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A Rapid Cold Method for the Preparation of Bullard's and Harris' Hematoxylin

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A frequent cause of minor disaster in the laboratory is the addition of oxidants such as sodium iodate and mercuric oxide to boiling mixtures of hematoxylin.

After two or three episodes of having these solutions bubble over and stain laboratory table tops and floors, sometimes causing minor burns to personnel, we have devised the following method for the prevention of this rather common laboratory accident.

It differs from the usual standard formulas for the preparation of Bullard's and Harris' hematoxylin[1,2] in that we substitute a magnetic stirrer for heat. The stirrer is used for dissolving all ingredients and the boiling of solutions is avoided.

Bullard's hematoxylin is the stain we use routinely at Bellevue Hospital. It is the strongest hematoxylin formula available and stains quite strongly in seven minutes. It has a long staining life (we stain approximately 3,000 slides per 400 ml of stain) and does not need filtering more than once a week.

Method

Because of the great variation in dye content of various brands of hematoxylin due to the present shortage, it may be necessary to increase the amount of hematoxylin used in order to obtain proper staining results. Some hematoxylin crystals we have obtained recently have required double the usual amount for good staining reactions.

Bullard's Hematoxylin

1. Using a magnetic stirrer at high speed, dissolve 8 gm of hematoxylin in 150 ml of 70% ethanol.
2. Again, using a magnetic stirrer, dissolve 20 gm of aluminum ammonium sulphate in 250 ml of distilled water.
3. Mix the two solutions and, while stirring at high speed, add 800 mg of sodium iodate. Allow the solution to mix thoroughly (at least 5 minutes).
4. Add the following in this sequence, 275 ml of 95% alcohol, 330 ml of pure glycerol, 34 ml of glacial acetic acid, and 40 gm of aluminum ammonium sulphate. Stir at high speed for at least 10 minutes before using.

Harris' Hematoxylin

1. Using a magnetic stirrer at high speed, dissolve 5 gm of hematoxylin in 75 ml of 70% ethanol.
2. Again, using the stirrer at high speed, dissolve 100 gm of ammonium alum in 1000 ml of distilled water. This solution is almost at the saturation point and if some of the ammonium alum remains undissolved, it will have no deleterious effect on staining.
3. Mix the two solutions, stirring at high speed, and add 500 mg of sodium iodate. Allow to mix at high speed for about 10 minutes before using.

A method has been presented for the preparation of Bullard's and Harris' hematoxylin which employs no heat. The method is quicker and safer than those which require boiling.

References

1. 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, pg. 34, 1968.
2. Lillie, R. D.: Histopathologic Technic and Practical Histochemistry, 3rd ed., New York, McGraw-Hill Book Co., pg. 174, 1965.

Modified Alizarin Red-S Staining Method for Fetal Bones

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Solutions

3% Potassium Hydroxide

Potassium hydroxide . . . . 3.0 gm
Distilled water . . . . 100.0 ml

0.1% Alizarin Red-S

Alizarin Red-S . . . . 0.1 gm
Distilled water . . . . 100.0 ml

Procedure

1. After fixing a fresh specimen in Lillie's acetic-alcohol, formalin, or 95% alcohol, free it of skin, fat, viscera, and brain, and rinse in water.
2. Bleach in a mixture of 9 volumes of 95% alcohol to 1 volume of 3% aqueous hydrogen peroxide for 2-5 days. (Time depends on size of specimen.)
3. Wash in water 10 to 20 minutes.
4. Place specimen in 3% aqueous potassium hydroxide for 1 to 2 weeks. Change solutions every day. Remove when tissue begins to become transparent.
5. Transfer specimen to a freshly prepared 3% aqueous solution of potassium hydroxide to which has been added 10 drops of 0.1% Alizarin Red-S per 100 ml of solution. Transfer the specimen to fresh solution each day for 3 to 4 days, or until the bones have reached the desired intensity of red.
6. Place specimen in fresh potassium hydroxide for 2 days to clear soft tissues.
7. When the tissue is clear, place specimen in Methyl-Salicylate for 3 days, changing solution every day.
8. Store specimen in Methyl-Salicylate.

