edge will exhibit no serrations at 100 magnifications and produce very little, if any, "bite" when tested by the tactile method. Experience indicates that knives which feel dullest, in reality are the sharpest. For this reason, extreme care must be taken.



Golden Forceps Award

The first Golden Forceps Award will be presented at the October, 1973 AFIP Histopathologic Symposium. The winner will also receive an expense paid trip to Washington, D.C. to attend the symposium.

All articles accepted from July 1, 1971 through March 1, 1973 will be considered. Originality, clarity, and scientific contribution will be the criteria for winning. All articles become the property of HISTO-LOGIC.

Methods for Obtaining Fungus, Bacteria and Acid-Fast Controls [1]

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Excellent special stain controls for gram-positive and gram-negative microorganisms, fungi, and acid-fast bacteria can be produced with the aid of microbiology cultures.

Gram-Positive:
Innoculate four blood agar plates heavily with Staphylococcus aureus and incubate at 37° C for 18 hours (overnight). The heavy growth is harvested with a stiff wire loop and re-suspended in a test tube containing a few ml of phosphate-buffered 10% neutral formalin. Wait one hour to allow for fixation, centrifuge for 5 minutes at 2,500 R.P.M. and decant the supernatant. Add 1.5 ml of molten agar-agar and re-suspend the button in the agar. Allow to solidify and remove the agar by inverting the tube and gently tapping the rim. Cut into strips, wrap in lens paper, label, place in cassette and process. The strips are arranged in half moons and embedded alongside a half moon of a gram-negative strip. Cut sections until the specimen is exhausted and mount on slides. Dry the slides in 37° C oven and store in a covered slide box.

Sections should be cut immediately. The agar block will absorb water if stored for any length of time. Dr. Bouton[2] suggests adding egg white after harvesting to eliminate this problem.

Gram-Negative:
The procedure is identical to that above. Klebsiella, a gram-negative bacillus, is an excellent choice.

Fungus Control:
Heavily innoculate four blood agar plates with Candida species. Harvest and prepare as above.

Acid-Fast Control:
A non-pathogenic mycobacterium such as M. Phlei is recommended. M. tuberculosis can be used. Add 5 ml of 40% formaldehyde to the culture tube surface and allow to fix for 24 hours. Harvest and suspend in 1.0 ml of agar. A critical acid-fast control is made by using Nocardia asteroides.

ED. NOTE: The method presented herein has been modified by Lorraine L. Quinn, DePaul Hospital, Norfolk, Virginia 23503.

References

1. Robinson, C.R.: Positive and Negative Bacteria and Fungus Controls for Gram Stain in Histopathology. Summary Report, *ASCP, 8:* No. 2, 1971.
2. Bouton, M.S.: Lynchburg Training School and Hospital, Lynchburg, Virginia. Personal communication.

Staining Fresh, Unfixed, Unembedded and Undecalcified Bone Sections

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Currently there are two staining methods for fresh, unfixed, unembedded and undecalcified bone; the Basic Fuchsin method of Frost[1] and the new differential bone stain called Osteochrome.[2] Both perform as a fixative and a stain. The techniques are simple and rapid, requiring little material and, perhaps most important, they are reliable and require no expensive or delicate equipment.

Method
Immediately, upon receipt, the bone is sawed to 2-3 mm thickness with a very fine jeweler's or coping saw. The specimen is held, during the sawing process, in a small vise or V-block fashioned out of wood. The bone slab is ground to a 50-100 micra thickness according to the method of Frost.[3] Average time for grinding a section is 2 minutes; larger sections will take longer, approximately 5-10 minutes.

Solutions

Frost's Basic Fuchsin

Basic fuchsin	1.0 gm
Ethyl alcohol, 70%	100.0 ml

Ensure that the basic fuchsin is dissolved thoroughly.

Staining Procedure
1. After grinding bone to 50-100 micra, rinse in distilled water.
2. Stain in 1% basic fuchsin; 24-48 hours for adult bone, 4-8 hours for infants, young children and immature animals.
3. Place sections in tap water then grind surface stains.
4. Wash with 0.1% zephiran chloride or in 0.01% mild household detergent to remove debris adhering to the section.
5. Wash with tap water several times and rinse in distilled water.
6. Place section between two slides, each wrapped with paper towels, and press together gently with a Hoffman clamp. Allow to dry overnight in a 42-45° C oven.
7. Mount with a neutral synthetic resin.

Results

Osteocytes and their canaliculae osteoblasts, osteoclasts	Red
Osteoid seams	Light pink
Bone with low mineralization density	Red
Bone with high mineralization density	Unstained

Osteochrome Method

Solutions

Osteochrome Solution*

Acetic Alcohol

Glacial acetic acid	0.01 ml
Alcohol, methanol 95%, reagent grade	100.0 ml

Staining Procedure
1. After section is ground to 50-100 micra, rinse in distilled water.
2. Stain in the Osteochrome solution; 90 minutes for quick diagnosis, or 48 hours for complete permeation of the tiny lacunae.
3. Transfer section to tap water and lightly grind surface stains.
4. Wash with 0.1% zephiran chloride or in 0.01% mild household detergent.
5. Wash section with tap water and rinse with distilled water.
6. Differentiate in 0.01% glacial acetic acid in 95% methanol:
   a. 90 minute stain, differentiate for 3-5 minutes.
   b. 48 hour stain, differentiate for 10-15 minutes.
7. Dehydrate as follows:
   a. 95% alcohol, 9 minutes.
   b. 100% alcohol, 8 minutes.
8. Clear as follows:
   a. Equal parts of 100% alcohol and xylol, 5 minutes.
   b. 1 part of 100% alcohol and 3 parts of xylol, 3 minutes.
   c. 1 part of 100% alcohol and 9 parts of xylol, 2 minutes.
   d. Xylol, 2 minutes.
   e. Xylol, 1 minute.
9. Mount with a neutral synthetic resin.

