

HISTO-LOGICTM

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A Simple Staining Procedure for Calcium Deposits

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Because of the difficulties encountered with traditional histochemical calcium methods, a new rapid, and simple staining procedure has been devised utilizing the principles of serum colorimetric determinations. Methods commonly used for selective staining reactions are difficult to control or quantitate. Carr's method requires polarized light. Von Kossa's determination of calcium depends on ultra-violet light or incandescent illumination. The Alizarin Red "S" solution used in Dahl's method is only stable for about a month. Each method seems to have its own particular drawback. Clearly, a better way to stain calcium deposits in tissues would be an asset to the histology laboratory. The spectrophotometric determination for minute particles of calcium in urine, serum, and spinal fluid, using Nuclear Fast Red (Kernechtrot) dye as a binding agent has been used for the past decade. Calcium methods of this kind have been reported by Baar,¹ Kingsley and Robnett,^{2,3} and Chilcote and Wasson.⁴

Nuclear fast red forms a colored complex with calcium in acids. It seems logical that calcium in tissue would also have an affinity for the dye. After experimentation, the staining technique described in this report proved to be a completely workable procedure. It is a very simple test, easily adaptable to the small, minimally-equipped laboratory and little previous experience with special staining techniques is needed. Reproducibility is excellent and the stock solution is stable for at least six months at room temperature. The staining reaction may be accomplished either in an oven or a water bath which will maintain a temperature of 56° C. A negative control is obtained by decalcifying a second section and staining it along with an undecalcified section.

Fixation

10% buffered neutral formalin.

Microtomy Paraffin sections at 6 micra. Process negative control, (see below).

Solution

Nuclear Fast Red Staining Solution

Alcohol, ethyl 50% 50.0 ml
Nuclear fast red (Kernechtrot) 0.5 gm

Staining Procedure

1. Decerate sections as usual to 95% ethyl alcohol.
2. Stain in nuclear fast red solution in a 56° C. oven for 30 minutes. (For best results, place staining solution in oven 15 minutes prior to staining.)
3. Rinse off excess stain with distilled water.
4. Dehydrate in 95%, 100% ethyl alcohol, 2 changes each.
5. Clear in xylene, 3 changes.
6. Coverslip.

Negative Control Slide

1. Decerate and hydrate to distilled water.

2. Place section in chelating agent or decalcifying fluid of choice for 15-30 minutes.
3. Wash slide in running tap water for 15 minutes.
4. Stain the undecalcified section beginning with step 2 of the staining procedure.

Results

Calcium Bright Red (Fig. 1)
Decalcified material
(control) No Stain to Light Pink (Fig. 2)
Background Very Light Pink

References

1. Baar, S.: *Clin. Chem. Acta*, 2: 567-575, 1957.
2. Kingsley, G.R., and Robnett, O.: *Ibid.*, 27: 223-230, 1957.
3. Kingsley, G.R., and Robnett, O.: *Analytical Chem.*, 33: 552, 1961.
4. Chilcote, M.E., and Wasson, R.D.: *Clin. Chem.*, 4: 200-210, 1958.

Figure 1
Dark area (arrow) represents calcium in kidney stained with nuclear fast red.



Figure 2
Higher power of Figure 1 to demonstrate calcium removal (arrow) after 30 minutes in a decalcifying fluid and subsequent staining in nuclear fast red.



Can You Help?

"Recently our Histology Department received a request for Bouin's solution as a fixative for a testicular biopsy. A sperm count on the specimen was also to be done by the surgeon. My question is whether Bouin's solution is applicable in such a case or if your readers know of a better method."

Mrs. Janice Armstrong, HT (ASCP)

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1860 Queen City Avenue
Cincinnati, Ohio 45214

NOTE: If you have a workable suggestion to this request, please send the information to the address provided above and send a carbon copy of the reply to the editor for possible subsequent publication in HISTO-LOGIC.

Did You Know?

An Editorial

... that paraffin embedded tissue specimens section better when frozen. Place blocks in the freezing compartment of a laboratory refrigerator or place them, tissue down, on ice cubes or your tissue embedding center prior to microtomy.

... that the micron thickness of a cut tissue section will increase if the microtome is operated at excessive speed.

... that the use of sodium sulfate in Zenker's fixation is to prevent hemolysis of red blood cells.

... that it is possible to over-abstract stainable dye from a staining solution by excessive filtration through fine filter paper, resulting in less available dye for coloration of tissue.

... that the word "DECERATE" is the proper term for removing paraffin from microscopic slides and should be used instead of "deparaffinized."

... that the staining properties of many solutions can be altered if they are in close proximity to a bottle of ammonium hydroxide or hydrochloric acid. These two chemicals should be kept far away from staining solutions or staining areas.

... that tissues fixed in formalin which has been buffered with sodium phosphate monobasic and dibasic salts will remain considerably softer than tissues fixed in formalin containing other neutralizing or buffering agents.

... that picric acid (Trinitrophenol) has potential explosive properties if kept in an excessively dry state. Picric acid must be maintained in a moist condition to prevent this hazard.