NOTE: Storing fresh Alizarin Red-S stained specimens in Methyl-Salicylate eliminates bubbles, prevents color from fading, and keeps them transparent.

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A New Technique for the Rapid Identification of Neoplastic Mast Cells

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The metachromatic characteristics of mast cell granules when stained with dyes such as toluidine blue are well recognized. Neoplastic mast cells, however, have variable metachromasia depending on their degree of cellular differentiation. This becomes quite important in the diagnosis and differentiation of mast cell tumors from morphologically similar but biologically benign tumors such as the cutaneous (juvenile) histiocytoma. Both of these tumors are common in dogs, and mast cell tumors are found in several animal species. We have recently found that the granules of neoplastic mast cells are argyrophilic – a characteristic not shared by their normal counterparts.[1] While the degree of cellular differentiation affects the metachromasia of granules, it does not affect the argyrophilia. Taking advantage of this latter characteristic, we have combined a methenamine silver[2] and toluidine blue technique for the rapid diagnosis of mast cell tumors regardless of their degree of cellular differentiation.

Fixation
10% phosphate buffered neutral formalin.

Microtomy
Paraffin sections cut 4 to 6 micra.

Solutions

5% Chromic Acid

Chromic acid . . . . 5.0 gm
Distilled water . . . . 100.0 ml

1% Sodium Bisulfite

Sodium bisulfite . . . . 1.0 gm
Distilled water . . . . 100.0 ml

5% Silver Nitrate

Silver nitrate. . . . . 5.0 gm
Distilled water . . . . 100.0 ml

5% Borax

Borax . . . . 5.0 gm
Distilled water . . . . 100.0 ml

3% Methenamine

Hexamethylenetetramine,
(Methenamine), USP . . . . 3.0 gm
Distilled water . . . . 100.0 ml

Methenamine-Silver Nitrate (stock)

5% silver nitrate solution. . . . . 5.0 ml
3% methenamine solution . . . . 100.0 ml
A white precipitate forms but immediately dissolves on shaking.

Methenamine-Silver Nitrate (working)

5% borax solution . . . . 2.0 ml
Distilled water . . . . 25.0 ml
Mix and add:
Methenamine-silver nitrate, stock solution, 25.0 ml

0.1% Gold Chloride

1% gold chloride . . . . 0.1 ml
Distilled water . . . . 100.0 ml

2% Sodium Thiosulfate

Sodium thiosulfate. . . . . 2.0 gm
Distilled water . . . . 100.0 ml

0.2% Light Green (stock)

Light green S.F. (yellowish). . . . . 0.2 gm
Distilled water . . . . 100.0 ml
Glacial acetic acid . . . . 0.1 ml

Light Green (working)

Light green (stock) . . . . 10.0 ml
Distilled water . . . . 50.0 ml

0.5% Toluidine Blue

Toluidine blue . . . . 0.5 gm
20% ethyl alcohol . . . . 100.0 ml
Combine. When dye is completely dissolved, filter. Stain improves on standing.

Staining Procedure

1. Decerate and hydrate in distilled water.
2. Oxidize in 5% chromic acid for 1 hour.
3. Wash in running tap water for a few seconds.
4. Rinse in 1% sodium bisulfite to remove any residual chromic acid.
5. Wash in running tap water for 10 minutes.
6. Wash with 3 or 4 changes of distilled water.
7. Place in working methenamine-silver nitrate solution in oven at 58° to 60°C for 30 to 60 minutes until sections turn yellowish-brown. Dip slide in distilled water and check for adequate silver impregnation with microscope.
8. Rinse in 6 changes of distilled water.
9. Tone in 0.1% gold chloride for 5 minutes.
10. Rinse in distilled water.
11. Remove any unreduced silver with 2% sodium thiosulfate for 2 minutes.
12. Wash thoroughly in tap water.
13. Stain in 0.5% toluidine blue for 1 minute.
14. Rinse in tap water.
15. Counterstain with working light green for 1 minute.
16. Dehydrate with 95% alcohol, absolute alcohol, clear with xylene and mount with resinous media.

