METHOD OF THE HISTOCHEMICAL STAINS

&

DIAGNOSTIC APPLICATION

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ROCHESTER, NEW YORK

Second Web Edition (2009)

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Table of Contents

PREFACE

This manual is the main reference source used by the technical staff and personnel of the Department of Pathology and Laboratory Medicine at the University of Rochester Medical Center, Rochester, New York. The staining methods included in the manual are those which we have found to be reliable, and consistent. The format of these staining methods is patterned after the Manual of

Histologic Staining Methods of the Armed Forces Institute of Pathology, third Edition, 1968, The McGraw-Hill Book Company, Blakiston Division, New York, Edited by Lee G. Luna.

In this revised edition several of the staining procedures have been modified in order to improve the staining results and/or simplify the methods. Many of the methods were developed in our Special Stains Laboratory and have been published in various journals including The Journal of Histotechnology, Stain Technology, Biotechnic and Histochemistry, Laboratory Medicine and Theory and Practice of Histological Techniques. Research and development of new staining procedures and the improvement of existing ones has and will continue to be one of our objectives.

Several methods included in this manual utilized microwave irradiation. The use of microwave irradiation greatly reduces the time required to perform the methods without compromising the quality of the staining results. In fact in some of the methods, the staining results are improved by the use of microwave irradiation. Some investigators (Boon and Kok in Microwave Cookbook of Pathology) recommend the use of expensive microwave ovens to heat solutions more uniformly. We have used low-priced microwave ovens manufactured by General Electric and Litton with good staining results. Numerous other makes are available which will, with the proper adjustments in times and power settings, give equally good results.

Included in this manual is a section on the stability of dye and chemical solutions that are used in most of the staining procedures in this manual. These solutions are listed under the staining methods and in separate lists of dye and chemical solutions. A list of the dyes certified by the Biological Stain Commission and their Color Index numbers is provided. Also, the manual contains methods for removing stains from hands, clothing and glassware. There is also a list for the sources of controls for special stains and a list of where to order chemicals and dyes.

It is my sincere desire that all who use this manual will achieve a greater degree of success in the art of Special Stains.

Charles J. Churukian

Supervisor, Special Stains Laboratory

PURPOSE, PROCEDURES, QUALITY ASSURANCE, AND

SAFETY PROCEDURES IN THE SPECIAL STAINS LABORATORY

1. Purpose of the Special Stains Laboratory – The Special Stains Laboratory is a service-oriented laboratory where special stains and histochemical procedures are performed on surgical, autopsy, and other tissues for diagnostic and research purposes. Special stains are also performed on material received from cytology.

2. Procedure for Requesting Special Stains – Special stain requests can be made via electronic ordering or by using a Histopathology Special Stain Request form. Special stain requests on surgical and autopsy tissue sections must be countersigned by a senior staff member or by a senior resident. The request form must be properly and completely filled out and should indicate the diagnosis which the special stain is expected to help confirm.

3. Procedure for Processing Special Stain Requests – The request form should be taken to the main Histopathology Laboratory. The request form should be placed in the proper request container. If electronically ordered the order will be retrieved periodically throughout the day. Rush requests should be given directly to the laboratory supervisor or the technologist who is responsible for cutting special stains.

4. Procedure for Cutting Special Stain Requests – All special stain requests are cut in the main Histopathology Laboratory. Surgical requests take priority over autopsy and research requests. Sections for special stains are cut routinely on liver, kidney, bone marrow, and other biopsy tissues. After the special stain sections are cut, they are placed in a 60o-65o C. drying oven for a minimum of one hour. The cut off time for same day requests is 11:00 a.m. If a request is received before 12 noon you must verbally indicate if it is a rush for that day or can be microwaved dry.

5. Procedure for Handling Special Stain Slides and Requests – When the special stain slides and request forms are received in the Special Stains Laboratory, the slides are placed in a 60o-65o C. oven. The required information on the request forms is then recorded in the surgical, autopsy, cytology, or research record books.

6. Procedure for Performing Special Stains – Special stains are performed on liver, kidney, bone marrow, and other biopsies the same day they are received. All acid-fast bacilli, G.M.S., and bacterial requests are done the day they are received. Most of the surgical special stain requests are done on sections which have been cut by 11:00 a.m. Autopsy and research requests are done when corresponding surgical special stains are performed or when they can be worked into the daily work schedule. Cytology requests are done the same day they are received.

7. Procedure for Handling Rush Special Stain Requests – Requests for rush special stains are done on surgical and cytology specimens. Most of these rush requests are for microorganisms such as fungi, pneumocystis carinii and acid fast bacteria. Whenever a rush case is pending the supervisor or a senior technologist should be notified as soon as possible. When the slides on the rush case are received the special stains are done as soon as possible.

8. Procedure for Signing Out Special Stain Slides – The stained slides are checked with a microscope in order to determine if the quality of the stains are acceptable. Any stain which does not meet the standards of quality control will be repeated. All control slides are checked to make sure that they are positive. Any stain on which a control slide comes out negative will be repeated. A record is kept on all the stains that are repeated on the Special Stain Quality Assurance Monitoring Form. The slides that are signed out in the record book are delivered to the person who made the request. Rush requests are delivered when they are completed.

9. Procedure for Changing Solutions – The solutions (xylene, alcohols and distilled water) in the hydration and dehydration set-up are changed once a week (usually on Monday). The alcohols and xylene in coplin jars are changed as needed. The staining solutions in the coplin jars are changed on a regular basis. Those changed weekly are Weigert's iron hematoxylin, 2% sodium thiosulfate, 0.2% gold chloride, and 0.3% sodium borate. The others are changed every other week except for Masson trichrome, cardiac trichrome, amyloid, Zenker's, Schiff's, and Kreyberg's which are changed once a month. The Strong-Fast solutions are changed every other month. All the solutions in coplin jars and those in the solution cabinets and refrigerator are dated when changed or prepared, and the initials of the persons who changed or prepared them are indicated on the labels. Outdated solutions are discarded and fresh ones prepared. The shelf-life of dye and chemical solutions is indicated in the section of Special Staining solutions.

10. Procedure for Changing, Revising, Adding and Deleting Staining Methods –

1. New staining methods are added when the need or request for them arises. These methods are thoroughly tested on appropriate material and evaluated by the Laboratory Director and senior pathologists before they are implemented.

2. Old staining methods are deleted when they are no longer requested or are replaced by new or modified techniques. The deleted methods are filed with the reason, date of deletion and approval of the Laboratory Director.

Continuing research is conducted to improve staining methods and/or reduce the time required to perform the techniques. The modified staining methods are thoroughly tested to make sure that they perform at least as well as the original procedures. Many of these modified methods have been published in various journals.

11. Quality Assurance in the Special Stains Laboratory –

1. The solutions used in the various staining procedures are changed at regular intervals.

2. Only reagent grade or high quality chemicals are used in preparing solutions. Dyes which are certified by the Biological Stain Commission are used to prepare the staining solutions. Many chemicals and solutions are stored in the refrigerator at 3°-6°C. or in the freezer at -15° to -18° C.

3. When a new dye or chemical is used in a staining method, the staining results are compared with those obtained with the old dye or chemical. If they are satisfactory, the bottle is labeled with an S, the date used and the initials of the person who used it.

4. The temperature of the ovens, water baths and refrigerator are checked daily and recorded with date and initials of the person who checked them.

5. The technologists and trainees on special stains have their own copies of the staining methods. Some of these are revised and updated from time to time.

6. Only those staining methods which give consistently reliable results are used.

7. Whenever problems occur with any of the special stains, the cause of the problem is investigated and the necessary corrective measures taken. This is recorded on the Quality Assurance Monitoring Form.

8. Independent research to improve existing staining methods and develop new ones is maintained on a continuing basis.

9. Meetings are held with the Laboratory Director and members of the Biological Stain Commission regarding dyes, staining procedures, and problems with special stains.

10. Positive controls are included with stains for acid fast bacteria, aluminum, amyloid, argentaffin, argyrophil, bacteria, bile, calcium, central nervous system, chromaffin, copper, DNA, RNA, enzymes, fibrin, fungi, glycogen, hemoglobin, hepatitis B, surface antigen, iron (ferric), keratin, mast cells, reticulum, melanin, mercury, mucopolysaccharides, nerve fibers, neutral fats and lipids, Nissl substance, pancreatic islet cells, pituitary cells, pneumocystis carinii, and spirochetes. See pages 191A and 191B for a list of those stains that require controls.

11. Controls are not included for the following special stains: Gomori's trichrome, Jones' basement membrane, Masson's trichrome, Periodic Acid-Schiff (PAS), and Verhoeff's elastic. The reason for not using controls for these stains is that they all contain the substance for which they are being stained which perform as internal controls. Therefore, when these stains are viewed with a microscope it is easy to determine if they are properly stained.

12. When the special stain slides are completed they are viewed by the technologist or trainee and the supervisor or a senior staff member with a dual-viewing microscope. The following purposes are achieved by this joint viewing:

a. The slides are critically evaluated as to the quality of the special stains to determine if they meet the standards of acceptability. If they are unacceptable the stains are repeated.

b. Control slides are determined to be either positive or negative. After the stains and controls have been evaluated a record of the results is recorded in the Special Stains Record Book as follows: S = satisfactory for those cases where the control is internal, * = control positive and delivered with the case, and 3= the control was positive but was delivered with another case stained at the same time.

c. Some basic histology is taught.

d. Some histopathology is taught.

12. Laboratory Safety Procedures in the Special Stains Laboratory –

1. All workers are instructed in proper laboratory safety practices as required by the Occupational Safety and Health Administration (OSHA), New York State, and other agencies.

2. All workers are instructed in the procedure for handling biohazardous material as described in a memo dated December 1996.

3. Medical Emergencies

a. Acid or chemicals splashed into eyes. Flush with water using the eye wash located next to the refrigerator. Get medical treatment in the Emergency Department or University Health.

b. Glass in the eye. Do not rub the eye. Go immediately to the Emergency Department.

c. For all other medical emergency situations call Security at X13.

4. Chemicals, Dyes, and Solutions

a. Bottles containing chemicals, dyes, and solutions are clearly labeled.

b. Material Safety Data Sheets on chemicals and dyes are kept in the laboratory.

c. Reagents and solutions must contain a label clearly staining the contents with the date and initials of the person who prepared them and the expiration date.

d. Solutions must be pipetted by automatic pipetting devices. Mouth pipetting is prohibited.

e. Acids are stored on the lower shelf in the Acid Storage Cabinet.

f. Acids, bases, and chemicals that will react with one another are stored separately.

g. Flammable liquids are stored in the Flammable Storage Cabinet located in room G-5405A.

h. Flammable liquids or materials are never stored in the refrigerator.

i. Disposal of chemical, flammable liquids, and waste solutions is accomplished through the Hazardous Waste Management Unit.

j. Gloves and lab coats are required when weighing chemicals and dyes, in the use of acids, and when preparing solutions.

k. The fume hood is required when working with acids and volatile solutions.

l. Trace amounts of acid, chemical, and dye solutions may be flushed down the drain using large amounts of water.

5. Acid or Chemical Spills

a. For large spills of acid or chemicals, call Health and Safety at X53241 and evacuate the area.

b. For small spills of acid treat with sodium bicarbonate (located under the test tube rack by the window) and then mop up with paper towels. Call the Hazardous Waste Management Unit at X52056 for pickup and disposal.

6. Smoking and Open Flames

a. Smoking is not allowed in the laboratory and a No Smoking sign is posted.

b. Open flames are never used or allowed.

7. Food and Beverages

a. Food or beverages are never stored in the refrigerator.

b. The use or storage of food or beverages in the laboratory is prohibited.

8. Fume Hoods

a. Two fume hoods are available and are used daily. Coverglassing of slides is done under one and the other houses the solutions (xylene and alcohol) used in the hydration and dehydration of the slides.

b. The fume hood housing the solutions is also used when working with acids and volatile liquids.

9. Broken Glassware

a. Broken glassware must never be placed in the regular trash cans.

b. Place broken glassware in the Glass Disposal Can.

10. Biohazardous Material

a. Gloves are required when handling biohazardous or unfixed slides.

b. Gloves and containers used in the transport and handling of biohazardous slides may only be disposed of in the Biohazardous Waste Can in room G-5405A.

c. For additional information see memo dated January 1992 – Procedure for Handling Biohazardous Material in the Special Stains Laboratory.

11. Maintenance of Equipment

a. All electrical equipment is checked annually by Engineering.

b. Fume hoods are tested annually for adequate face velocity by Medical Engineering.

c. Microwave ovens are checked annually for microwave leakage.

d. Balances are cleaned and adjusted annually.

e. Microscopes are cleaned and adjusted annually.

f. The temperatures of the refrigerator, waterbath, and ovens are checked and recorded daily with the date and initials of the person who checked them.

LIST OF STAINS FOR BACTERIA

MICROWAVE ZIEHL-NEELSEN METHOD FOR ACID-FAST BACTERIA

MICROWAVE MODIFICATION OF TRUANTS

GRIDLEYS METHOD FOR ENDAMOEBA HISTOLYTICA

MODIFIED BROWN AND BRENN METHOD

GRAM METHOD FOR MICROORGANISMS IN SMEARS

CRESYL VIOLET METHOD FOR HELICOBACTER

MICROWAVE WOLBACHS GIEMSA METHOD

GIEMSA METHOD FOR BONE MARROW SMEARS

RAPID GOMORIS METHENAMINE-SILVER NITRATE METHOD

MICROWAVE AMMONIACAL SILVER METHOD

MICROWAVE AMMONIACAL SILVER METHOD-UNFIXED

AMMONIACAL SILVER METHOD FOR FUNGI IN UNDECALCIFIED BONE MARROW SECTIONS

MICROWAVE MODIFICATION OF THE WARTHIN-STARRY METHOD FOR BACTERIA

MICROWAVE STEINER METHOD FOR SPIROCHETES AND BACTERIA

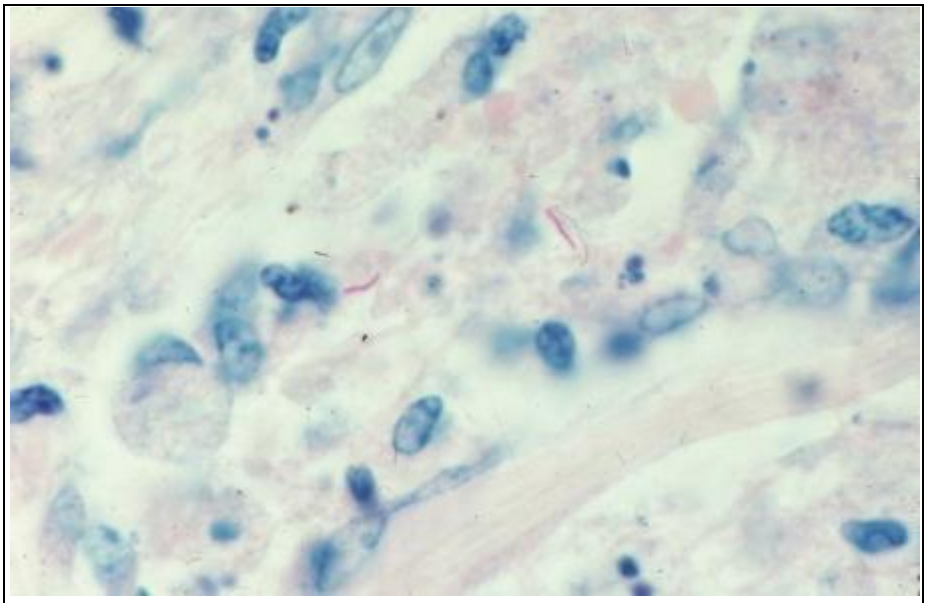
LENDRUMS METHOD FOR INCLUSION BODIES

MODIFIED ZIEHL-NEELSEN METHOD FOR CRYPOSPORIDIUM OOCYST

Charles Churukian MICROWAVE ZIEHL-NEELSEN METHOD FOR ACID-FAST BACTERIA

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Ziehl-Neelsen method for Mycobacteria Avium-intracellular.

Diagnostic Application:

Diagnosis for mycobacterial infection

Highlights lipofuscin's in the liver for distiguishing them from hemosiderin granules.

Material and Solutions:

FIXATION: 10% buffered neutral formalin

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Carbol Fuchsin Solution

Basic fuchsin, C.I. 42510 ---------- - 0.8 gm
Isopropyl alcohol ---------- 14.0 ml
Phenol, liquid, approximately 90% ---------- 8.5 ml
Distilled water ---------- 140.0 ml

Dissolve the basic fuchsin in the isopropyl alcohol and the phenol in the distilled water. Mix the two solutions. Add two drops of 25% Tween 20 to 45 ml of this solution and mix well. Filter through Whatman #1 filter paper before use.

25% Tween 20

TWEEN 20 (Polysorbate 20) ---------- - 25.0 ml

Distilled water ---------- 75.0 ml

0.5% Acid Alcohol Solution

Alcohol, 70 % ---------- 1000.0 ml
Hydrochloric acid, concentrated ---------- 5.0 ml

Methylene Blue Solution

Methylene blue, C.I. 52015 ---------- 0.25 gm
Distilled water ---------- 100.00 ml
Acetic acid, glacial ---------- 1.00 ml

Staining Procedure:

- Deparaffinize and hydrate to distilled water
- Place in 45 ml of carbol fuchsin in a glass Coplin jar and microwave at power level 1 (60W) for 1 1/2 minutes. Dip the slides up and down several times and allow them to remain in the warm solution for 5 minutes.
- Wash well in running water to remove excess stain.
- Decolorize with acid alcohol until sections are pale pink.
- Wash in running water for 1 minute and rinse in two changes of distilled water.
- Counterstain with methylene blue solution for 15 seconds.
- Rinse in three changes of distilled water.
- Dehydrate in graded alcohols.
- Clear in three or four changes of xylene.
- Mount with synthetic resin.

Staining Results:

Acid fast bacilli including M.avium intracellulare ---------- red
Erythrocytes ---------- pink
Mast cells ---------- blue
Nuclei ---------- blue

Comment:

Ziehl-Neelsen or similar carbol fuchsin solutions are usually prepared with basic fuchsin. The dye sold as "basic fuchsin" is usually a mixture of pararosanilin (C.I. 42500), rosanilin (C.I. 42510),

magenta II and new fuchsin (C.I. 42520). Carbol fuchsin prepared with any of these dyes (or mixtures thereof) stains acid fast bacteria well with the above procedure.

The addition of Tween 20 to the carbol fuchsin solution causes the acid fast bacteria to stain a more intense red and reduces background staining.