... that Synasol alcohol (Union Carbide) can be used as a substitute for ethyl alcohol in dehydrating hematoxylin- and eosin-stained slides. (It is not used in hydrating slides or for special stains.) Thanks to: Harold S. Bolt, Saginaw Osteopathic Hospital, Saginaw, Michigan 48602.

... that muscle will not stain properly with Masson's trichrome stain unless sections are adequately mordanted in Bouin's solution. Chromatically, the muscle will range from blue to deep purple depending on the degree of mordanting.

... that synthetic orcein is a chromatically acceptable substitute for natural orcein for demonstrating elastic fibers.

... that one of the major reasons for the use of picric acid in Bouin's fixative mixtures is to counteract the swelling properties of acetic acid.

... that we are most anxious to compile a list of names and addresses of histologists who are willing to present lectures in histology seminars, symposiums or workshops. (A title and short abstract of the proposed lecture should be sent to the editor.) This information will be made available to anyone requesting it.

A Modified Movat Pentachrome Stain

Jeffrey S. Silverman

Westbury, New York 11590

Fixation

Bouin's fluid or 10% buffered neutral formalin.

NOTE: If formalin fixative is used, decerated tissue sections must be mordanted in Bouin's fluid for one hour in a 56° C. oven. Wash well in running water to remove picric acid deposits.

Microtomy

Paraffin sections at 4-6 micra.

Solutions

1% Alcian Blue

Alcian blue	1.0 gm
Distilled water	98.0 ml
Acetic acid	1.0 ml

Ammonium Hydroxide

Ammonium hydroxide, 58%	5.0 ml
Ethyl alcohol, 95%	95.0 ml

Orcein-Verhoeff Stock Solutions

Solution A

Orcein	1.0 gm
Ethyl alcohol, 70%	498.0 ml
Hydrochloric acid, concentrated	1.0 ml

Solution B

Hematoxylin	8.0 gm
Ethyl alcohol, 100%	160.0 ml

Solution C

Ferric chloride	9.6 gm
Distilled water	90.0 gm

Solution D

Iodine	1.0 gm
Potassium iodide	2.0 gm
Distilled water	97.0 ml

Orcein-Verhoeff Working Solution

Immediately before use, combine above solutions in following order:

Solution A	25.0 ml
Solution B	8.0 ml
Solution C	5.0 ml
Solution D	5.0 ml

Use working solution once and discard.

Woodstain Scarlet-Acid Fuchsin Stock Solutions

Solution A

Woodstain scarlet, N.S. concentrated	0.1 gm
Distilled water	99.5 ml
Acetic acid, glacial	0.5 ml

Solution B

Acid fuchsin	0.1 gm
Distilled water	99.5 ml
Acetic acid, glacial	0.5 ml

Woodstain Scarlet-Acid Fuchsin Working Solution	
Solution A	40.0 ml
Solution B	10.0 ml

0.5% Acetic Acid Water

Acetic acid, glacial	0.5 ml
Distilled water	100.0 ml

5% Phosphotungstic Acid

Phosphotungstic acid	5.0 gm
Distilled water	95.0 ml

6% Saffron Solution

Saffron (Spanish) C.I. No. 75100	6.0 gm
Ethyl alcohol, 100%	94.0 ml

To allow proper dye extraction, solution must be placed in an airtight bottle in a 56-60° C. oven for 48 hours.

Staining Procedure

1. Decerate section and hydrate to distilled water.
2. Stain in 1% alcian blue solution for 20 minutes.
3. Dip five times in distilled water.
4. Place in ammonium hydroxide solution in a 56° C. oven for 10 minutes.
5. Wash in running tap water for 2 minutes.
6. Stain in Orcein-Verhoeff working solution for 2 hours.
7. Wash in running tap water for 3 minutes.
8. Stain in Woodstain scarlet-acid fuchsin working solution for 2½ minutes.
9. Place in 0.5% acetic acid water for 30 seconds.
10. Differentiate in 5% phosphotungstic acid for 5-10 minutes. Well differentiated sections demonstrate colorless collagen and blue-green mucopolysaccharides.
11. Rinse in 0.5% acetic acid water for 30 seconds.
12. Three changes of 100% ethyl alcohol, 1 minute each.
13. Stain in 6% Saffron solution for 8 minutes.
14. Dehydrate in 100% ethyl alcohol, two changes.
15. Clear in xylene, three changes.
16. Coverslip.

Results

Nuclei	Black
Cytoplasm	Red
Elastic fibers	Purple to Black
Collagen and bone	Yellow
Mucopolysaccharides	Blue-green
Muscle	Red

Cross striations and intercalated discs in cardiac muscle are clearly stained.

NOTE: The author wishes to thank Mr. Richard J. Schroeder for helpful criticism and guidance during the development of this technique.

References

1. Movat, H.Z.: Demonstration of all Connective Tissue Elements in a Single Section. *Arch. Path.*, 60: 289-295, 1955.
2. Roman, N., et al: Orcein-hematoxylin in Iodized Ferric Chloride as a Stain for Elastic Tissue. *Stain Techn.*, 42: 199-202, 1967.