Results

Osteoid seams	Red or green
Osteocytes and canaliculae, osteoblast, osteoclast, nuclei	Red to bluish-purple
Cytoplasm	Faint green to pink
Mast cells granules	Reddish
Eosinophil granules and periosteum	Bluish-green
Cartilage cells	Dark purple to red
Bone with low mineralization density	Orange
Bone with medium mineralization density	Red
Periosteal cells and Sharpey's fibers	Red

*Harleco

References

1. Frost, H. M.: Staining of fresh, undecalcified thin bone sections, Stain Technol. **34**: 135-146, 1959.
2. Villanueva, A. R.: An improved stain for fresh, mineralized bone sections – useful in the diagnosis of certain metabolic diseases of bone, Amer. J. Clin. Pathol. **47**: 78-84, 1967.
3. Frost, H. M.: Preparation of thin, undecalcified bone sections by rapid manual method, Stain Technol. **33**: 273-277, 1958.

Method of Eliminating Wrinkles From Paraffin Sections

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Difficulty with wrinkled sections in a busy histopathology laboratory is common. A dull knife, improperly fixed or processed tissue, the clearance angle, and poor equipment are contributory. Regardless of the cause, most wrinkles can be removed during floatation by the addition of alcohol to the bath.

The floatation bath is filled with 20-30% ethanol instead of plain water. Ribbons are floated on the water bath in the usual manner. Wrinkles, when present, will spread and flatten. This is probably due to the surface tension phenomenon.

This method has been found to produce no ill effects on sections from all types of tissue, during a period of one year.

Combined Silver Methenamine - One - Step Trichrome Procedure

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Grocott's[1] method for demonstrating fungi does not allow sufficient tinctorial differentiation of morphologic features in tissue sections. Additional stains are usually required to correlate morphology with fungal location.

The procedure presented herein will demonstrate fungi with good tissue morphology in a single preparation, reducing the need for additional stains.

Fixation

10% phosphate buffered neutral formalin.

Microtomy

Cut paraffin or frozen sections 6 to 8 micra.

Solutions

Solutions for Grocott's method for fungi are prepared according to instructions provided on page 230 of the AFIP Staining Manual.[2]

0.5% Periodic Acid

Periodic acid	0.5 gm
Distilled water	100.0 ml

Groat's Iron Hematoxylin

Distilled water	50.0 ml
Sulfuric acid (sp.gr. 1.84, 94% H_2SO_4)	0.8 ml
Ferric alum ($FeNH_4(SO_4)_2 \cdot 12H_2O$)	1.0 gm
95% ethyl alcohol	50.0 ml
Hematoxylin	0.5 gm

Mix in order given at room temperature and filter before use.

Trichrome Stain

Chromatrope 2R	0.6 gm
Light green, SF yellowish	0.3 gm
Glacial acetic acid	1.0 ml
Phosphotungstic acid	0.8 gm
Distilled water	100.0 ml

Aniline blue may be substituted for light green if blue collagen is desired.

Staining Procedure

1. Decerate and hydrate to water.
2. Oxidize in periodic acid for 10 minutes.
3. Wash in running tap water for 3 minutes.
4. Oxidize in chromic acid for 45 minutes.
5. Wash in tap water for 5 minutes.
6. Rinse in distilled water, 3 changes, 5 minutes total.
7. Place in working methenamine-silver nitrate, 56-58° C for 1 hour. Use coated metal forceps. Observe the tissue section macroscopically during the staining procedure. The tissue should become a yellowish brown. If this color change does not occur, incubate until it shows tan coloration (1-4 hours).
8. Wash thoroughly in 3 changes of distilled water, 3 minutes each.
9. Tone in gold chloride (0.1% solution) until sections turn purplish gray and fungi are black.
10. Wash thoroughly in 3 changes of distilled water, 3 minutes each.
11. Fix in sodium thiosulfate (5% solution) for 3 minutes.
12. Wash in running tap water for 5 minutes.
13. Place in Bouin's solution for 60 minutes.
14. Wash in running tap water for 5 minutes or until yellow color disappears.
15. Stain in Groat's iron hematoxylin for 10 minutes.
16. Wash in running tap water for 5 minutes.
17. Place in trichrome stain for 15 minutes.
18. Place in acetic acid water (0.5% solution) for 30 seconds. If sections are too dark, differentiate in 1% acetic acid water to which 0.7 gm of phosphotungstic acid has been added. Place in distilled water for 30 seconds.
19. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.
20. Mount coverglass with a synthetic resin.

Results

Fungi	Black
Basement membrane, cellulose, chitin, mucin, glycogen	Black
Muscle fibers	Red
Collagen	Green (blue if aniline blue is used)
Nuclei	Blue to black

References

1. Grocott, R. J.: A Stain for Fungi in Tissue Sections and Smears. Am. J. Clin. Path., **25**: 975-979, 1955.
2. 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.

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Editor's Corner

Histology Workshop in Milwaukee

A one-day histology workshop will be held in Milwaukee, Wisconsin on May 5, 1973. This excellent program is being presented by the Milwaukee Histology Society.
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Additional Information On Glass Ink

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In HISTO-LOGIC, Vol. II, No. 1, reference was made to the very good permanency of "Labink" for marking slides. In our laboratory we have found Paragon Glass and Metal Ink even easier to work with. The ink flows more smoothly onto the glass, even as the level gets lower. Paragon ink does not fade and is impervious to staining procedures. Paragon ink can be purchased from:

Paragon C & C Company, Inc.
Bronx, New York 10454

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, write: Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 N. Aurora Rd., Naperville, Illinois 60540.