In developing this staining method we exposed the carbol fuchsin to varying amounts of microwave radiation. We observed that optimal staining results are obtained when the solution is heated as described above. The final temperature of the solution will be about 46˚ C. However, the temperature of the solution is not uniform throughout the Coplin jar. The top portion of the solution is warmer than that near the bottom of the Coplin jar. Therefore, in order to equalize the temperature of the solution the slides are dipped up and down. This assures uniformity of staining throughout the tissue sections.

The stain may be performed at room temperature by staining the sections for 15 minutes in the carbol fuchsin solution.

References:

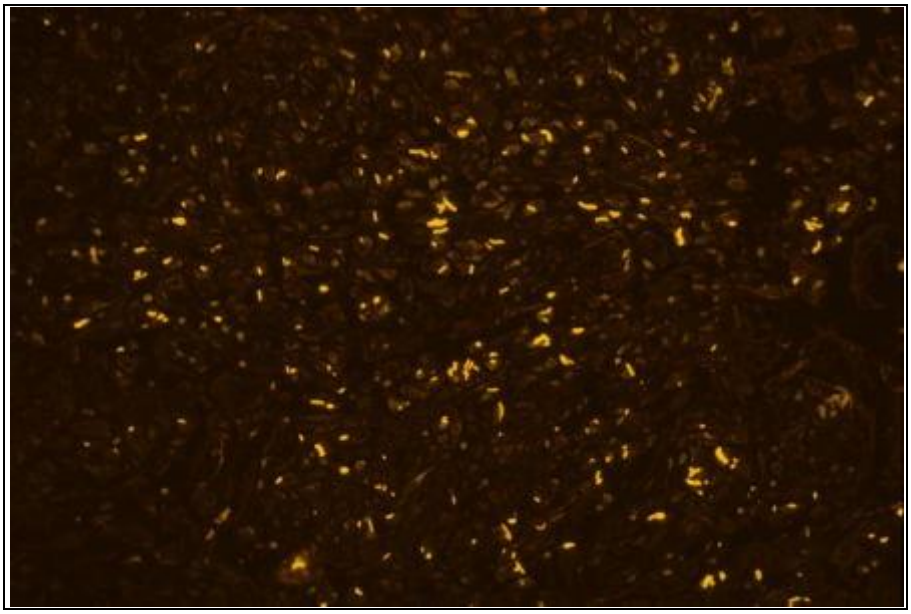
Lillie, R.D., and Fullmer, H.M.: Histopathologic Technic and Practical Histochemistry, New York, McGraw-Hill, 1976, pp.734-737.

MICROWAVE MODIFICATION OF TRUANTS

FLUORESCENT METHOD FOR ACID-FAST BACTERIA

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Fluorescent method for acid fast bacteria

(Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Auramine-Rhodamine Solution

Auramine 0, C.I. 41000 ---------- 0.45 gm

Rhodamine B, C.I. 45170 ---------- 0.03 gm

Glycerine ---------- 90.00 ml

Phenol, liquid, approximately 90% ---------- 13.20 ml

Ethyl alcohol ---------- 15.00 ml

Distilled water ---------- 60.00 ml

Combine the liquids in a 250 ml flask and place on a magnetic stirrer. Add the dyes to the solution, apply gentle heat, and allow to mix for about 30 minutes. Filter through #4 Whatman filter paper, while warm, before use.

0.5% Acid Alcohol Solution

Alcohol, 70% ---------- 1000.0 ml

Hydrochloric acid, concentrated ---------- ---- 5.0 ml

0.3% Eriochrome Black T

Eriochrome black T, C.I. 14645 ---------- 0.3 gm

Distilled water ---------- 100.0 ml

Staining Procedure:

Use Positive and negative control slides.

1. Deparaffinize and hydrate to distilled water.

2. Place in 45 ml of auramine-rhodamine solution in a glass Coplin jar. Place in a microwave oven and microwave at power level 1 (60W) for 3 minutes. Agitate for about 15 seconds and allow to set in the warm solution for 2 minutes.

3. Rinse with three changes of distilled water.

4. Differentiate in two changes of acid alcohol, 1 minute in each change.

5. Rinse in four changes of distilled water.

6. Place in 0.3% eriochrome black T solution for 20 seconds.

7. Rinse with three changes of distilled water.

8. Stand slide on end and thoroughly air dry.

9. Dip in xylene and mount with synthetic resin.

Staining Results:

Acid fast bacteria, including leprosy and M. avium intracellulare, will fluoresce orange-yellow on a dark background.

Comment:

Always use a positive and a negative control slide. The negative control slide is important because tap water, and on occasion staining solutions, may contain acid fast bacilli. Look at the slide away from the tissue section for such contaminants. False positive staining in our laboratory was found on culture to be due to Runyon Group II scotochromogenic acid fast bacteria present in tap water.

Remember that this stain is not specific for M. tuberculosis; it will stain several types of acid fast organisms. Also, it does not appear to be affected by the state of viability of the organisms. Dead or dying organisms will stain.

The use of heat is necessary to obtain satisfactory staining results. Most texts recommend staining for 10 minutes in the auramine-rhodamine solution, which has been preheated to 60˚ C. For many years we did the staining in a 58˚ C water bath for 30 minutes with good results. This microwave oven method saves time and produces consistently good staining results.

Usually a plastic Coplin jar is used when staining in the microwave oven. This is because there is a tendency for glass Coplin jars to break when solutions in them are rapidly heated with microwaves. However, when the lowest power setting is used the solution heats up slowly and there is no danger of the Coplin jar cracking. The main reason for using a glass Coplin jar in this method is that it is easier to clean than a plastic Coplin jar. Cleaning is accomplished with the acid alcohol solution.

References:

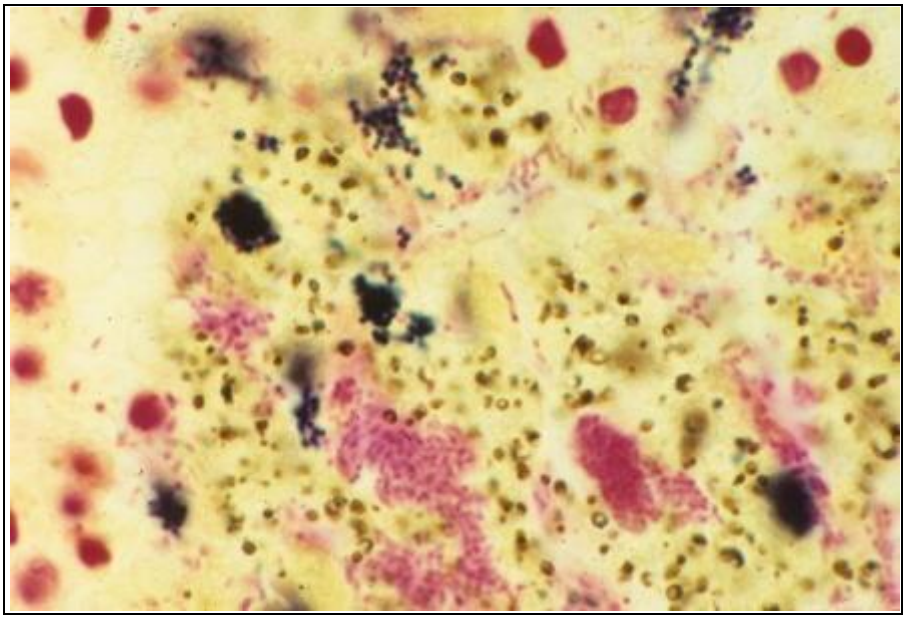
Truant, J.P.: Fluorescence microscopy of tubercle bacilli stained with auramine and rhodamine. Henry Ford Hospital Med. Bull. 10:287-296, 1962.

Churukian, C.J.: Demonstration of mycobacteria: a brief review with special emphasis on fluorochrome staining. J. Histotechnol. 14:117-121, 1991.

MODIFIED BROWN AND BRENN METHOD FOR GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Brenn Method for gram positive and gram negative bacteria

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Hucker-Conn Solution, Modified

Crystal violet, C.I. 42555	0.8 gm
Ethyl alcohol, absolute	8.0 ml
Ammonium oxalate	4.0 gm
Distilled water	392.0 ml

Dissolve the crystal violet in the ethyl alcohol, and the ammonium oxalate in the distilled water. Add the ammonium oxalate to the crystal violet and mix. Filter through Whatman #1 filter paper before use.

Modified Grams Iodine Solution

Iodine	2.0 gm
Potassium iodide	4.0 gm
Distilled water	400.0 ml

Dissolve the potassium iodide in a little of the distilled water, add the iodine and dissolve. Add the remainder of the distilled water.

Ethyl Alcohol-Acetone Solution

Ethyl alcohol, absolute ---	50.0 ml
Acetone	50.0 ml

0.5% Basic Fuchsin Solution (Stock)

Basic fuchsin or pararosanilin, (C.I. 42500) ---------- 0.5 gm

Distilled water ---------- 100.0 ml

Dissolve with the aid of heat and a magnetic stirrer.

Basic Fuchsin Solution (Working)

Basic fuchsin solution (stock) ---------- --- 5.0 ml

Distilled water ---------- 35.0 ml

Picric Acid-Acetone Solution

Picric acid ---------- 0.05 gm

Acetone ---------- 100.0 ml

Acetone- Xylene solution

Acetone ---------- 50.0 ml

Xylene ---------- 50.0 ml

Staining Procedure:

Use both positive and negative control slides.

1. Deparaffinize and hydrate to distilled water.
2. Hucker-Conn solution for 2 minutes.
3. Rinse quickly with two changes of distilled water.
4. Gram's iodine solution for 1 minute.

5. Rinse quickly with two changes of distilled water. Blot the slide but not the tissue section.

6. Decolorize by dipping in the ethyl alcohol-acetone solution until the blue color stops coming off.

7. Rinse in two changes of distilled water, several dips in each.
8. Working basic fuchsin solution for 30 seconds.

9. Rinse in two changes of distilled water, three dips in each. Blot the slide but not the tissue section.
10. Place in acetone for 5 seconds.

11. Differentiate and decolorize by placing in picric acid-acetone for 10 seconds.

12. Dip several times in acetone-xylene.

13. Clear in xylene, four changes.

14. Mount with synthetic resin.

Staining Results:

Gram positive bacteria ---------- blue

Gram negative bacteria ---------- red

Filaments of Nocardia and Actinomyces ---------- blue or red

Nuclei ---------- red

Other tissue elements ---------- yellow

Comment:

This staining method is a modification of Taylors modification of the Brown-Brenn stain for bacteria. Not only does it reduce the staining time, but it also eliminates the use of ether; a laboratory safety hazard.

The tissue section should not be allowed to dry out at any point during the staining procedure. If the section is allowed to dry out after treatment with the modified Grams iodine, decolorization with alcohol-acetone will be difficult and inadequate. If the section is allowed to dry out after staining with basic fuchsin, differentiation with picric acid-acetone will be inadequate. Nothing can be done to remedy these problems.

References:

Brown, J.H., and Brenn, L.: A method for the differential staining of Gram-positive and Gram-negative bacteria in tissue sections. Bull. Johns Hopkins Hosp. 48:69-73, 1931.

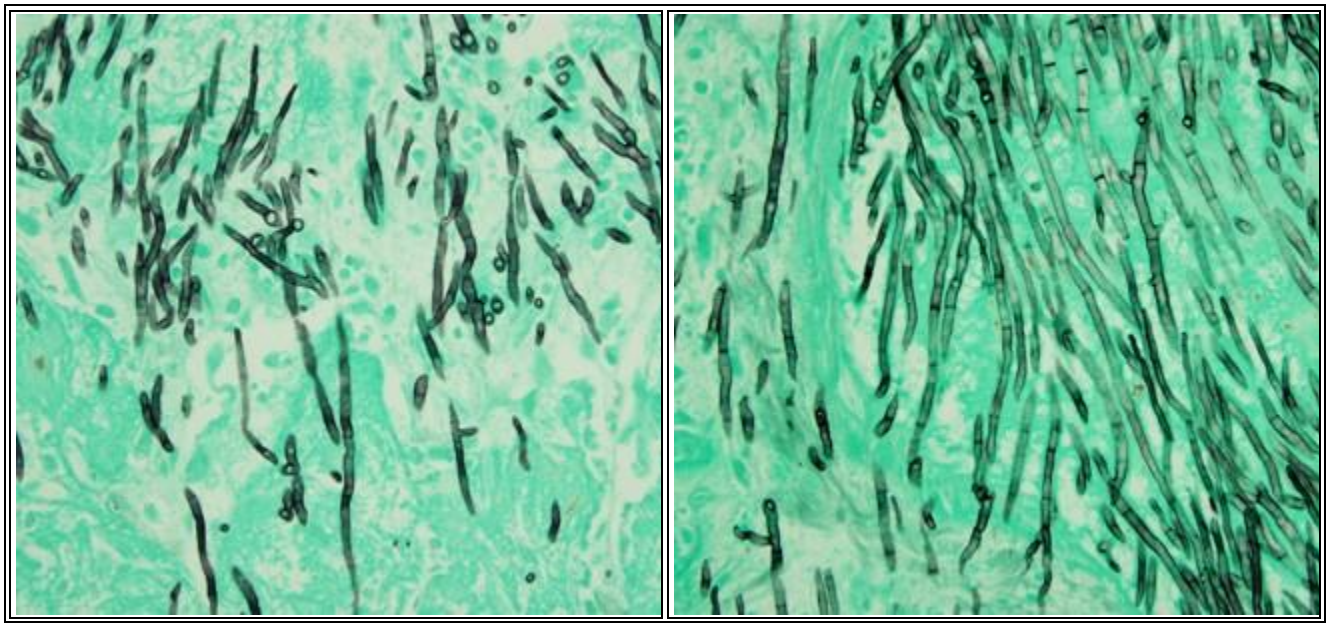
Taylor, R.D.: Modification of the Brown-Brenn Gram stain. Amer. J. Clin. Path. 46: 472-474, 1966.

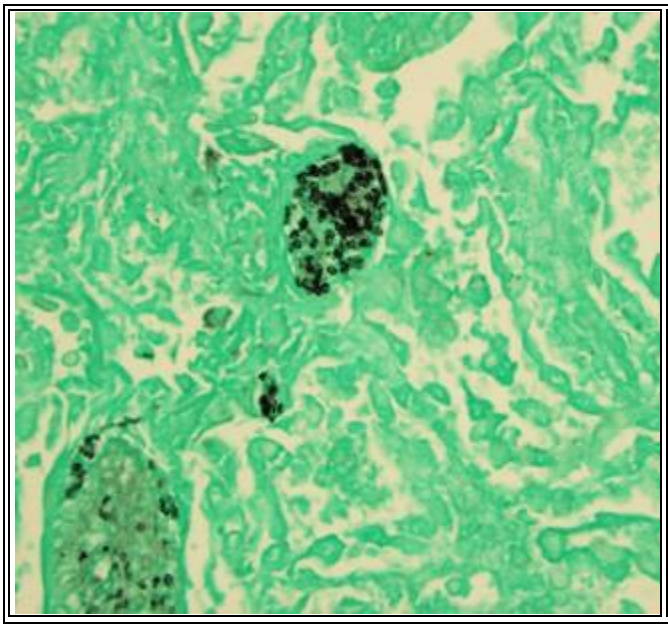
Churukian, C.J., and Schenk, E.A.: A method of demonstrating Gram-positive and Gram-negative bacteria. J. Histotechnology 5:127-128, 1982.

MICROWAVE AMMONIACAL SILVER METHOD FOR DEMONSTRATING FUNGI AND PNEUMOCYSTIS CARINII

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:





Images A-C Courtesy of Dr. Zhenhong Qu

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin or frozen sections cut at 5 µm.
Smears and touch preparations may be done.

SOLUTIONS:

Modified Chromic Acid Solution

Chromium trioxide ---------- 20.0 gm

Potassium dichromate ---------- 20.0 gm
Distilled water ---------- 360.0 ml

Sulfuric Acid ---------- 40.0 ml

1% Sodium Bisulfite Solution

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

Ammoniacal Silver Solution

To 10 ml of 10% silver nitrate, add 5.0 ml of 4% lithium hydroxide, monohydrate. Then add concentrated ammonium hydroxide, drop by drop with constant shaking, until the precipitate just dissolves. Make up the solution to 1000 ml with distilled water and store in a refrigerator at 3° C. This solution is stable for about one month.

0.2% Gold Chloride Solution

Gold chloride	0.2 gm
Distilled water	100.0 ml

2% Sodium Thiosulfate Solution

Sodium thiosulfate	2.0 gm
Distilled water	100.0 ml

Fast Green, Stock Solution

Fast Green FCF C.I. 42053	0.2 gm
Distilled water	100.0 ml
Acetic acid, glacial	0.2 ml

Fast Green Solution, Working Solution

Fast green, stock solution	100.0 ml
Distilled water	35.0 ml

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Place in 45 ml of modified chromic acid solution for 5 minutes.

3. Rinse with three changes of distilled water.
4. Treat with 1% sodium bisulfite for 30 seconds.
5. Wash in running water for 30 seconds.
6. Rinse in three changes of distilled water.
7. Microwave 40 ml of refrigerated cold ammoniacal silver in a plastic Coplin jar at

power level 7 (420W) for I minute. Immediately place the slides in this hot solution, return to the microwave oven and microwave at power level 2 (120W) for 30 seconds. Dip the slides up and down several times and allow them to remain in the hot solution for 1-2 minutes or until the sections turn a light brown.

8. Rinse in four changes of distilled water.
9. Tone in 0.2% gold chloride for 30 seconds.

10. Rinse in two changes of distilled water.
11. Place in 2% sodium thiosulfate for 30 seconds.
12. Rinse in four changes of distilled water.
13. Counterstain with working fast green solution for 30 seconds.
14. Rinse in three changes of distilled water.
15. Dehydrate through graded alcohols.
16. Clear in three or four changes of xylene and mount with synthetic resin.

Staining Results:

Fungi and pneumocystis carinii ---------- sharply delineated in black

Mucin and glycogen ---------- black

Melanin ---------- black

Background ---------- green

Comment:

We prefer this microwave ammoniacal silver method over the microwave methenamine silver procedure for the following reasons: 1) Dilute ammoniacal silver is much more stable at high temperatures (above 80° C) than methenamine silver. 2) When methenamine silver is heated to high temperatures the solution rapidly breaks down and sometimes produces a deposit of silver on the slides and the Coplin jar. This never occurs when dilute ammoniacal silver is heated to temperatures even above 90° C. 3) Dilute ammoniacal silver is ready for immediate use and does not need to be diluted and buffered like methenamine silver.