Results

Granules in well-differentiated mast cells will take up toluidine blue in favor of the methenamine. Undifferentiated mast cells will readily take up the methenamine-silver.

We have examined sections prepared by this technique from 19 different tumors of the mast cell – histiocytoma type. By blind examination, a rapid and correct diagnosis was made in every case.

References

1. Lillie, R. D.: Histopathologic Technic and Practical Histochemistry, 3rd ed., McGraw-Hill Book Co., New York, p. 516, 1965.
2. Gomori, G.: A New Histochemical Test for Glycogen and Mucin, Tech. Bull. Reg. Med. Technol., 7:177-179, in Am. J. Clin. Path., 16, 1946.

Alarm System for Tissue Processors

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Mechanical and/or electrical failures of tissue processors result in hard, dried, and often ruined tissue specimens. There are

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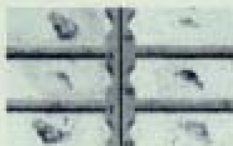

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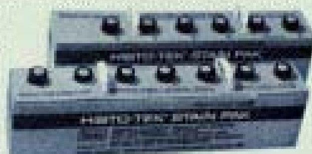

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many reasons why tissue processors "hang up". Many of the processors' "hang ups" are caused by: (1) forgetting to plug in a paraffin pot, (2) a basket not hooked securely, (3) electrical problems with the paraffin pot which allows paraffin to solidify and subsequently prevents tissue basket from submerging into the paraffin, (4) electric failure resulting in a hanging basket allowing tissue to dry or paraffin to solidify.

Because all of the above have occurred in our laboratory, we have developed a solution to the problem. An alarm system, set to operate within two minutes after processor malfunction, is connected with the hospital switchboard. On notification of the malfunction problem, the operator calls the individual designated. Those wanting additional information about this system may write to Mr. Meyers.

Identification of Vasectomies During Processing

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Vasa Deferentia are fixed in 10% formalin. After fixation, dip the right vas in mercurochrome and blot dry with a paper towel. The left vas is left unstained. Both can now be processed together. Label slide L & R for identification of specimens. Pick up section so that red stained vas is immediately below "R" marked on slide.

Special Stains Under Non-Special Conditions

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> "I do that stain only once or twice a year; why waste all those solutions each time I'm required to do it?"
>
> "I don't have triple-distilled or glass-distilled water, therefore I can't do special stains."
>
> "With everything else I have to do, who has time to acid-clean glassware?"

If any of the above sound familiar, then this article is for you. It might also be titled: "How to keep your sanity in a small laboratory."

Let's answer the last statement first. You don't have to acid-clean glassware. I haven't acid-cleaned glassware in years and have excellent results with special stains. Use common household bleach to clean your glassware. According to Dr. Raymond Gambino, Editorial Consultant for "Medical Laboratory Observer," a bleach is an under-used chemical in the laboratory. It effectively removes protein deposits from glassware as well as stains and many other contaminants. However, make sure the glassware is well rinsed after using a bleach.

The use of glass or triple-distilled water is not an absolute necessity except when the most exacting work is concerned. Our laboratory staff has used only de-ionized water from a commercial de-ionizer for the last several years. Some private laboratories have used distilled water, purchased from a grocery store, with good success.

In recent years, I have heard much talk about the "shelf-life" of staining solutions. I believe personal experience is the best teacher.

The stains at my laboratory (with a very few exceptions) are now five years old. They were made in bleach-cleaned glassware with distilled water purchased at a grocery store. I stained sections with my 5-year-old stains, and adjacent sections with fresh stains. Microscopic comparison by my pathologist indicated there wasn't much difference in the staining quality, except that one set of slides seemed a bit more delicate. They were the slides stained with the old solutions. A few of the chemical solutions, such as potassium metabisulfite, lost their potency and had to be replaced, but all the staining solutions, including the silver nitrate and gold chloride, remained good.