Substitute Dye Source for Movat's Pentachrome

An Editorial

In 1955, Movat published a very useful pentachrome stain for the demonstration of all connective tissue elements in a single section. Woodstain Scarlet, N.S., a dye used in the procedure, was formerly obtainable from E.I. du Pont de Nemours and Company. Mr. Keith Russell, Histopathology Laboratories Division, Armed Forces Institute of Pathology, recently compared the staining results of the similarly-named substitute, Crocein Scarlet MOO, and found that it was chromatically comparable. Crocein Scarlet MOO can be substituted for Woodstain Scarlet N.S. in Movat's pentachrome stain.

Dye

Crocein Scarlet MOO, CI 27290

Sources

J. T. Baker Chemical Company
Phillipsburg, New Jersey

Allied Chemical

National Biological Stain and Reagent Department
Morristown, New Jersey

Harleco

Philadelphia, Pennsylvania

Reference

Movat, H.Z.: Demonstration of all Connective Tissue Elements in a Single Section. *A.M.A., Arch. Path.*, 60: 289-294, 1955.



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HISTOTECH BUTTON...
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BE SURE TO PICK ONE UP
WHEN YOU REGISTER
FOR THE ASMT MEETING THIS JUNE

Starch in Tissue Sections

An Editorial

A most common artifact seen microscopically in tissue sections is starch from surgical dusting powder. The classic appearance is a hexagonal crystal. The crystal stains gray with hematoxylin and eosin, is PAS positive and stains green with Gomori-type preparations. A Maltese cross is observed under polarized microscopy. Powdered rubber gloves used for grossing tissue (macrosectioning) are the most frequent means by which the starch crystal is introduced. Starch can also be deposited on tissue during surgical procedures. These crystals pose no particular problem but are objectionable if seen in large numbers. This is especially true if the slide is to be photographed or if polarized microscopy is required for the identification of other crystalline materials. This artifact cannot be removed from tissue sections, and little can be done about its presence after surgery, but particular care should be taken during macrosectioning to eliminate its introduction during this procedure.

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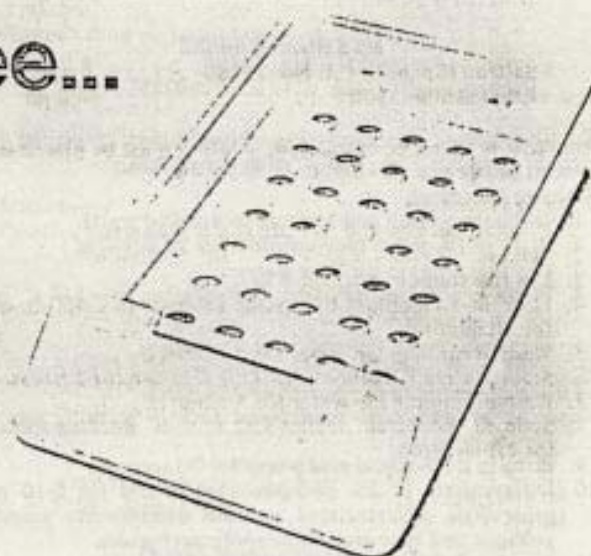
why our newly improved TISSUE-TEK-II Cassette makes tissue processing and embedding more efficient:

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In ordering the new-style Cassette, use the same #4191.

The new Cassette utilizes the same metal cover #4194.

In order to write on the new Cassette, a soft-leaded art pencil is recommended.



NOTE: Tissue-Tek II Processing/Embedding Cassette is a plastic "disposable" designed for one-time use. Exposure to chloroform and/or molten paraffin contaminated with an excess of any clearing agent may cause Cassette distortion.

Editor's Corner

Pennsylvania Society of Medical Technologists will meet May 4-6, 1972. An all day Tissue Identification Seminar will be conducted for histotechnologists. For information contact: Mrs. Deana C. Sheehan, Laboratory of Pathologic Anatomy, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104.

The Histology Section of ASMT will conduct a comprehensive histological technique program during the 40th Annual Convention in Minneapolis, Minnesota, June 11-16, 1972. For information contact: American Society of Medical Technologists, Suite 1000, Hermann Professional Building, Houston, Texas 77025.

A Tri-State Spring Histopathology Seminar will be conducted on April 27, 1972 in Elizabeth, New Jersey. For information contact: Mary Lou DiGiovanni, 153 Stiles Street, Apartment 11-A, Elizabeth, New Jersey, 07208.

We Want to Help

HISTO-LOGIC will advertise your meeting dates. If you are conducting a seminar, workshop or related educational program on histologic technique, send salient information to the editor for inclusion in HISTO-LOGIC. Remember, HISTO-LOGIC is published in January, April, July and October. Information should reach the editor at least 60 days prior to publication. Announcements will provide histopathology technicians with an idea of the many national and regional programs being conducted annually in this laboratory discipline.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo Logic, Box 882, Hyattsville, Maryland 20782. 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., 39 E. Burlington, Westmont, Illinois 60559.