The volume and temperature of the ammoniacal silver used in this method is important. We remove the ammoniacal silver form the refrigerator and pour 40 ml of the cold solution into a plastic Coplin jar immediately before use. The temperature of the ammoniacal silver is 3-6° C before and about 85° C after the second exposure to microwave irradiation.

When heating a solution with microwave irradiation the top portion of the solution is warmer by 10-15° C than that near the bottom of the Coplin jar. Therefore, in order to equalize the temperature of the solutions the slides are dipped up and down in steps #2 and #7 of the staining procedure. This assures uniformity of staining throughout the tissue sections.

We have found that silver nitrate has a shelf-life of several years when stored in refrigerator. Chromic acid is a strong oxidizing agent and should be handled carefully with gloves and it is recommended that safety glasses be worn.

It is recommended that gloves and safety glasses be worn when preparing ammoniacal silver solutions because of solution instability during preparation.

References:

Gomori, G.: Silver impregnation of reticulum in paraffin sections. Amer. J. Clin. Path. 13:993-1002, 1937.

Churukian, C.J. and Schenk, E.A.: Rapid Grocotts methenamine-silver nitrate method tor fungi and Pneumocystis carinii. Amer. J. Clin. Path. 68:427-428, 1977.

Churukian, C.J. and Schenk E.A.: Dilute ammoniacal silver - a substitute for methenamine silver for demonstrating Pneumocystis carinii and fungi. Lab Med. 17:87-90, 1986.

AMMONIACAL SILVER METHOD FOR FUNGI

IN UNDECALCIFIED BONE MARROW SECTIONS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Undecalcified glycol methacrylate embedded bone marrow sections cut at 4 µm.

SOLUTIONS:

4% Periodic Acid

Periodic acid, H5IO6	4.0 gm
Distilled water	100.0 ml

Ammoniacal Silver Solution

To 10 ml of 10% silver nitrate, add 5.0 ml of 4% lithium hydroxide, monohydrate. Then add concentrated ammonium hydroxide, drop by

drop with constant shaking, until the precipitate just dissolves. Make up the solution to 1000 ml with distilled water and store in a refrigerator at

3-6° C. This solution is stable for about one month.

0.2% Gold Chloride

Gold chloride	0.2 gm
Distilled water	100.0 ml

2% Sodium Thiosulfate

Sodium thiosulfate	2.0 gm
Distilled water	100.0 ml

Fast Green, Stock Solution

Fast green FCF, C.I. 42053	0.2 gm
Distilled water	10.0 ml
Acetic acid	0.2 ml

Fast Green, Working Solution

Fast green, stock solution	10.0 ml
Distilled water	35.0 ml

Staining Procedure:

- Use control slide.

1. Place in 4% periodic acid for 5 minutes.

2. Rinse in five changes of distilled water.

3. Pour 40 ml of ammoniacal silver in a glass Coplin jar and allow to warm to near

room temperature. Place slides in the solution and set the Coplin jar in a 43° C waterbath for 3 minutes, then transfer to a 58° C waterbath for 25-35 minutes or until the sections appear light brown. Check the slides after 25 minutes.

4. Rinse in five changes of distilled water.

5. Place slides on a hot plate at 60-65° C for 5 minutes. This step is necessary to

prevent the loss of sections in the proceeding steps.

6. Tone in 0.2% gold chloride for 30 seconds.
7. Rinse in two changes of distilled water.
8. Place in 2% sodium thiosulfate for 30 seconds.
9. Rinse in four changes of distilled water.
10. Counterstain with working fast green solution for 3 minutes.
11. Rinse in three changes of distilled water.
12. Dehydrate through graded alcohols.
13. Clear in three or four changes of xylene and mount with synthetic resin.

Staining Results:

Fungi---------- sharply delineated in grey to black

Background ---------- green

Comment:

In the staining method of Castro and Maynard for plastic embedded sections chromic acid is used as the oxidizing agent. We found that this causes the plastic to stain varying shades of grey and brown but, even so, the fungi stain a rather intense greyish brown. However, because the plastic is stained the fungi are somewhat difficult to locate. Oxidizing the sections with periodic acid prevents the plastic from staining, but the fungi are less intensely stained than when they are oxidized with chromic acid. Even so, the fungi are stained well enough to be easily located and identified.

References:

Churukian, C.J., and Schenk, E.A.: Dilute ammoniacal silver as a substitute for methenamine silver to demonstrate pneumocystis carinii and fungi. Lab. Med. 17:87-90, 1986.

Churukian, C.J.: A more stable ammoniacal silver using lithium hydroxide. Lab. Med. 17:260, 1986.

Churukian, C.J., and Schenk, E.A.: Staining pneumocystis carinii and fungi in unfixed specimens with ammoniacal silver using a microwave oven. J. H~stotechnol. I 1:19-21, 1988.

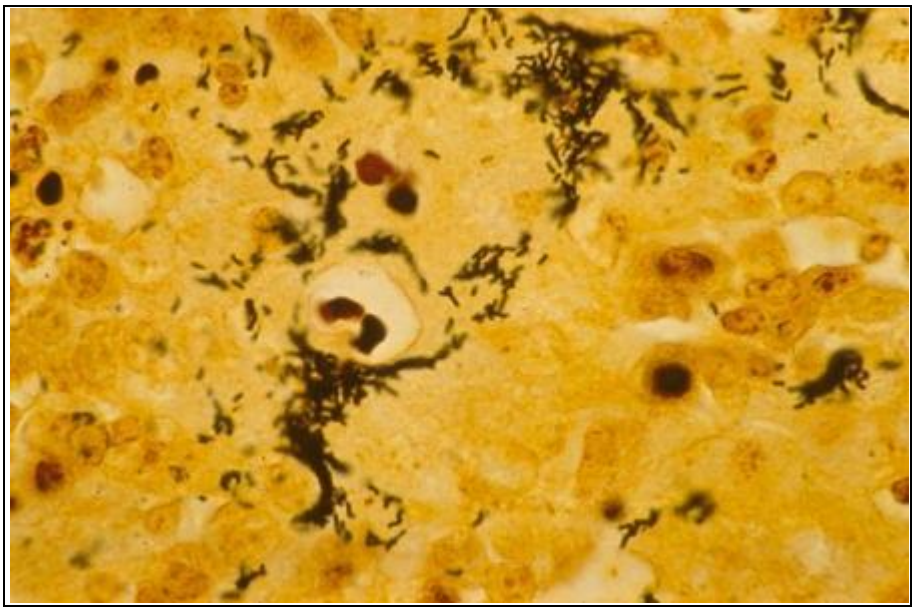
Castro, M.D., and Maynard, J.H.: Routine and special staining techniques in glycol methacrylate. Presented at the 1988 NSH Symposium/Convention in Louisville, Kentucky.

Churukian, C.J: Ammoniacal silver method for fungi in undecalcified bone marrow sections. Histologic 20:163-164, 1990.

MICROWAVE MODIFICATION OF THE WARTHIN-STARRY METHOD FOR BACTERIA

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Warthin-Starry Method for Spirochetes.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin. Do not use dichromate fixatives.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Glycine-Acetic Acid (Stock)

Glycine ---------- 1.2 gm
Acetic acid, glacial ---------- ---------- 0.3 ml
Distilled water ---------- 100.0 ml

The pH of this solution is approximately 3.5. Store in a refrigerator at 3-6o C.

Glycine-Acetic Acid (Working)

Glycine-acetic acid (stock) ---------- 3.0 ml

Distilled water ---------- 300.0 ml

The pH of this solution is approximately 3.5.

0.25% Silver Nitrate Solution

Silver nitrate ---------- 0.1 gm
Glycine-acetic acid, working solution ---------- ----- 40.0 ml

Store in a refrigerator at 3-6° C.

2% Silver Nitrate Solution

Silver nitrate---------- 0.2 ml
Glycine-acetic acid, working solution ---------- ---- 10.0 ml

Prepare fresh.

0.2 % Hydroquinone Solution

Hydroquinone ---------- 0.2 gm
Glycine-acetic acid, working solution ---------- ----- 10.0 ml

4% Gelatin Solution

Gelatin ---------- 1.0 gm
Glycine-acetic acid, working solution ---------- ------ 25.0 ml

Dissolve by placing on a magnetic stirrer and applying gentle heat.

0.2% Hydroquinone Solution

Hydroquinone ---------- 0.02 gm

Glycine-acetic acid, working solution ---------- 10.00 ml

Place the 2% silver nitrate, 4% gelatin, and the 0.2% hydroquinone solutions in separate flasks in a 55°-58° C

oven. These solutions and the 0.25% silver nitrate are prepared immediately before use.

Silver Nitrate-Gelatin-Hydroquinone Developer

Silver nitrate, 2% ---------- 10.0 ml

Gelatin, 4% ---------- 25.0 ml

Hydroquinone, 0.2% ---------- 10.0 ml

Combine in order given making certain the solutions are mixed well after each addition. Prepare immediately

before use.

0.1% Tartarazine Solution

Tartarazine, C.I. 19140 ---------- 0.1 gm
Distilled water ---------- 100.0 ml
Acetic acid ---------- 0.2 ml

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to working glycine-acetic acid solution.

2. Place in 40 ml of 0.25% silver nitrate in a plastic Coplin jar, with the cap loosely applied, and microwave at power level 6 (360W) for 40 seconds. Gently agitate the Coplin jar for about 15 seconds. Microwave again at power level 6 for 20 seconds. Gently agitate the Coplin jar again for about 15 seconds. Allow the slides to remain in the hot solution (75°-80°C) for 2 minutes.

3. Place in freshly prepared silver nitrate-gelatin-hydroquinone developer in a plastic Coplin jar, with the cap loosely applied. Place in a microwave oven and microwave at power level 1 for one minute. Gently agitate the Coplin jar for about 15 seconds. Allow the slides to remain in the solution until the sections appear gray. This usually takes about 1 minute but may take longer.

4. Wash quickly and thoroughly in hot running water.
5. Rinse in two changes of distilled water.
6. Counterstain with tartarazine solution for 15 seconds.
7. Rinse in two changes of distilled water.
8. Dehydrate in graded alcohols.
9. Clear in three or four changes of xylene.
10. Mount with synthetic resin.

Staining Results:

Alipia felis (cat-scratch bacillus)---------- black
Legionnaires bacteria---------- black
Klebsiella---------- brown to black

Helicobacter (pylori) ---------- black

Nuclei ---------- brown-black

Background---------- yellow

Comment:

We prefer this microwave oven method over the regular Warthin-Starry, Deterle and Steiner methods for demonstrating bacteria, namely, because it is easier to do, less time consuming, and gives equally good if not superior results. Remember that all bacteria are stained by these silver methods, they are not specific for Legionella.

The original Warthin-Starry method required the use of 1% silver nitrate to impregnate the tissues prior to placing them in the developer. We found that a 0.1% silver nitrate solution works equally well with less background staining.

The type of gelatin used in this method is important in obtaining satisfactory results. We tried samples obtained from different suppliers including types I-IV from the Sigma Chemical Co., types A and B from Fisher Scientific Products. The results were satisfactory with al1 of these samples except Sigma type IV and Fisher type B. However, we prefer using Fisher type A gelatin because when this gelatin is used there is less background staining.

The use of xylene substitutes in this method is not recommended. We tried Hemo-De (obtained from Fisher Scientific) as the clearing agent and found that the stain faded within 24 hours to the extent that some bacteria and spirochetes were no longer visible.

References:

Kerr, D.A.: Improved Warthin-Starry method of staining spirochetes in tissue sections. Amer. J. Clin. Pathol. 8:63-67, 1938.

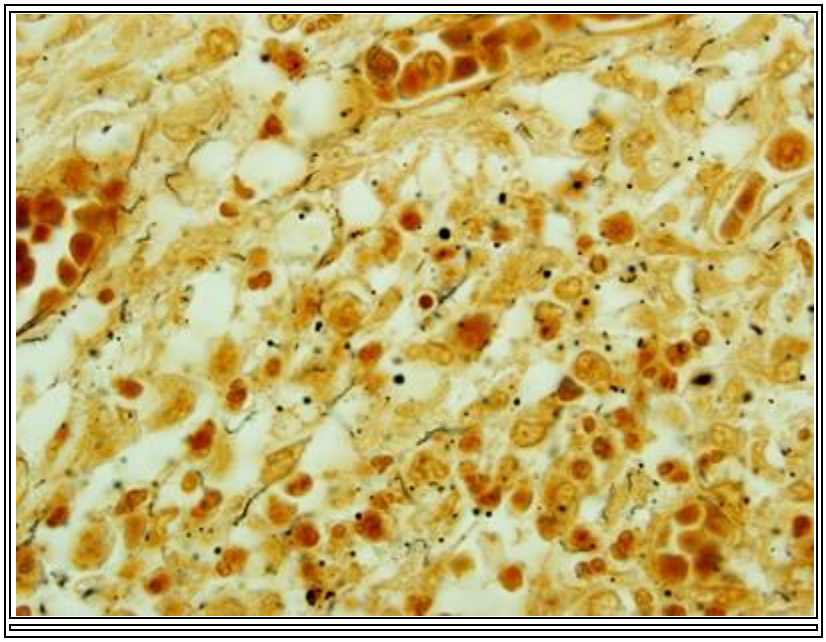
Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 238-240.

Churukian, C.J. and Schenk, E.A.: A Warthin-Starry method for spirochetes and bacteria using a microwave oven. J. Histotechnol. 11:149-151, 1988.

MICROWAVE STEINER METHOD FOR SPIROCHETES AND BACTERIA

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin. Do not use fixatives which contain chromium or mercury.

TECHNIQUE: Paraffin sections cut at 4-5 um.

SOLUTIONS:

1.0% Uranyl Nitrate-1% Copper Nitrate

Uranyl nitrate ---------- 0.4 gm

Copper nitrate ---------- 0.4 gm
Distilled water ---------- 40.0 ml

0.5% Silver Nitrate

Silver nitrate ---------- 0.205 gm
Distilled water ---------- 41.000 ml

2.5% Gum Mastic

Gum mastic ---------- 2.5 gm
Absolute alcohol ---------- 100.0 ml

Allow the gum mastic to dissolve for 24 hours. Filter through Whatman #4 filter paper and store in a refrigerator at 3-6° C.

2% Hydroquinone

Hydroquinone ---------- 0.5 gm
Distilled water ---------- 25.0 ml

Prepare fresh each time.

Reducing Solution

2.5% Gum mastic ---------- 15.0 ml
2% Hydroquinone ---------- 25.0 ml
0.5% Silver nitrate ---------- 0.7 ml

The 0.7 ml of 0.5% silver nitrate is obtained from the 41 ml of

0.5% silver nitrate.

Prepare just before use. The solution will have a milky appearance

but does not need to be filtered.

Staining Procedure:

- Use control slide.

l . Deparaffinize and hydrate to distilled water.

2. Place in 40 ml of l % uranyl nitrate and 1% copper nitrate in a plastic Coplin jar, with the cap loosely applied, and microwave at power level 6 (360W) for 40 seconds. Gently agitate the Coplin jar for about 15 seconds. Microwave again at power level 6 for 25 seconds. Gently agitate the Coplin jar for about 15 seconds. Allow the slides to remain in the hot solution for 2 minutes.

3. Rinse in five changes of distilled water.

4. Place in 40 ml of 0.5% silver nitrate in a plastic Coplin jar, with the cap loosely applied, and microwave at power level 6 (360W) for 40 seconds. Gently agitate the Coplin jar for about l 5 seconds. Microwave again at power level 6 for 20 seconds. Allow the slides to remain in the hot solution (80° C) for 2 minutes.

5. Rinse in three changes of distilled water.
6. Dehydrate in two changes of 95% ethanol and two changes of absolute ethanol.
7. Place in 2.5% gum mastic for 1 minute.

8. Place slides in freshly prepared reducing solution in a plastic Coplin jar with the cap loosely applied, and microwave at power level 4 (240W) for 30 seconds. Gently agitate the Coplin jar for about 15 seconds. Microwave again at power level 4 for 30 seconds. Gently agitate the Coplin jar again for about 15 seconds. Allow the slides to remain in the hot solution (75° C) for 3 minutes. The sections should appear yellow-brown at the end of the impregnation time. If the sections still appear too light, allow them to remain in the reducing solution for 3 more minutes. If they are still too light, microwave again at power level 4 for 30 seconds.

9. Rinse in three changes of distilled water.
10. Dehydrate in graded alcohols.
l1. Clear in three or four changes of xylene.
12. Mount with synthetic resin.

Staining Results:

Spirochetes, Legionella pneumophila, Helicobacter (formerly Campylobacter pyloridis), Alipia felis (cat-scratch bacillus) and other bacteria stain dark brown to black.

Comment:

The most commonly used staining methods for demonstrating spirochetes and some Gram negative bacteria which stain poorly with tissue Gram stains, are the Deterle, WarthinStarry and Steiner methods. All of these methods require the use of heat to impregnate tissue sections with silver nitrate followed by a reducing solution which converts the silver nitrate to deposits of black metallic silver on the spirochetes and bacteria. The staining time required to perform these methods has been greatly reduced by the use of the microwave oven. We have tried all of these methods and found that they all usually give satisfactory staining results. However, for demonstrating spirochetes the Steiner method produces the most consistent and reliable staining results.

Garvey modified Steiners method and was able to reduce the staining time required to perform the procedure. She used a new developing solution which is easier to prepare than Steiners original formula. The staining time was reduced more by Swisher who used a microwave oven in performing the method. We have been able to further reduce the staining time by performing the reducing step in a microwave oven rather than a hot water bath.

In Swishers method the uranyl and silver nitrate solutions are heated to the boiling point. We have found that when this is done there is the danger of the solutions boiling over. In the described method the uranyl nitrate is not heated in order to reduce non-specific background staining. The silver nitrate is heated to only 80˚ C which eliminates the possibility of the solution boiling over.

It is necessary to add a very small amount of silver nitrate to the reducing solution in order to achieve satisfactory staining results. If no silver nitrate is added none of the spirochetes will stain. If the amount of silver nitrate is increased there will be an increase in background staining which may obscure the organisms.

When the solutions are heated in a microwave oven there can be up to a 15° C difference in temperature between the top portion of the solution as compared to that in the lower part of the Coplin jar. Therefore, in order to equalize the temperature of the solutions, the slides are gently agitated in steps 2, 4 and 8. This assures uniformity of staining throughout the tissue sections.