I have proved to myself that one *can* do special stains, and do them successfully, in a small laboratory under somewhat primitive conditions.

Getting the Most Out of Special Stain Solutions in the Small and Moderate-Sized Histopathology Laboratory

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Many articles are written about the problems of a large histopathology laboratory. The small and moderate-sized histopathology laboratory faces problems of obsolescence of special staining solutions not found in a large one.

One problem is the deterioration of chemicals and reagents used in compounding special staining solutions. This is wasteful because chemicals and reagents which have deteriorated with age must be discarded.

We have alleviated this problem by ordering chemicals in quantities which can be used within the known shelf-life of the chemical. Also, we date the labels on delivery so we know exactly when a chemical was received.

Some reagents cannot be ordered in small enough quantities to prevent deterioration from long storage. In this case, a check of other departments in the Clinical Laboratory may prove helpful since they may be able to supply the chemical you need.

A second problem for the small or moderate-sized laboratory, is deterioration of solutions compounded for special stains. Such solutions are not discarded because they have been used to their capacity, but rather because they no longer perform satisfactorily.

Several ways of solving this problem are suggested. First, make up smaller quantities of the solutions; e.g., 100 ml rather than 1000 ml. The staining solution formulas found in most staining manuals were compiled in large histopathology laboratories for large slide volumes. Reduce the quantities of the chemicals and solutions to the amount needed for your laboratory.

Second, keep your solutions in brown glass or polyethylene bottles. This will prevent, to a large degree, solution deterioration from exposure to light.

Third, and most important, make a label for each bottle with the following information: (1) Name of the solution. (2) Name of the special stain. (3) Initials of the person who made the solution. (4) Date the solution was made up. (5) Approximate date it should be replaced.

A final point to consider when evaluating a new stain is to make enough solution to try the procedure several times, allowing you to determine its quality. Do not make a large quantity which would have to be discarded if the procedure proves unacceptable. Additional quantities can be made if the procedure is found acceptable. Adopting these few suggestions can save time, space, and money for a small to moderate-sized histopathology laboratory, and help make the laboratory function more effectively.

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Editor's Corner

New Jersey Seminar

The New Jersey Society for Histotechnology will present its Second Annual Seminar and Workshop, November 1-3, 1974, in Atlantic City. For information contact: Robert M. Bingaman, 102 Maxwell Avenue, Atlantic City, New Jersey 08401.

ASCP Regional Training Program

Applications of chemistry to histotechnology, co-sponsored with National Society for Histotechnology, January 24-25, 1975, Lansing, Michigan. For information contact: ASCP Regional Training Program, 2100 West Harrison Street, Chicago, Illinois 60612.

Knife Sharpening Workshop

The Maryland State Society of Histology Technicians is presenting a "Knife Sharpening Workshop," on December 7, 1974, at the Good Samaritan Hospital, Baltimore, Maryland. In addition to the workshop, a guest speaker will discuss "Certification of Histology Laboratories." For information contact: Donald L. Meyers, Johns Hopkins Hospital, Baltimore, Maryland 21205.

Washington Conference

On July 15 and 16 in Washington, D.C., a conference of Temporal Bone Histology Technicians was held. The technicians present expressed interest in an organization of those involved in histology of the ear for exchange of information and ideas, and, it is hoped, to continue meeting at an annual conference. If you are interested in learning more about this group, or know of a laboratory that would like to participate, please advise Dr. John Bordley, National Temporal Bone Bank, Center of the Deafness Research Foundation, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Seminar for Continuing Education

Birmingham's annual Southeastern Histological Seminar for Continuing Education will be conducted December, 1974. For information: Mr. Ken Alexander, Baptist Medical Center-Montclair, 800 Montclair Road, Birmingham, Alabama 35213.

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.

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