References:

Garvey, W. and Fathi, A.: Modified Steiner for the demonstration of spirochetes. J. Histotechnol. 8:15-17,1985.

Swisher, B.L.: Modified Steiner procedure for microwave staining of spirochetes and nonfilamentous bacteria. J. Histotechnol. 10:241-243, 1987.

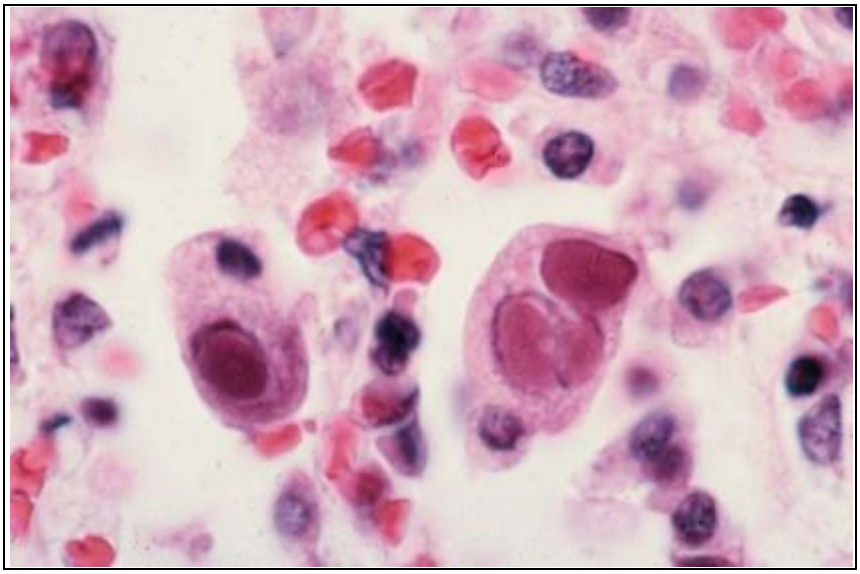
Churukian, C.J. and Garvey, W.: Microwave Steiner method for spirochetes and bacteria. J. Histotechnol. 13:45-47, 1990.

Garvey W., Fathi A., Bigelow F. and Wynnchuk M.: Revised modified Steiner to enhance visability of spirochetes. J. Histotechnol. 18:57-60, 1995.

LENDRUMS METHOD FOR INCLUSION BODIES

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Lendrum's Method for Cytomegalovirus (CMV).

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.5% Phloxine Solution

Phloxine B, C.I. 45410	0.5 gm
Alcohol, 70%	100.0 ml
Calcium chloride	0.5 gm

2.5% Tartrazine Solution

Tartrazine, C.I. I 9140	2.5 gm
Cellosolve	100.0 ml

0.3% Sodium Borate

(see PAS Method)

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Acidified Lillie-Mayer or Harris hematoxylin for 1 minute.
3. Rinse in three changes of distilled water.
4. Blue hematoxylin in 0.3% sodium borate for 15 seconds.
5. Rinse in four changes of distilled water.
6. Phloxine solution for 30 minutes.

7. Rinse with three changes of distilled water and drain slides well. Wipe excess water from around tissue. If this is not done, the section will not differentiate uniformly.

8. Differentiate in tartrazine solution until inclusion bodies stand out a bright red on a yellow background.

9. Dehydrate in 95% alcohol and absolute alcohol, three changes each.
10. Clear in three to four changes of xylene.
11. Mount with synthetic resin.

Staining Results:

Inclusion bodies ---------- red

Erythrocytes ---------- red

Nuclei ---------- blue

Background ---------- yellow

Comment:

Another method for demonstrating inclusion bodies is Wolbachs Giemsa method. The phloxine-tartrazine method is excellent for demonstrating Paneth cell granules in small intestine mucosa.

References:

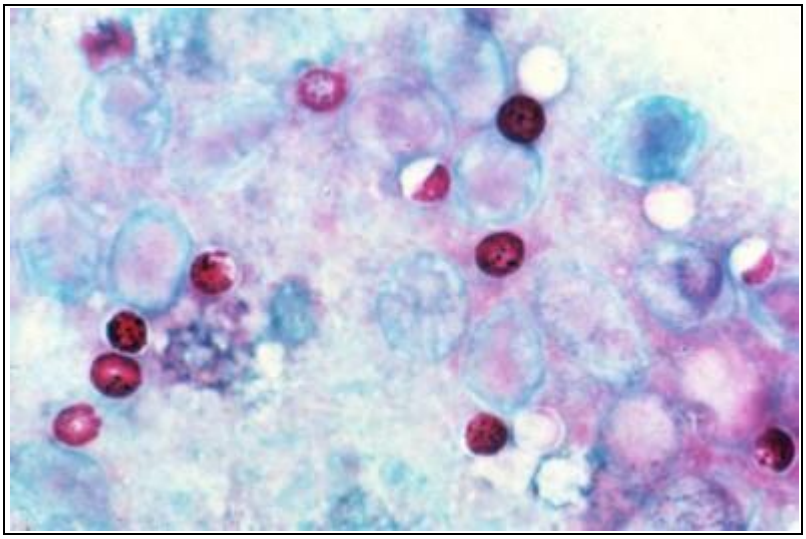
Lendrum A. C.: The phloxine-tartrazine method as a general histological stain for demonstration of inclusion bodies. J. Path. Bact. 59:399-404, 1947.

MODIFIED ZIEHL-NEELSEN METHOD

FOR CRYPTOSPORIDIUM OOCYST

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Ziehl-Neelsen Method for Cryptosporidiosis.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm. Smears and touch preparations may

be done. These are fixed in 10% buffered neutral formalin for 15 minutes.

SOLUTIONS:

Carbol Fuchsin Solution

Pararosanilin, C.I. 42500 or Basic fuchsin, C.I. 42510 ---------- 0.8 gm
Isopropyl alcohol ---------- 14.0 ml
Phenol ---------- 7.5 ml
Distilled water ---------- 140.0 ml

Dissolve the pararosanilin or basic fuchsin in the isopropyl alcohol

and the phenol in the distilled water. Mix the two solutions. Filter

through Whatman #1 filter paper before use.

0. 1% Hydrochloric Acid

Alcohol, 70%	100.0 ml
Hydrochloric acid, concentrated	0.1 ml

Fast Green. Stock Solution

Fast green FCF, C.I. 42053	0.2 gm
Distilled water	100.0 ml
Acetic acid, glacial	0.2 ml

Fast Green, Working Solution

Fast green, stock solution	10.0 ml
Distilled water	35.0 ml

Staining Procedure:

- Use control slide.

l . Deparalfinize and hydrate to distilled water.
2. Stain in carbol-fuchsin solution for 15 minutes.
3. Rinse with three changes of distilled water.

4. Decolorize in the acid-alcohol solution until no more color comes off (approximately 30 - 60 seconds.)

5. Wash in running tap water for 30 seconds and rinse in two changes of distilled water.

6. Counterstain with working fast green solution for 30 seconds.
7. Rinse in three changes of distilled water.
8. Dehydrate in graded alcohols.
9. Clear in three or four changes of xylene.
10. Mount with synthetic resin.

Staining Results:

Cryptosporidium oocysts	red
Background	green

References:

Henriksen, S.A. and Pohleuz, J.F.L.: Staining of cryptosporidia by modified ZichlNeelsen technique. Acta. Vet. Scand. 22:594-596, 1981.

Human Cryptosporidiosis: New Engl. J. Med. 309:1325-1327, 1983.

LIST OF STAINS FOR CARBOHYRDATES AND MUCOPOLYSACCHARIDES

MICROWAVE ALCIAN BLUE METHOD

MICROWAVE ALCIAN BLUE WITH HYALURONIDASE DIGESTION

MICROWAVE ALCIAN BLUE - PAS METHOD

MICROWAVE COLLOIDAL IRON METHOD

PERIODIC ACID SCHIFF METHOD

MICROWAVE PERIODIC ACID-SCHIFF WITH DIASTASE (DiPAS)

BESTS CARMINE METHOD FOR GLYCOGEN

SOUTHGATES MUCICARMINE METHOD

PERIODIC ACID, THIONIN, KOH PAS METHOD (PAT)

MICROWAVE KREYBERG METHOD FOR KERATIN AND MUCIN

MODIFIED PUCHTLER CONGO RED AMYLOID METHOD WITH/WITHOUT POTASSIUM PERMANGANATE OXIDATION

LIEBS CRYSTAL VIOLET METHOD FOR AMYLOID

MODIFIED PUCHTLER CONGO RED AMYLOID METHOD

MICROWAVE ALCIAN BLUE METHOD

(pH 2.5)

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Images will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

3% Acetic Acid Solution

Acetic acid, glacial ---------- - 3.0 ml
Distilled water ---------- 97.0 ml

1.0% Alcian Blue Solution

Alcian blue 8GX, C.I. 74240 ---------- 1.0 ml

3% acetic acid ---------- 100.0 ml

Filter and add a few crystals of thymol.

Nuclear Fast Red Solution

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with aid of heat. Cool, filter and add a few grains of thymol as a preservative.

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in 3% acetic acid for 3 minutes.

3. Place in 40 ml of 1.0% alcian blue solution in a glass Coplin jar and microwave at power level 1 (60W) for 3 minutes. Dip the slides up and down several times and allow them to remain in the hot solution (65° C) for 5 minutes.

4. Wash in running tap water for 1 minute and rinse in distilled water.
5. Nuclear fast red solution for 3 minutes.
6. Rinse in three changes of distilled water.
7. Dehydrate in graded alcohols.
8. Clear in three or four changes of xylene.
9. Mount with synthetic resin.

Staining Results:

Acidic sulfated mucosubstances ---------- blue

Cryptococcus ---------- blue

Nuclei---------- red

Comment:

No single staining solution of alcian blue colors all polyanions maximally, but the above procedure, done at pH 2.5, stains all except the most strongly sulfated mucopolysaccharides, and is very useful for demonstrating goblet cell mucins in the respiratory tract and in small and large bowel, as well as various components of skin, heart valve.s, large arteries and cartilage.

Alcian blue probably find sits greatest usefulness in combination stains such as Alcian blue, PAS, and the Kreyberg method.

For a more detailed discussion of various uses of alcian blue see: Schenk, E.A. and Mowry, R.W.: Alcian Blue. J. Histotech., June 1983, pp. 65-69.

When heating the alcian blue solution with microwave irradiation the top portion of the solution is warmer by 10-15° C than that near the bottom of the Coplin jar. Therefore, in order to equalize the temperature of the solution the slides are dipped up and down. This assures uniformity of staining throughout the tissue sections.

The stain may be performed at room temperature by staining in the alcian blue solution for 30 minutes.

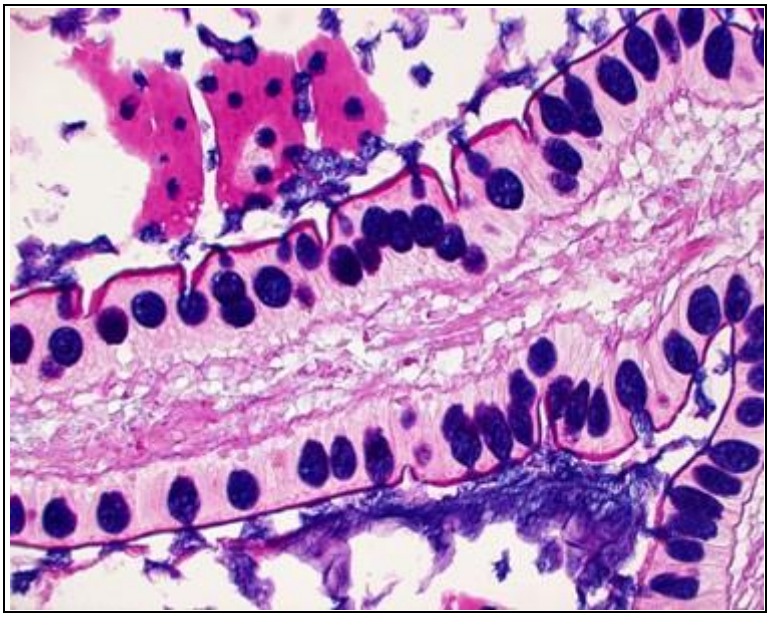
References:

McManus, J.F. and Mowry, R.W.: Staining Methods Histologic and Histochemical, New York, Paul B. Hoeber, 1960, pp. 137-138.

MICROWAVE ALCIAN BLUE WITH HYALURONIDASE DIGESTION

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Small intestine (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Phosphate Buffer. pH 6.0

Sodium phosphate, dibasic ----------	0.128 gm
Potassium phosphate, monobasic ----------	0.872 gm
Distilled water ----------	100.0 ml

Hyaluronidase Digestion Solution

Hyaluronidase, testicular (Sigma) ---------- 0.02 gm
Phosphate buffer, pH 6.0 ---------- 40.00 ml

Mix just before use.

3% Acetic Acid Solution

(see Microwave Alcian Blue Method)

1.0% Alcian Blue Solution

(see Microwave Alcian Blue Method)

0. 1% Nuclear Fast Red Solution

(see Microwave Alcian Blue Method)

Staining Procedure:

- Use control slide (duplicate section).

1. Deparaffinize and hydrate duplicate sections to distilled water.

2. Place the control and requested section in 40 ml of hyaluronidase digestion solution in a glass Coplin jar and microwave at power level 1 (60W) for 1 minute. Transfer to a 37° C oven for 1 hour. Place the duplicate control and requested slides in 40 ml of phosphate buffer, pH 6.0 in a glass Coplin jar and microwave at power level 1 for 1 minute. Transfer to a 37o C oven for 1 hour.

3. Rinse all the slides in four changes of distilled water.
4. Place in 3% acetic acid solution for 3 minutes.

5. Place in 40 ml of 1.0% alcian blue solution in a glass Coplin jar and microwave at power level 1 (60W) for 3 minutes. Dip the slides up and down several times and allow them to remain in the hot solution (65° C) for 5 minutes.

6. Rinse in three changes of distilled water.
7. Nuclear fast red solution for 3 minutes.
8. Rinse in three changes of distilled water.
9. Dehydrate in graded alcohols.
10. Clear in three or four changes of xylene.
11. Mount with synthetic resin.

Staining Results:

Staining attributable to hyaluronic acid, chrondrotin 4 sulfate, or chrondrotin 6 sulfate is selectively eliminated.

Comment:

The alcian blue method with and without hyaluronidase digestion has some value in differentiating mesotheliomas from carcinomas. Some mesotheliomas contain hyaluronic acid and therefore can be stained with alcian blue. If the alcian blue positivity is destroyed following prior incubation with hyaluronidase this helps in establishing the diagnosis of mesothelioma.

When heating the alcian blue solution with microwave irradiation the top portion of the solution is warmer by 10-15° than that near the bottom of the Coplin jar. Therefore, in order to equalize the temperature of the solution, the slides are dipped up and down. This assures uniformity of staining throughout the tissue sections.

The stain may be performed at room temperature by staining in the alcian blue solution for 30 minutes.

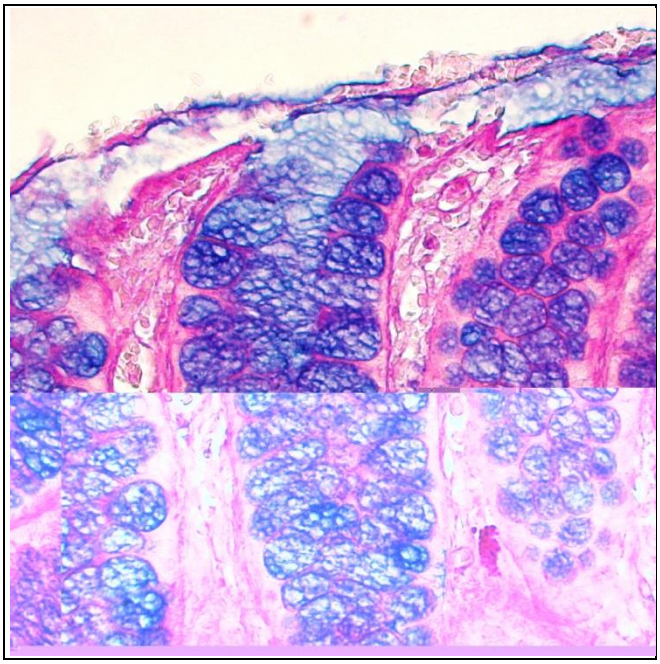
References:

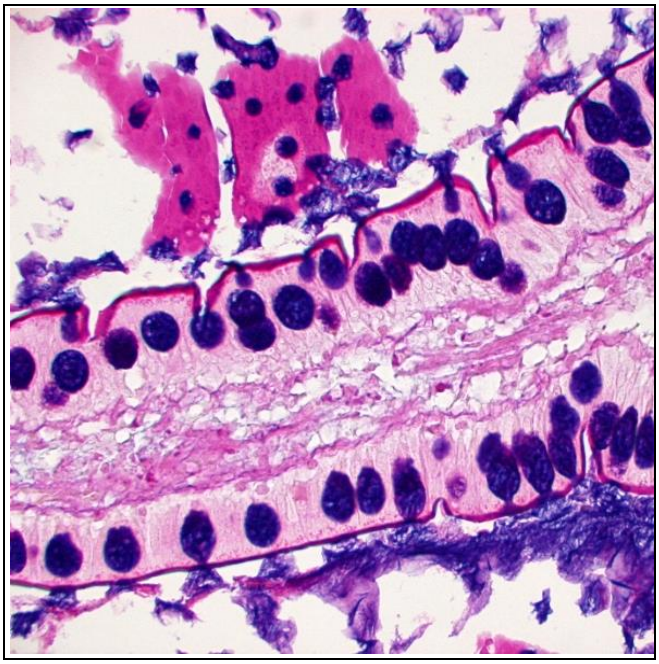
Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 171-172.

MICROWAVE ALCIAN BLUE - PAS METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



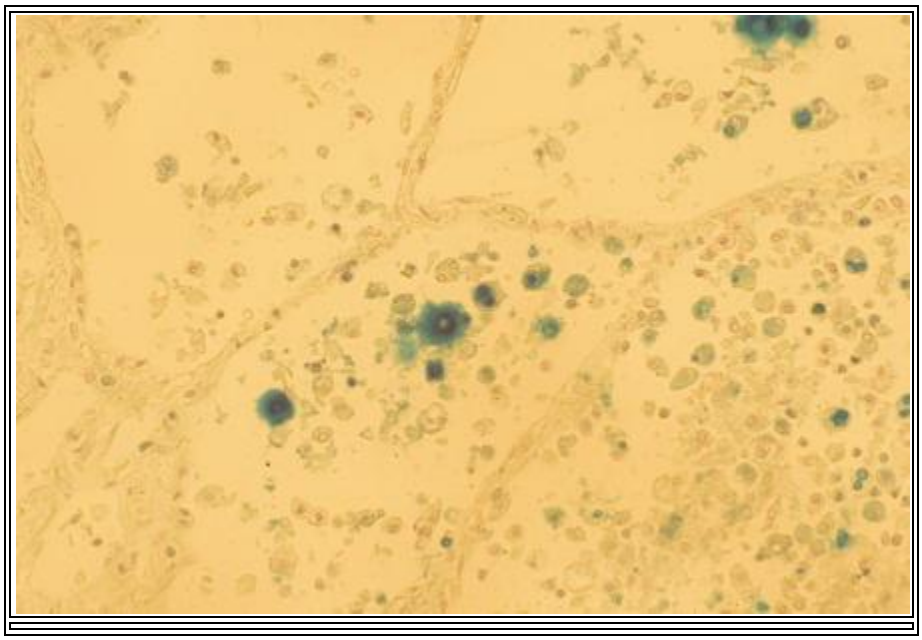


Small intestine (Courtesy of Dr. Zhenhong Qu)

MICROWAVE COLLOIDAL IRON METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin. Avoid the use of dichromate fixatives.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

20% Ferric Chloride Solution

Ferric chloride, FeC13 6H2O ---------- 2.0 gm
Distilled water ---------- 10.0 ml

Colloidal Iron Solution (Stock)

Distilled water ---------- 250.0 ml

Ferric chloride, 20% ---------- 10.0 ml

Bring the distilled water to a boil and then add 10.0 ml of 20%

ferric chloride. When the solution turns dark red remove from

the heat and cool. This solution is stable for at least one year.

Colloidal Iron-Acetic Acid Solution (Working)

Colloidal iron (stock) ---------- 20.0 ml
Distilled water ---------- 15.0 ml
Acetic acid, glacial ---------- 5.0 ml

12% Acetic Acid Solution

Acetic acid, glacial ---------- 12.0 ml
Distilled water ---------- 88.0 ml

2% Hydrochloric Acid Solution

Hydrochloric acid, concentrated ---------- 2.0 ml
Distilled water ---------- 98.0 ml

10% Triton X-100

Triton X-100 ---------- 10.0 ml
Distilled water ---------- 90.0 ml

1% Potassium Ferrocyanide Solution

Potassium ferrocyanide ---------- 1.0 gm
Distilled water ---------- 96.0 ml

10% Triton X-100---------- 4.0 ml

Hydrochloric Acid-Potassium Ferrocvanide Solution

4% Hydrochloric acid ---------- 20.0 ml
2% Potassium ferrocyanide ---------- 20.0 ml
10% Triton X-100 ---------- 0.4 ml

Prepare just before use.

Nuclear Fast Red Solution

Dissolve 0.1 gm of nuclear fast red in 100 ml of 5% aqueous solution of aluminum sulfate with the aid of heat. Cool, filter and add a few grains of thymol as a preservative.

Staining Procedure:

- Use control slide.

1 . Deparaffinize and hydrate to distilled water.
2. Place in 12% acetic acid for I minute.
3. Place in working colloidal iron-acetic acid solution in a glass Coplin jar and

microwave at power level 1 (60W) for 3 minutes. Dip the slides up and down several times and allow them to remain in the warm solution for 10 minutes.

4. Rinse in 12% acetic acid, four changes, 1 minute each.

5. Place in the hydrochloric acid-potassium ferrocyanide solution in a glass Coplin jar and microwave at power level 1 (60W) for 2 minutes. Dip the slides up and down several times and allow them to remain in the warm solution for 2 minutes.

6. Wash in running tap water for 1 minute.
7. Rinse in two changes of distilled water.
8. Nuclear fast red solution for 3 minutes.
9. Rinse in three changes of distilled water.
10. Debydrate through graded alcohols.
11. Clear in three or four changes of xylene.
12. Mount with synthetic resin.

Staining Results:

Acidic mucopolysaccharides ---------- deep blue

Acidic epithelial mucus ---------- deep blue

Cryptococcus ---------- deep blue

Nuclei ---------- red

Comment:

The colloidal iron method stains essentially the same acidic mucopolysaccharides that are stained with alcian blue, except that the color with colloidal iron is a more intensive blue.

It appears that cryptococcus is the only fungus which can be demonstrated by methods for acid mucopolysaccharides such as Mowrys alcian blue, Southgates and Mayers mucicarmine and Mowrys colloidal iron. Most texts recommend Southgates or Mayers mucicarmine for demonstrating cryptococcus but, based on our experience, this colloidal iron method gives the best results.

When heating a solution with microwave irradiation the top portion of the solution is warmer by l 0-15° C than that near the bottom of the Coplin jar. Therefore in order to equalize the temperature of the solutions the slides are dipped up and down in steps #3 and #5 of the staining procedure. This assures uniformity of staining throughout the tissue sections.

The stain may be performed at room temperature by staining the sections for l hour in the colloidal iron-acetic acid working solution and for 30 minutes in the hydrochloric acid-potassium ferrocyanide solution.

References:

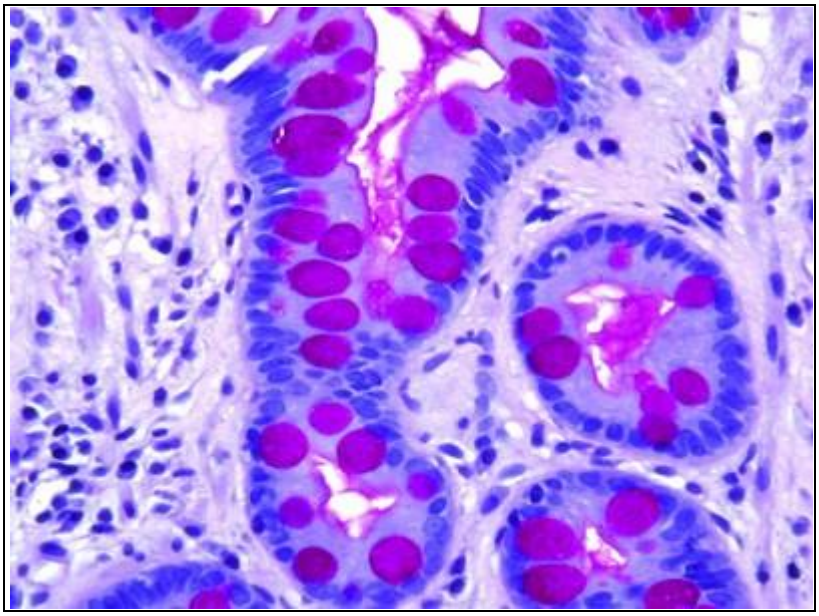
Mowry, R.W.: Improved procedure for the staining of acidic polysaccharides by Mullers colloidal-hydrous-ferric oxide and its combination with the Feulgen and the periodic acid-Schiff reactions. Lab. Invest. 7:566-576, 1958.

McManus, J.F.A. and Mowry, R.W.: Staining Methods Histologic and Histochemical, New York, Paul B. Hoeber, 1960, pp. 133-136.

PERIODIC ACID SCHIFF METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Small intestine (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin, Zenkers or any good fixative.

TECHNIQUE: Paraffin sections cut at 4 µm.

SOLUTIONS:

0.5% Periodic Acid Solution

Periodic acid ----------	0.5 gm
Distilled water ----------	100.0 ml

Modified Lillies Schiff Solution

Dissolve 0.3 gm of pararosanilin, in 20 ml of 20% ethyl alcohol. Add 80 ml

of distilled water, 2.0 gm of sodium metabisulfite, 0.15 gm of sodium hydrosulfite,

and thoroughly mix. Then add 1.0 ml of hydrochloric acid and 0.3 gm of activated charcoal. Stir for 3 minutes and filter through Whatman #1 filter paper. The solution should be water-white. Store Schiffs reagent in a refrigerator at 3-6° C. When stored in a refrigerator, the solution has a shelf-life of at least one year.

0.3% Sodium Borate

Sodium borate (Na2 B4 O7 10H2O) ----------	0.3 gm
Distilled water ----------	100.0 ml

Staining Procedure:

1 . Deparaffinize and hydrate to distilled water.

2. Oxidize in periodic acid solution for 10 minutes. For kidney, skin and diastase digested liver biopsies, 15 minutes.

3. Rinse in four changes of distilled water.

4. Place in modified Lillies Schiff solution for 15 minutes. For kidney and skin biopsies, 30 minutes.

5. Rinse in four changes of distilled water.
6. Place in 0.3% sodium borate for 15 seconds.

7. Rinse in four changes of distilled water.

8. Stain in acidified Lillie-Mayer hematoxylin for 45 seconds.

9. Rinse in three changes of distilled water.

10. Blue hematoxylin in 0.3% sodium borate for 15 seconds.

11. Rinse in four changes of distilled water.

12. Dehydrate through graded alcohols.

13. Clear in three or four changes of xylene.

14. Mount with synthetic resin.

Staining Results:

Glycogen, mucin, reticulum, colloid droplets, hyaline deposits in glomeruli, most basement membranes, colloid of pituitary stalks and thyroid give a rose to purple reaction; some types of fungi - red, nuclei - blue.

Comment:

Treatment of tissue sections with 0.5% periodic acid results in the formation of aldehyde groupings by cleavage of 1,2 glycols found in various carbohydrates. The presence of the newly formed aldehyde groups is detected by Schiff's reagent (or leucofuchsin). The first stage reaction involves the formation of a colorless, unstable dialdebyde addition compound that is transformed to the colored final product by restoration of the quinoid chromophoric grouping of pararosanilin or other basic fuchsin. This color restoration is usually accomplished by washing in running tap water for 10 minutes after treatment with Schiff's reagent. We found that treatment with 0.3% sodium borate for 15 seconds accomplishes the same purpose.

The formula for Lillies Schiff reagent has been modified. This modified solution requires less basic fuchsin (pararosanilin) to prepare, can be prepared much faster, yields equally good staining results, and appears to have greater stability than Lillies formula.

Schiff's reagent after decolorization with activated charcoal may be either water clear or have a pale straw color. Some believe that the quality of the activated charcoal determines the final color of the Schiff's reagent. Fresh, activated charcoal is supposed to result in a water clear Schiff's reagent. From our experience this is not necessarily true as we have found that Schiff's reagent prepared with different samples of basic fuchsin will not all decolorize completely even when fresh activated charcoal is used. The final color of the Schiff's reagent does not appear to affect the quality or intensity of the Schiff's reaction.

Schiff's reagent can be tested by pouring a few drops of the reagent into 10 ml of reagent grade formaldehyde (37-40%) in a small beaker. If the solution turns reddish purple rapidly, it is good. If the reaction is delayed and the resulting color deep blue-purple, the solution is breaking down and should be discarded.

References:

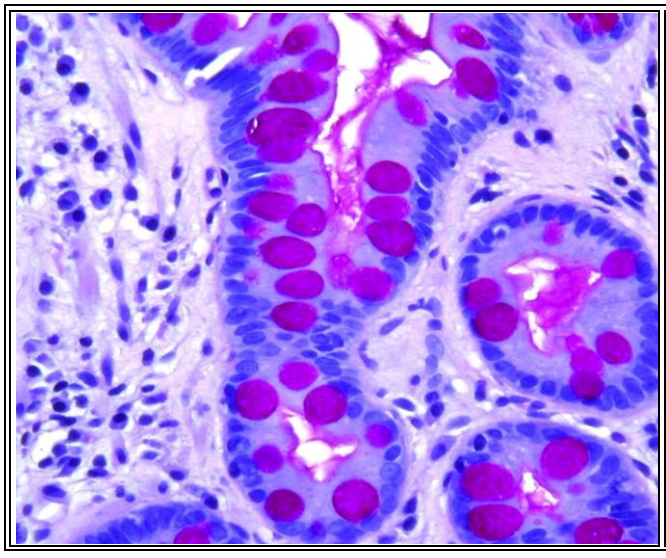
Garvey, W. et al: Combined modified periodic acid-Schiff and batch staining method. J. Histotechnol. 15:117-120, 1992.

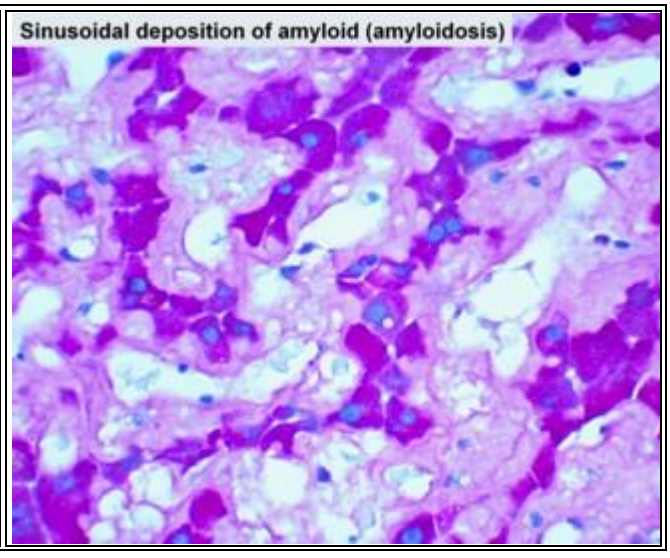
McManus, J.F.A. and Mowry, R.W.: Staining Methods Histologic and Histochemical, New York, Paul B. Hoeber, 1960, pp. 126-128.

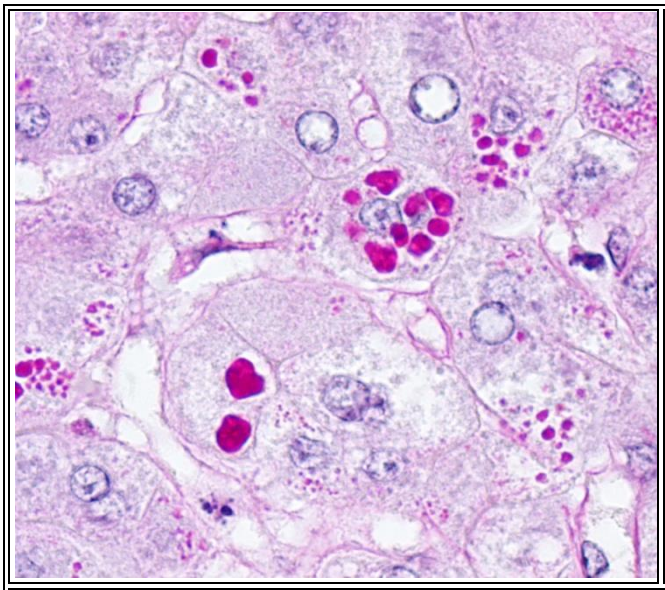
MICROWAVE PERIODIC ACID-SCHIFF WITH DIASTASE

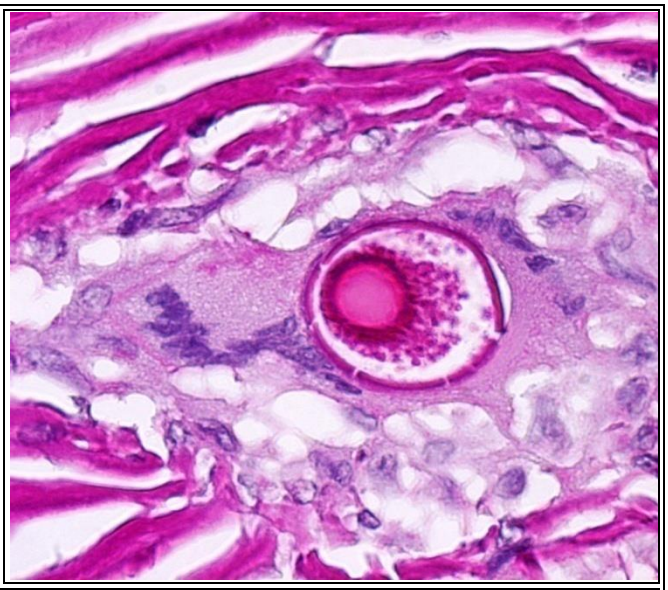
Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:









Mucin in intestine (upper left), hepatocytes in liver with amyloidosis (upper right), intracytoplasmic hyaline globues in liver of alpha-1-antitrypsin deficiency (lower left), and coccidioides in lung (lower right). Small intestine (Courtesy of Dr. Zhenhong Qu).

2ndPAS-D

Diagnostic Application:

Tumor of glandular origin (e.g., adenocarcinoma) contains polysacride glycoprotein stained pink or red.

Highlights alpha-1-antitrypsin in hepatocytes, a feature of alpha-1-antitrypsin deficiency.

Many fungal microorganisms are also stained by this methods.

Material and Solutions:

FIXATION: Absolute alcohol or 10% formalin - alcohol is preferred. If 10% buffered neutral formalin is used, there will be a significant loss of glycogen.

TECHNIQUES: Paraffin sections cut at 4 μm.

SOLUTIONS:

Phosphate Buffer Solution pH 6.0*

Sodium chloride ---------- 8.000 gm

Sodium phosphate, dibasic Na_2HPO_4 ---------- 0.282 gm

Sodium phosphate, monobasic $NaH_2PO_4 \cdot H_2O$ ---------- 1.970 gm

Distilled water ---------- 1000.000 ml

*The pH of the solution without the sodium chloride is 6.0.

With the sodium chloride the pH will be approximately 5.7.

Diastase Digestion Solution

Phosphate buffer solution ---------- 40.00 ml.

Diastase of malt ---------- 0.04 gm

0.5% Periodic Acid Solution

(See PAS Method)

Modified Lillie's Schiff Solution

(See PAS Method)

0.3% Sodium Borate

Sodium borate (Na2 B4 O7 10H2O ---------- -- 0.3 gm

Distilled water ---------- 100.0 ml

Staining Procedure:

- Run two slides. Diastase digest one of the slides and then do the PAS stain on both slides.

1. Deparaffinize and hydrate to distilled water.

2. Place in diastase digestion solution in a glass Coplin jar. Place in a microwave oven and microwave at power level 1 for one minute. Transfer to a 37o C oven for 30-60 minutes. Leave the PAS slide in distilled water.

3. Wash in tap water for 3 minutes and rinse in distilled water, two changes.

4. Place in 0.5% periodic acid for 10 minutes.

5. Rinse in four changes of distilled water.

6. Place in modified Lillies Schiff solution for 15 minutes.

7. Rinse in four changes of distilled water.

8. Place in 0.3% sodium borate for 15 seconds.

9. Rinse in four changes of distilled water.

10. Stain in acidified Lillie-Mayer hematoxylin for 45 seconds.

11. Rinse in three changes of distilled water.

12. Blue hematoxylin in 0.3% sodium borate for 15 seconds.

13. Rinse in four changes of distilled water.

14. Dehydrate in graded alcohols.

15. Clear in three or four changes of xylene and mount with synthetic resin.

Staining Results:

Staining attributable to glycogen is selectively eliminated in the diastase digested slide, but will be stained magenta in the undigested slide.

Nuclei ---------- blue

References:

Garvey, W. et al: Combined modified periodic acid-Schiff and batch staining method. J. Histotechnol. 15:117-120, 1992.

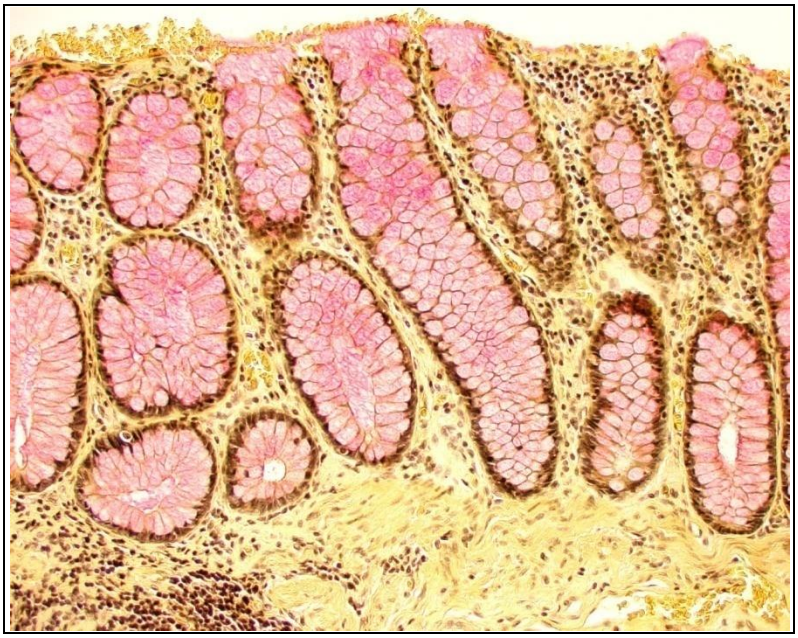
Lillie, R.D.: Histopathologic Technic and Practical Histochemistry, 3rd Edition, New York, McGraw-Hill, 1965, pp. 496-497.

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Edition, New York, McGraw-Hill, 1968, pp. 159, 171.

BEST'S CARMINE METHOD FOR GLYCOGEN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Intestines (Courtesy of Dr. Zhenhong Qu)

Rectum colon mucicarmine.

Diagnostic Application:

Material and Solutions:

FIXATION: Carnoy's or alcoholic-formalin. 10% buffered neutral formalin may be used.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Weigert's Iron Hematoxylin

(See Southgate's mucicarmine method)

Carmine Solution (Stock)

Carmine, C.I. 75470 ---------- 2.0 gm.

Potassium carbonate ---------- 1.0 gm

Potassium chloride ---------- 5.0 gm

Distilled water ---------- 60.0 ml

Boil gently and cautiously for 5 minutes. When cool add 20 ml

of 28% ammonium hydroxide. Store in the refrigerator.

Carmine Solution (Working)

Carmine solution (stock) ----------	10.0 ml
Ammonium hydroxide, 28% ----------	15.0 ml
Methyl alcohol ----------	15.0 ml

Differentiating Solution

Alcohol, absolute ----------	20.0 ml
Methyl alcohol ----------	10.0 ml
Distilled water ----------	25.0 ml

Staining Procedure:

- Two slides are required. One of the slides is diastase digested (see PAS-diastase method) and then both slides are stained.

1. Deparaffinize and hydrate to distilled water.
2. Weigert's iron hematoxylin for 1 minute.
3. Wash briefly in running water.
4. Decolorize with 0.5% hydrochloric acid in 70% alcohol for 10 seconds.
5. Wash in running water for 5 minutes.
6. Rinse in distilled water.
7. Working carmine solution for 30 minutes.
8. Place in differentiating solution for 3 seconds.
9. Rinse quickly in 70% alcohol.
10. Dehydrate in graded alcohols.
11. Clear in xylene, three or four changes.

12. Mount with synthetic resin.

Staining Results:

Staining attributed to glycogen is selectively eliminated in the diastase digested slide, but will be stained pink to red in the undigested slide.

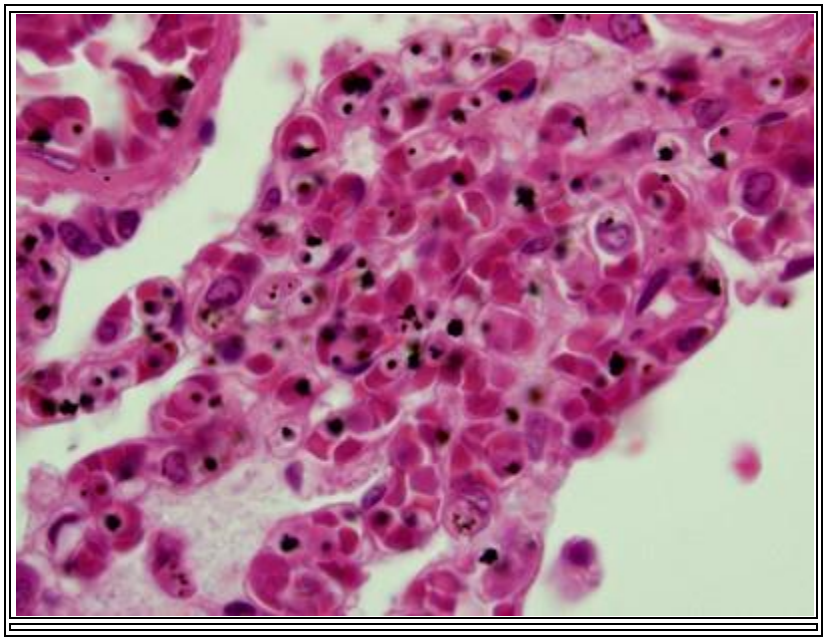
References:

Mallory, F.B.: Pathological Technique, New York, Hafner Publishing Co., 1961, pp. 126-128.

SOUTHGATE'S MUCICARMINE METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Weigert’s Iron Hematoxylin (Concentrated)

Solution A

Hematoxylin crystals, C.I. 75290 ---------- 2.0 gm

Alcohol, 90% ---------- 100.0 ml

Solution B

Ferric chloride, Fe Cl3 6 H20 62% ---------- 4.0 ml

Distilled water ---------- 95.0 ml

Hydrochloric acid, concentrated ---------- 1.0 ml

For use mix equal parts of each solution.

Mucicarmine Solution (Stock)

Carmine, C.I. 75470 ---------- 1.0 gm

Aluminum hydroxide ---------- 1.0 gm

50% alcohol ---------- 100.0 ml

Mix and then add:

Aluminum chloride hexahydrate ---------- 1.0 gm

Heat and boil 3 minutes. Cool and make up to original volume with 50% alcohol. Filter through Pyrex glass wool. This stock solution is stable for several months when stored in a refrigerator at 3-6o C.

To prepare the working solution add 30 ml. of distilled water to 10 ml. of the stock solution.

0.2% Tartrazine Solution

Tartrazine, C.I. 19140 ---------- 0.2 gm

Distilled water ---------- 100.0 ml

Acetic acid ---------- 0.2 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Weigert's iron hematoxylin for 1 minute.

3. Wash briefly in running water.

4. Decolorize with 0.5% hydrochloric acid in 70% alcohol for 10 seconds.

5. Wash in running tap water for 1 minute and rinse in distilled water.

6. Place in diluted mucicarmine solution for 90 minutes or longer (2-3 hours) if a more intense stain is desired.

7. Rinse quickly in distilled water.

8. Place in tartrazine solution for 10 seconds.

9. Rinse quickly in distilled water.

10. Dehydrate in graded alcohols.

11. Clear in xylene, three or four changes.

12. Mount with synthetic resin.

Staining Results:

Mucin ---------- deep rose to red

Capsule of Cryptococcus ---------- deep rose to red

Nuclei ---------- black

Other tissue elements ---------- yellow or orange

Comment:

This method gives better staining results than Mayer's mucicarmine method. It will demonstrate acidic mucopolysaccharides similar to those stained with Alcian blue (pH 2.5) and with colloidal iron. For many years we used metanil yellow as the counterstain in this method. This counterstain has a slight masking effect upon the stained mucin. We found that tartrazine produces a lighter background stain which allows for better visualization of the mucin.

References:

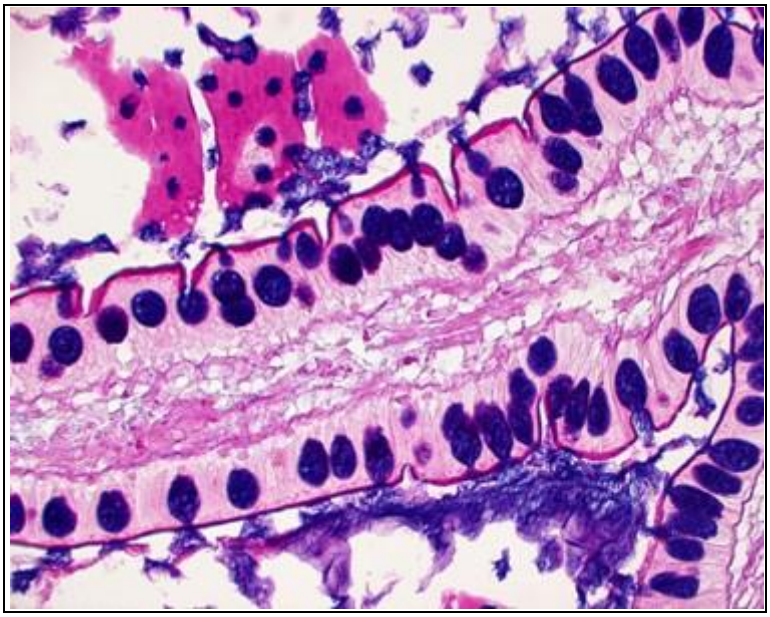
Culling, C.F.A.: Handbook of Histopathological Technique, 3rd ed., Butterworths, London, 1974, pp. 304-305.

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 161-162.

PERIODIC ACID, THIONIN, KOH PAS METHOD (PAT)

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Small intestine (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

1% Periodic Acid

Periodic Acid ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Thionin Schiff Reagent

Thionin C.I. 52000 ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Sodium hydrosulfite ---------- 1.0 gm

Add the thionin to the distilled water and heat to near boiling. When the solution has cooled add the sodium hydrosulfite. A grey precipitate forms. Filter through Whatman #4 filter paper. The solution is not stable at room temperature and should be prepared shortly before use. If refrigerated the solution is good for one or two days.

0.5% Potassium Hydroxide in 70% Alcohol

Potassium hydroxide ---------- 0.5 gm

Alcohol, 70% ---------- 100.0 ml

Modified Lillie's Schiff Solution

(See PAS Method)

Staining Procedure:

Use control slide (colon).

1. Deparaffinize and hydrate to distilled water.
2. Place in 1% periodic acid for 15 minutes.
3. Rinse with four changes of distilled water.
4. Place in thionin Schiff reagent for 30 minutes.
5. Wash in running tap water for 10 minutes and rinse in two change of distilled water.
6. Place in 70% alcohol for 30 seconds.

7. Treat with 0.5% potassium hydroxide in 70% alcohol for 5 minutes.
8. Wash gently in running tap water and rinse with two changes of distilled water.
9. Place in 1% periodic acid for 10 minutes.
10. Rinse in four changes of distilled water.
11. Place in modified Lillie's Schiff solution for 15 minutes.
12. Rinse with three changes of distilled water.
13. Place in 0.3% sodium borate for 15 seconds.
14. Rinse with four changes of distilled water.
15. Dehydrate in graded alcohols.
16. Clear in xylene, three or four changes.

Mount with synthetic resin.

Staining Results:

Normal small bowel mucosal mucins ---------- blue

Normal colonic mucin ---------- red

Adenocarcinomas of the colon ---------- blue to purple or non-stained

Comment:

This is an excellent stain for differentiating colonic mucins from those found in other areas of the GI tract. It is also helpful in demonstrating the loss of normal mucins in colonic adenocarcinomas, although the specificity of this is as yet undetermined. Colonic polyps may show similar loss of normal (red) mucin staining.

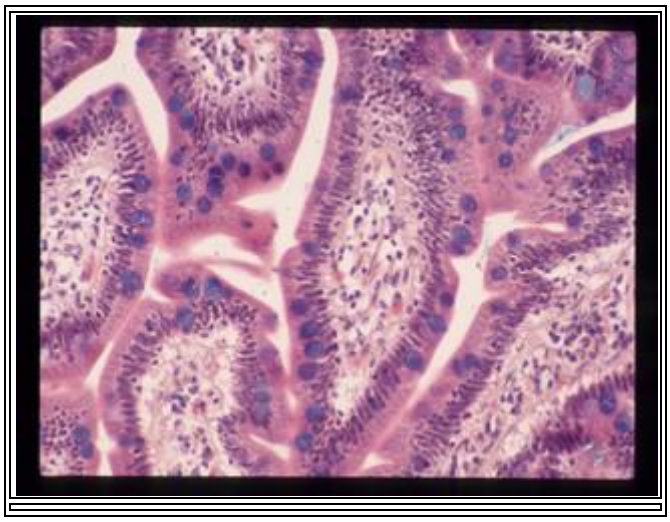
References:

Culling, C.F.A., Reid, P.E. and Dunn, W.L.: A new histochemical method for the identification and visualization of both side chair acylated and non-acylated sialic acids. J. Histochem. Cytochem. 24:1225-1230, 1976.

MICROWAVE KREYBERG METHOD FOR KERATIN AND MUCIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.3% Acetic Acid Solution

(See Microwave Alcian Blue Method)

1% Alcian Blue Solution

Alcian blue 8 GX, C.I. 74240 ----------	1.0 gm
Distilled water ----------	100.0 ml
Acetic acid ----------	1.0 ml

Ammonia-Alcohol Solution

Alcohol, 95% ---------- 90.0 ml

Ammonium hydroxide, 28% ---------- 10.0 ml

Weigert's Iron Hematoxylin, Concentrated

Solution A

Hematoxylin crystals, C.I. 75290 ---------- 2.0 gm

Alcohol, 90% ---------- 100.0 ml

Solution B

Ferric chloride, 62% aqueous ---------- 4.0 ml

Distilled water ---------- 95.0 ml

Hydrochloric acid, conc. ---------- 1.0 ml

To make up the working solution, mix equal parts of Solution A and Solution B.

Acid Alcohol Solution

Alcohol, 70% ---------- 100.0 ml

Hydrochloric acid, conc. ---------- 0.5 ml

1% Erythrosin B Solution

Erythrosin B, C.I. 45430 ----------	1.0 gm
Distilled water ----------	100.0 ml

Alcoholic Metanil Yellow Solution

Metanil yellow, C.I. 13065 ----------	0.5 gm
Alcohol, absolute ----------	100.0 ml
Filter and add acetic acid ----------	1.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in 3% acetic acid for 3 minutes.
3. Place in 40 ml of 1% alcian blue solution in a glass Coplin jar and microwave at power level 1 (60W) for 3 minutes. Dip the slides up and down and allow them to remain in the hot solution for 5 minutes.
4. Rinse in three changes of distilled water.
5. Place in ammonia-alcohol solution for 10 minutes.
6. Wash well with tap water and rinse in distilled water.
7. Place in Weigert's iron hematoxylin solution for 45 seconds.
8. Wash with distilled water.
9. Acid alcohol solution for 10 seconds.
10. Rinse in four changes of distilled water.
11. Place in erythrosin B solution for 5 minutes.
12. Rinse quickly in one change of distilled water.
13. A few quick dips in two changes of 95% alcohol followed by two changes of absolute alcohol.
14. Place in alcoholic metanil yellow for 30 seconds.

15. Dehydrate with four changes of absolute alcohol.

16. Clear in three or four changes of xylene and mount with synthetic resin.

Staining Results:

Keratin ---------- red or orange red

Mucin ---------- green or bluish green

Nuclei ---------- black

Collagen ---------- orange

Muscle ---------- various shades of red and orange

Erythrocytes ---------- red

Comment:

This method has been useful in distinguishing between keratinizing and mucus producing tumors and in identifying tumors which contain both elements. This method has been used most often to stain tumors of the lung, gastrointestinal tract, breast, bladder, prostate and female genital tract. It is used to stain cytologic smears, especially bronchial aspirations.

The modification of Weigert's iron hematoxylin used in this method results in clear, sharp nuclear detail which is not affected by treatment with the other staining solutions. Following staining with alcian blue the method uses ammonia-alcohol which converts the alcian blue into the insoluble pigment monastral fast blue. If the sections are not treated with ammonia-alcohol after being stained with alcian blue, there will be a significant loss of stain from the acid mucopolysaccharides.

For several years we used saffron in this staining method. During the past few years the cost of saffron has greatly increased, therefore, a search was made for a substitute dye. Metanil yellow was found to be satisfactory in alcoholic solution when it was acidified with acetic acid.

The stain may be performed at room temperature by staining the sections in the alcian blue solution for 30 minutes.

References:

Churukian, C.J. and Schenk, E.A.: A modification of Kreyberg's method for demonstrating keratin and mucin. J. Histotechnology 7:146-148, 1984.

Elbadawi, A.: Hexachrome modification of Movat's stain. Stain Technol. 51:249-253, 1976.

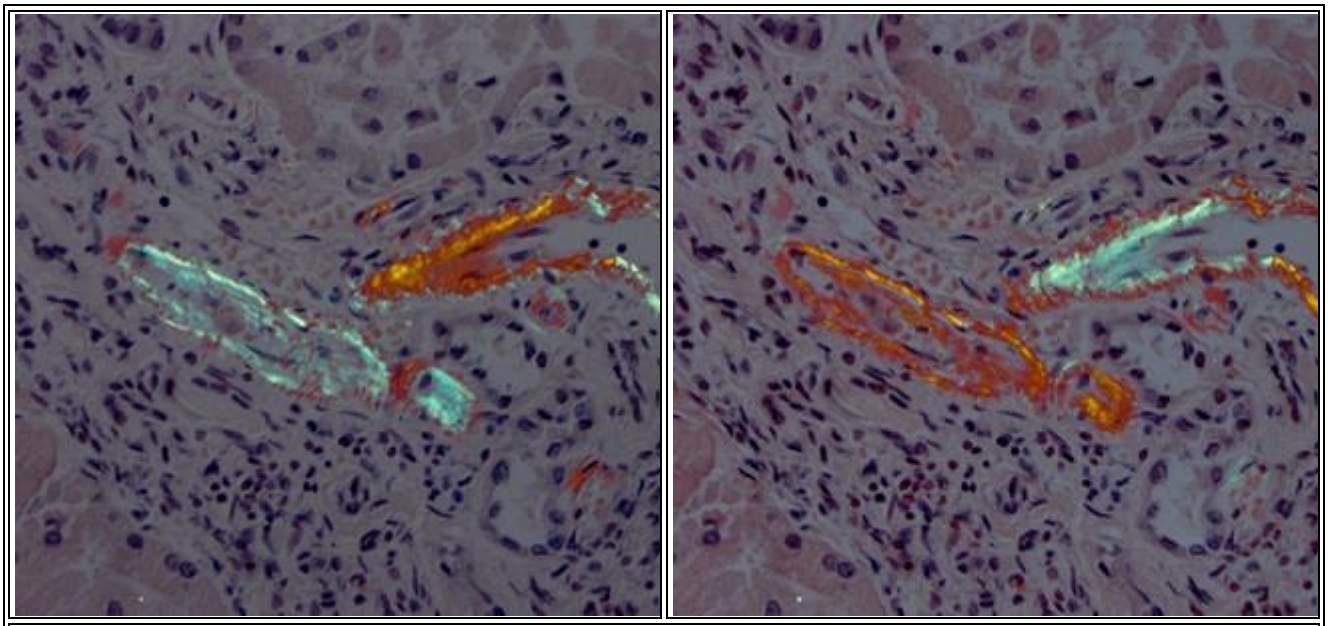
Kreyberg, L.: Main histological types of primary epithelial lung tumors. Brit. J. Cancer 25:206-210, 1961.

MODIFIED PUCHTLER CONGO RED AMYLOID METHOD

WITH/WITHOUT POTASSIUM PERMANGANATE OXIDATION

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Amyloid material deposition in small vessels viewed in two different phase of light show reciprocal color patter ("apple green" and orange) (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 6 µm.

SOLUTIONS:

Acidified Potassium Permanganate Solution

Potassium permanganate ---------- 0.3 gm

Distilled water ---------- 100.0 ml

Sulfuric acid ---------- 0.2 ml

1.0% Oxalic Acid

Oxalic acid ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Modified Weigert's Iron Hematoxylin

Solution A

Hematoxylin, C.I. 75290 ---------- 2.0 gm

Alcohol, 90% ---------- 100.0 ml

Solution B

Ferric chloride, $FeCl_3 6H_2O$, 62% aqueous ---------- 4.0 ml

Distilled water ---------- 95.0 ml

Hydrochloric acid, concentrated ---------- 1.0 ml

For use mix equal parts of Solution A and Solution B.

0.5% Acid Alcohol Solution

Alcohol, 70% ---------- 1000.0 ml

Hydrochloric acid, concentrated ---------- 5.0 ml

Sodium Chloride-Alcohol Solution

Sodium chloride ---------- 2.5 gm

Distilled water ---------- 50.0 ml

Absolute alcohol ---------- 50.0 ml

Dissolve the sodium chloride in the distilled water and then add the absolute alcohol. This solution is stable for several months.

1% Potassium Hydroxide Solution

Potassium hydroxide ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Congo Red Solution

Sodium chloride – alcohol solution ---------- 50.0 ml

Congo red, C.I. 22120 ---------- 0.1 gm

1% Potassium hydroxide ---------- 0.5 ml

Dissolve the Congo red in the sodium chloride-alcohol solution, then add the potassium hydroxide. Filter through Whatman #4 filter paper. Prepare just before use. This solution is stable for about one month.

Staining Procedure:

- Stain duplicate slides. If only one Congo red is requested, do just one slide and omit steps 2-4.

1. Deparaffinize and hydrate all the slides to distilled water.

2. Oxidize one of each duplicate slide (one from each specimen, one from control block) for 5 minutes in acidified potassium permanganate solution. Hold other duplicate slides on water.

3. Rinse in two changes of distilled water.

4. Decolorize oxidized slides in 1% oxalic acid solution for 5 minutes.

5. Wash oxidized slides in running tap water for 1 minute and rinse in two changes of distilled water.

6. Stain all the slides with modified Weigert's iron hematoxylin for 10 seconds.

7. Wash briefly in running tap water and rinse in two changes of distilled water.

8. Place in acid alcohol solution for 5 seconds.

9. Wash well in running tap water and rinse in two changes of distilled water.

10. Place in 95% alcohol for 5 seconds.

11. Place in Congo red solution for 20 minutes.

12. Dehydrate in three changes of 95% alcohol and four changes of absolute alcohol.

13. Clear in xylene, three or four changes.

14. Mount with synthetic resin.

Staining Results:

In unoxidized sections, amyloid stains pink to red and demonstrates apple-green birefringence with polarizing microscopy and fluoresces orange to red when viewed with fluorescence microscopy. In oxidized sections, secondary (AA) amyloid shows diminished staining and fluorescence and does not exhibit green birefringence. Primary (AL) amyloid will retain the stain.

Comment:

Amyloidosis is a group of diseases involving the deposition of insoluble polymerized protein filaments (amyloid) in the interstitial spaces of blood vessels and in various organs including heart, kidneys, lung and liver. Two major types of amyloid exist: primary (AL) and secondary (AA) amyloid. Primary amyloid is characterized by deposition of enzymatically altered immunoglobulin kappa or lambda light chains. Secondary amyloid comprises an enzymatically altered serum protein, which is made in the liver. Deposition of AA amyloid is usually seen in response to chronic inflammatory processes including pulmonary infection, tuberculosis, rheumatoid arthritis, osteomyelitis, leprosy and an occasional neoplasia.

Amyloid can be stained with several dyes including thioflavin T, thioflavin S, crystal violet, methyl violet, sirius red, and the dye of choice, Congo red. Congo red stains amyloid-oriented filaments by deposition of the linear dye molecules with their long axis parallel to protein filaments. This

subsequently shows an apple-green birefringence when viewed by polarizing microscopy. We have found that the optimal way of viewing Congo red stained amyloid is by fluorescence microscopy. Small or weakly stained deposits of amyloid, which are often difficult to see by light or polarization microscopy, are readily apparent by fluorescence microscopy. We have, furthermore, not seen significant orange-red fluorescence from any tissue components other than amyloid, except for elastic fibers and coarse collagen fibers.

In this method a modified Weigert's iron hematoxylin is used as the counterstain instead of Harris' alum hematoxylin as prescribed by Puchtler. Differentiation of the iron hematoxylin with acid alcohol results in a clear background that allows better visualization of amyloid by light microscopy. The iron hematoxylin also quenches background fluorescence, thus facilitating viewing with a fluorescence microscope.

References:

Churukian, C.J.: Improved Puchtler's Congo Red Method for Demonstrating Amyloid. J. Histotechnol. 23:139-141, 2000.

Churukian, C.J. and Schenk, E.A.: Eastwood Congo red method for demonstrating amyloid. J. Histotechnol. 11:105-107, 1988.

Wright, J.R., Calkins, E. and Humphrey, R.L.: Potassium permanganate reaction in amyloidosis. Lab. Invest. 36:274-281, 1977.

Puchtler, H., et al.: On the binding of Congo red by amyloid. J. Histochem. Cytochem. 10:355-363, 1962.

LIEB'S CRYSTAL VIOLET METHOD FOR AMYLOID

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Cut paraffin sections at 6 µm.

SOLUTIONS:

Crystal Violet Solution

Crystal violet, C.I. 42555	1.4 gm
Alcohol, 95%	10.0 ml
Distilled water	300.0 ml
Hydrochloric acid, concentrated	1.0 ml

*Apathy's Mounting Media

Acacia (gum arabic) ---------- 50.0 gm

Sucrose (can sugar) ---------- 50.0 gm

Distilled water ---------- 150.0 ml

Sodium chloride ---------- 10.0 gm

Thymol ---------- 0.1 gm

Mix the acacia, sucrose and distilled water in a 500 ml beaker using a hot plate stirrer. Heat the solution to 58o-60o C. It usually takes 5-6 hours for the acacia to completely dissolve. Add distilled water as needed to maintain the original volume of the solution. Dissolve the sodium chloride and thymol in the solution. Filter through Pyrex glass wool fiber. Store in a refrigerator at 3o-6o C. Sodium chloride prevents "bleeding" and thymol acts as a preservative.

Staining Procedure:

- Use control slide.
    1. Deparaffinize and hydrate to distilled water.
    2. Stain in working crystal violet solution for five minutes.
    3. Wash in running tap water for five minutes and rinse in distilled water.
    4. Mount with Apathy's mounting media.

Staining Results:

Amyloid ---------- purplish violet

Other tissue elements ---------- blue

Comment:

*"Bleeding" or diffusion into the surrounding mounting media of basic aniline dyes, tends to occur with aqueous mounting media. Because this Apathy's mounting media has been modified form the original formula by the addition of sucrose and sodium chloride, diffusion of the dye does not occur.

This crystal violet method does not stain all types of amyloid as well as Puchtler's Congo red technique. However, it usually better demonstrates amyloid in heart muscle than the Congo red method.

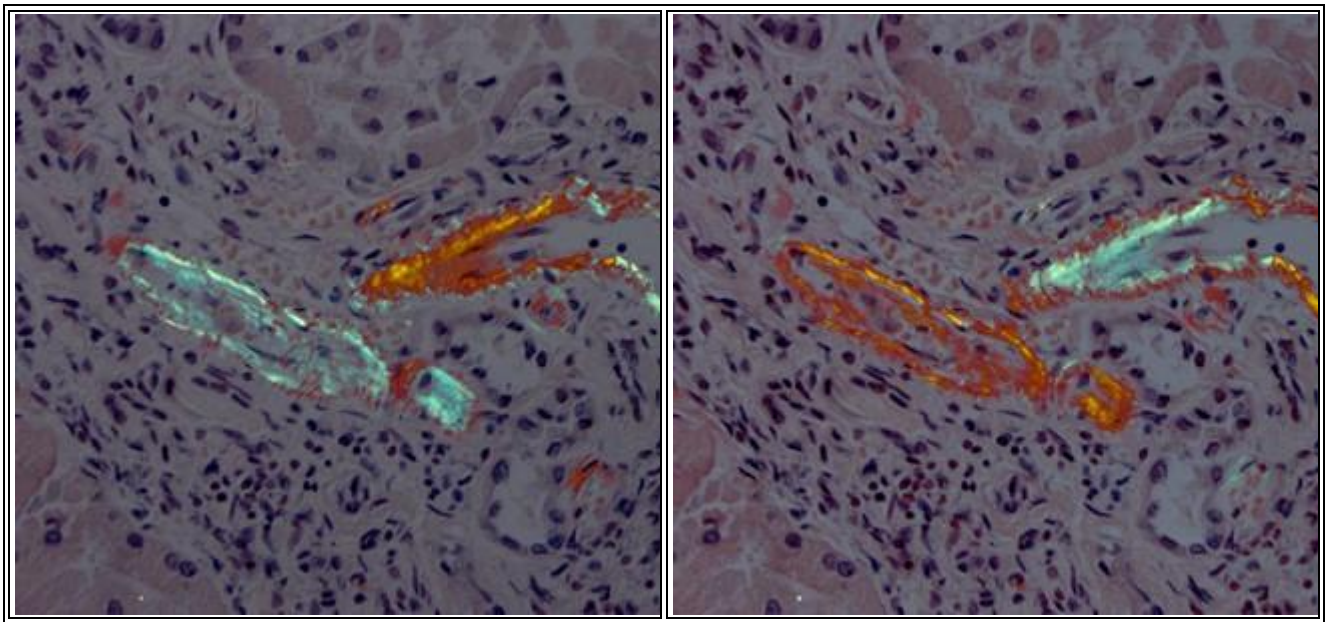
References:

Lieb, E.: Permanent Stain for Amyloid, Amer. J. Clin. Path. 17:413-414, 1947.

MODIFIED PUCHTLER CONGO RED AMYLOID METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Amyloid material deposition in small vessels viewed in two different phase of light show reciprocal color patter ("apple green" and orange) (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin

TECHNIQUE: Paraffin sections cut at 6 microns.

SOLUTIONS:

Modified Weigert's Iron Hematoxylin

(see Puchtler's Congo Red Amyloid Method)

0.5% Acid Alcohol Solution

(see Puchtler's Congo Red Amyloid Method)

Sodium Chloride-Alcohol Solution

Sodium chloride ---------- 2.5 gm

Distilled water ---------- 50.0 ml

Absolute alcohol ---------- 50.0 ml

Dissolve the sodium chloride in the distilled water and then add the absolute alcohol. This solution is stable for several months.

1% Potassium Hydroxide

Potassium hydroxide ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Congo Red Solution

Sodium chloride-alcohol solution ---------- 50.0 ml

Congo red, C.I. 22120 ---------- 0.1 gm

1% Potassium hydroxide ---------- 0.5 ml

Dissolve the Congo red in the sodium chloride-alcohol solution, then add the hydroxide. Filter through Whatman #4 filter paper. This solution is stable for about one month.

Staining Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Place in modified Weigert's iron hematoxylin for 10 seconds.
3. Wash briefly in running tap water and rinse in two changes of distilled water.
4. Place in acid alcohol solution for 5 seconds.
5. Wash in running tap water for 1 minute.
6. Rinse in two changes of distilled water.
7. Place in 95% alcohol for 5 seconds.
8. Place in Congo red solution for 20 minutes.
9. Dehydrate in three changes of 95% alcohol and four changes of absolute alcohol.
10. Clear in xylene, three or four changes.
11. Mount with synthetic resin.

Staining Results:

Amyloid ---------- pink or red

Nuclei ---------- black

Erythrocytes ---------- pale orange

Eosinophil granules ---------- reddish orange

Comment:

In Puchtler's Congo red method, alum hematoxylin is used as the counterstain. This tends to produce a faint, undesirable, pink background stain. By using the modified Weigert's iron hematoxylin, in place of alum hematoxylin, no background staining occurs.

Puchtler's Congo red solution is prepared with a saturated solution of sodium chloride in 80% alcohol. The sodium chloride suppresses non-specific staining of collagen, elastic and muscle thus

enhancing the selectivity of the stain for amyloid. In developing this modification of Puchtler's method it was found that a less than saturated solution of sodium chloride in alcohol worked equally well as a saturated one in suppressing non-specific staining. Congo red is more soluble in 50% alcohol than in 80% alcohol. The more concentrated solution produces better staining of amyloid and has much greater stability than Puchtler's Congo red solution.

In Puchtler's method the slides are treated with saturated sodium chloride in 80% alcohol for 20 minutes prior to staining with Congo red. We found this is unnecessary because it has no effect upon the final staining results.

References:

Churukian, C.J.: Improved Puchtler's Congo Red Method for Demonstrating Amyloid. J. Histotechnol. 23:139-141, 2000.

Puchtler, H. et al.: On the binding of Congo red amyloid. J. Histochem. Cytochem. 10:355-363, 1962.

Eastwood, H. and Cole, K.R.: Staining of amyloid by buffered Congo red in 50% ethanol. Stain Technol. 46:208-209, 1971.

Brown, G.: An Introduction to Histotechnology, New York, Appleton-Century-Crofts, 1978, pp. 318-319.

LIST OF STAINS FOR CONNECTIVE TISSUE

JONES METHOD FOR BASEMENT MEMBRANES

MICROWAVE ALCIAN BLUE PAS METHOD FOR KIDNEY SECTIONS

PAS METHOD FOR SKIN AND LIVER TRANSPLANT SECTIONS

GOMORIS ONE STEP TRICHROME METHOD

MASSONS TRICHROME STAIN

MASSONS TRICHROME STAIN FOR GMA PLASTIC BONE MARROW SECTIONS

MODIFIED MASSONS TRICHROME STAIN FOR CARDIAC TISSUES

MICROWAVE MALLORYS PHOSPHOTUNGSTIC ACID HEMATOXYLIN METHOD (PTAH)

VAN GIESONS METHOD FOR COLLAGEN

VERHOEFFS ELASTIC STAIN

MODIFIED FRAZER-LENDRUM METHOD FOR FIBRIN

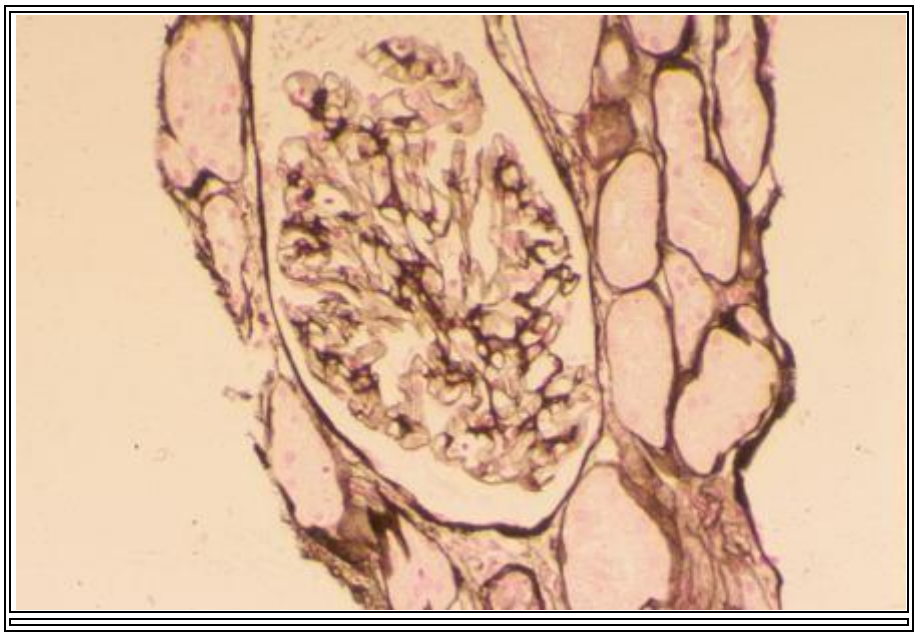
MODIFIED CARSTAIRS METHOD FOR FIBRIN AND PLATELETS

MODIFIED GOMORIS METHOD FOR RETICULUM

JONES' METHOD FOR BASEMENT MEMBRANES

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin or plastic sections cut at 2-3 μm.

SOLUTIONS:

0.5% Periodic Acid Solution

Periodic acid	0.5 gm
Distilled water	100.0 ml

0.5% Thiosemicarbazide

Thiosemicarbazide	0.5 gm
Distilled water	100.0 ml

5% Silver Nitrate Solution

Silver nitrate	1.0 gm
Distilled water	20.0 ml

3% Methenamine Solution

Hexamethylenetetramine (methenamine)	12.0 gm
Distilled water	375.0 ml
Ethylene glycol	25.0 ml

Methenamine-Silver Nitrate Solution (Stock)

Silver nitrate, 5% solution ---------- 20.0 ml

Methenamine, 3% solution ---------- 400.0 ml

This solution is good for about one month when stored in a

Refrigerator at 3o-6o C.

3% Sodium Borate (Borax) Solution

Sodium borate, Na2 B4 O710 H2O ---------- -- 3.0 gm

Distilled water ---------- 100.0 ml

Methenamine-Silver Nitrate Solution (Working)

Methenamine-silver nitrate solution (stock) ---------- ----- 20.0 ml

Distilled water ---------- 20.0 ml

Sodium borate, 3% solution ---------- 2.0 ml

Prepare fresh.

1% Gold Chloride (Stock)

Gold chloride ---------- 1.0 gm

Distilled water ---------- 100.0 ml

0.2% Gold Chloride (Working)

Gold chloride, 1%	20.0 ml
Distilled water	80.0 ml

2% Sodium Thiosulfate

Sodium thiosulfate	2.0 gm
Distilled water	100.0 ml

0.1% Nuclear Fast Red Solution

(See Gomori's Iron Stain)

Staining Procedure:

1. Deparaffinize and hydrate to distilled water. For plastic sections begin at Step 2.
2. Place in 0.5% periodic acid solution for 10 minutes for paraffin sections or for 15 minutes for plastic sections.
3. Rinse in four changes of distilled water.
4. Place in 0.5% thiosemicarbazide for 5 minutes.
5. Rinse with four changes of distilled water.
6. Place in working methenamine-silver nitrate solution and place in a 43o C water bath for 2 minutes. Transfer to a 58o C water bath for 15 minutes. Rinse in distilled water and check with a microscope. The basement membranes should be dark brown. If they are too light return to the hot methenamine-silver nitrate solution for 1 or 2 minutes. Check slides again. Because the staining reaction takes place rapidly care must be taken not to overstain the basement membranes.
7. Rinse with four changes of distilled water.
8. Tone in 0.2% gold chloride for 1 minutes.
9. Rinse in two changes of distilled water.
10. Place in 2% sodium thiosulfate for 1 minutes.

11. Rinse with four changes of distilled water.

12. Nuclear fast red solution for 3 minutes.

13. Rinse with three changes of distilled water.

14. Dehydrate paraffin processed sections in graded alcohols. Plastic sections must be air dried then dipped in xylene and mount.

15. Clear in xylene three or four changes and mount with synthetic resin.

Staining Results:

Basement membranes ---------- black

Reticulum fibers ---------- black

Nuclei ---------- red

Comment:

Treating the slides with thiosemicarbazide accelerates the reaction of methenamine silver with the glomerular basement membranes. This greatly reduces the time required to achieve satisfactory staining results in comparison with Jones original method. The addition of ethylene glycol to methenamine silver nitrate helps to stabilize the solution when it is heated. This usually prevents the formation of a mirror of silver on the slides and Coplin jar.

This stain is used mainly in kidney biopsies, which are sometimes embedded in plastic, to demonstrate glomerular and tubular basement membranes.

Plastic sections have a tendency to come off the slides with staining procedures which require heat. This problem can usually be avoided if the slides are laid flat on the bottom metal plate of a 60o C drying oven and are kept there for several hours. The use of alcohols for dehydrating plastic sections must be avoided as this causes the sections to either partially or completely come off the slide.

References:

Gomori, G.: A new histochemical test for glycogen and mucin. Amer. J. Clin. Path. 10:177-179, 1946.

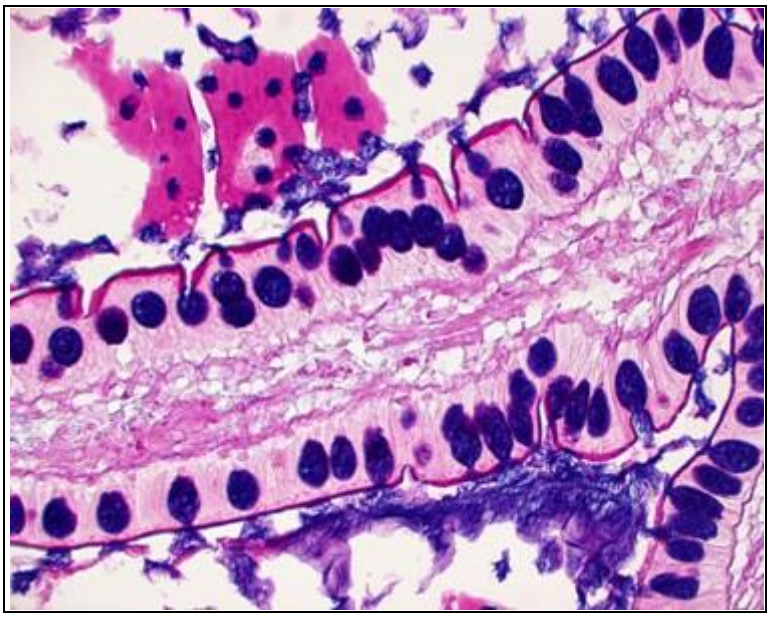
Jones, D.B.: Inflammation and repair of glomerulus. Amer. J. Path. 27:991-1009, 1951.

Hayashi, J.: Thiosemicarbazide used after periodic acid makes methenamine silver staining of renal glomerular basement membranes faster and cleaner. Stain Technol. 64:185-190, 1989.

MICROWAVE ALCIAN BLUE – PAS METHOD FOR KIDNEY SECTIONS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Intestine (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin or plastic sections cut at 3-4 μm.

SOLUTIONS:

3% Acetic Acid Solution

Acetic acid, glacial ---------- 3.0 ml

Distilled water ---------- 97.0 ml

1% Alcian Blue Solution

Alcian blue 8 GX, C.I. 74240 ---------- 1.0 gm

3% acetic acid ---------- 100.0 ml

Filter and add a few crystals of thymol.

0.5% Periodic Acid Solution

Periodic acid ---------- 0.5 gm

Distilled water ---------- 100.0 ml

Modified Lillie's Schiff Solution

(see PAS method)

0.3% Sodium Borate

Sodium borate (Na2 B4 O7 10H2O) ---------- 0.3 gm

Distilled water ---------- 100.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water. For plastic sections begin at Step 2.
2. Place in 3% acetic acid for three minutes.
3. Place in 40 ml of 1.0% alcian blue solution in a glass Coplin jar and microwave at power level 1 (100W) for 3 minutes. Dip the slides up and down several times and allow them to remain in the hot solution for 5 minutes.
4. Rinse in three changes of distilled water.
5. Place in 0.5% periodic acid for 15 minutes.
6. Rinse with four changes of distilled water.
7. Place in modified Lillie's Schiff Solution for 30 minutes.
8. Rinse in four changes of distilled water.
9. Place in 0.3% sodium borate for 15 seconds.

10. Rinse in four changes of distilled water.
11. Dehydrate in graded alcohols.
12. Clear in three or four changes of xylene.
13. Mount with synthetic resin.

Staining Results:

Normal and abnormal tubular and glomerular basement membranes, Bowman's capsule, elastic lamina of blood vessels, some renal casts, and juxtaglomerular granules are red. Epithelial cell mucoproteins and acid mucopolysaccharides in blood vessels are blue.

Comment:

When heating the alcian blue solution with microwave irradiation the top portion of the solution is warmer by 10-15o C than that near the bottom of the Coplin jar. Therefore, in order to equalize the temperature of the solution the slides are dipped up and down. This assures uniformity of staining throughout the tissue sections.

The stain may be performed at room temperature by staining the sections in the alcian blue solution for 30 minutes.

References:

Garvey, W.: Combined modified periodic acid-Schiff and batch staining method. J. Histotechnol. 15:117-120, 1992.

Lillie, R.D. and Fullmer, H.M.: Histopathologic Technic and Practical Histochemistry, 4th ed., New York, McGraw-Hill, 1976, pp. 307-308.

McManus, J.F. and Mowry, R.W.: Staining Methods Histologic and Histochemical, New York, Paul B. Hoeber, 1960, pp. 63-64.

PAS METHOD FOR SKIN AND LIVER TRANSPLANT SECTIONS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin

TECHNIQUE: Paraffin embedded sections cut at 4 μm.

SOLUTIONS:

0.5% Periodic Acid Solution

Periodic acid	0.5 gm
Distilled water	100.0 ml

Modified Lillie's Schiff Solution

(see PAS method)

0.3% Sodium Borate

Sodium borate (Na2 B4 O7 10H2O)	0.3 gm
Distilled water	100.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in 0.5% periodic acid for 15 minutes.
3. Rinse in four changes of distilled water.

4. Place in modified Lillie's Schiff solution for 30 minutes.
5. Rinse in four changes distilled water.
6. Place in 0.3% sodium borate for 15 seconds.
7. Rinse in four changes of distilled water.
8. Stain in acidified Lillie-Mayer's hematoxylin for 45 seconds.
9. Rinse in three changes of distilled water.
10. Blue hematoxylin in 0.3% sodium borate for 15 seconds.
11. Rinse in four changes of distilled water.
12. Dehydrate in graded alcohols.
13. Clear in three or four changes of xylene.
14. Mount with synthetic resin.

Staining Results:

PAS positive substances ---------- red

Nuclei ---------- blue

Comment:

This method gives more intense staining of PAS positive substances. This is accomplished by oxidizing longer than the usual time in periodic acid and staining longer than usual in modified Lillie's Schiff reagent. This intensified staining helps to better visualize the basement membrane of skin biopsies and the bile ducts of transplant liver biopsies.

References:

Garvey, W. et al: Combined modified periodic acid-Schiff and batch staining method. J. Histotechnol. 15:117-120, 1992.

Lillie, R.D. and Fullmer, H.W.: Histopathologic Technic and Practical Histochemistry, 4th Ed., New York, McGraw-Hill, 1976, pp. 206, 307-308.

GOMORI'S ONE STEP TRICHROME METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Bouin's Solution

Picric acid, saturated aqueous	75.0 ml
Formalin, concentrated, 37-40%	25.0 ml
Acetic acid, glacial	5.0 ml

Modified Weigert's Iron Hematoxylin

Solution A

Hematoxylin crystals, C.I. 75290	2.0 gm
90% ethyl alcohol	100.0 ml

Solution B

Ferric chloride, FeCl3, 6H20, 62% aqueous ---------- 4.0 ml

Distilled water ---------- 95.0 ml

Hydrochloric acid, concentrated ---------- 1.0 ml

For use mix equal parts of Solution A and Solution B.

Trichrome Stain

Chromotrope 2R, C.I. 16570 ---------- 0.6 gm

Aniline blue, C.I. 42755 ---------- 0.3 gm

Acetic acid, glacial ---------- 1.0 ml

Phosphotungstic acid ---------- 0.8 gm

Distilled water ---------- 100.0 ml

Fast green F.C.F. or light green S.F. may be substituted for aniline blue if it is more desirable to have collagen stained green.

0.5% Acetic Acid

Acetic acid, glacial ---------- 0.5 ml

Distilled water ---------- 99.5 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in Bouin's solution which has been preheated to 58o C for 10 minutes.
3. Wash in running tap water until the yellow color disappears and then rinse in distilled water.

4. Modified Weigert's iron hematoxylin for five minutes.
5. Wash briefly in running water, and rinse in two changes of distilled water.
6. Place in 0.5% hydrochloric acid in 70% alcohol for 5 seconds.
7. Wash in running tap water for 30 seconds and rinse in two changes of distilled water.
8. Trichrome stain for 15 minutes.
9. Acetic acid water, 0.5% for 10 seconds and rinse in two changes of distilled water.
10. Dehydrate in graded alcohols.
11. Clear in xylene, three or four changes.
12. Mount with synthetic resin.

Staining Results:

Muscle fibers	red
Collagen	blue
Nuclei	blue to black
Erythrocytes	red

Comment:

Treatment with hot Bouin's solution is necessary to obtain satisfactory staining of muscle and collagen. Without this pretreatment color differentiation of muscle and collagen will be very poor.

Plastic sections may be stained with this method, but the quality of the stain tends to be less satisfactory than with paraffin sections.

References:

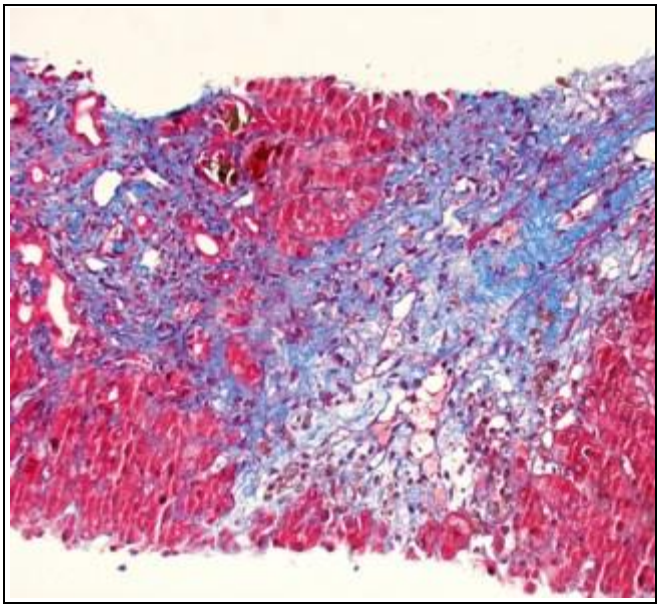
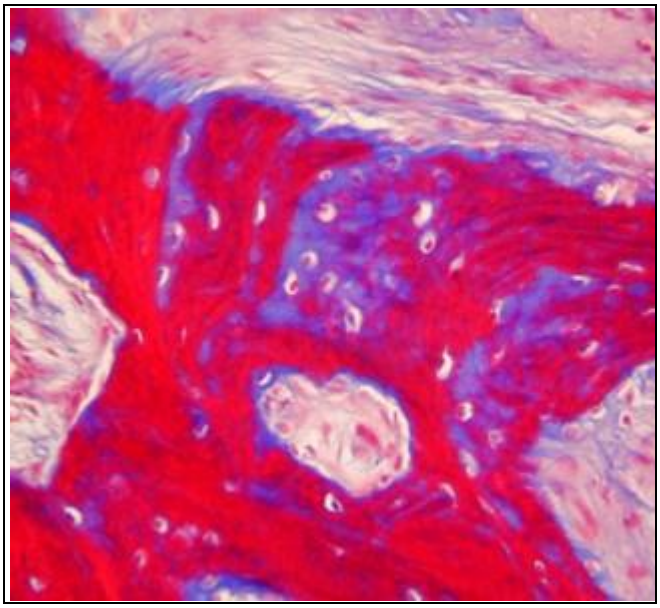
Elbadawi, A.: Hexachrome modification of Movat's stain. Stain Technol. 51:249-253, 1976.

Gomori, G.: A rapid one-step trichrome stain. Am. J. Clin. Path. 20:661-664, 1950.

MASSON'S TRICHROME

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

	
Fibrosis in a cirrhotic liver (Courtesy of Dr. Zhenhong Qu)	Bone (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin or Bouin’s.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Bouin’s Solution

Picric acid, saturated aqueous ---------- 75.0 ml

Formalin, concentrated, 37-40% ---------- 25.0 ml

Acetic acid, glacial ---------- 5.0 ml

Modified Weigert's Iron Hematoxylin

Solution A

Hematoxylin crystals, C.I. 75290 ---------- 2.0 gm

Alcohol, 90% ---------- 100.0 ml

Solution B

Ferric chloride, FeCl3, 6H20, 62% aqueous ---------- 4.0 ml

Distilled water ---------- 95.0 ml

Hydrochloric acid, concentrated ---------- 1.0 ml

Working Modified Weigert's Iron Hematoxylin

Equal parts of Solution A and Solution B

Biebrich Scarlet-Acid Fuchsin Solution

Biebrich scarlet, C.I. 26905 ---------- 0.45 gm

Acid fuchsin, C.I. 42685 ---------- 0.05 gm

Acetic acid, glacial ---------- 0.50 gm

Distilled water ---------- 50.0 ml

Phosphomolybdic-Phosphotungstic Acid Solution

Phosphomolybdic acid ---------- 2.5 gm

Phosphotungstic acid ---------- 2.5 gm

Distilled water ---------- 100.0 ml

Aniline Blue Solution

Aniline blue, C.I. 42755 ---------- 1.0 gm

Acetic acid, glacial ---------- 0.8 ml

Distilled water ---------- 40.0 ml

1% Acetic Acid Solution

Acetic acid, glacial ---------- 1.0 ml

Distilled water ---------- 100.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Mordant in Bouin's solution which has been preheated to 58o C for 15 minutes.
3. Wash in running water until the yellow color disappears and rinse in two changes of distilled water.
4. Modified Weigert's iron hematoxylin for 5 minutes.
5. Wash briefly in running water and rinse in two changes of distilled water.
6. Place in 0.5% hydrochloric acid in 70% alcohol for 5 seconds.
7. Wash in running tap water for 30 seconds and rinse in two changes of distilled water.
8. Biebrich scarlet-acid fuchsin solution for 5 minutes.
9. Rinse in three changes of distilled water.
10. Phosphomolybdic-phosphotungstic acid solution for 5 minutes.
11. Aniline blue solution for 20 minutes.

12. Rinse in distilled water.

13. Acetic acid solution for 10 seconds.

14. Rinse in two changes of distilled water.

15. Dehydrate in graded alcohols.

16. Clear in three or four changes of xylene.

17. Mount with synthetic resin.

Staining Results:

Nuclei ---------- black

Cytoplasm, keratin, muscle fibers and fibrin ---------- red

Collagen ---------- blue

Comment:

Treatment of the tissue sections with hot Bouin's solution is essential in order to obtain satisfactory results. Some authors recommend treating the tissue section for 30-60 minutes in hot Bouin's. This sometimes causes tissue sections to become detached from the slides. The 15-minute exposure to hot Bouin's is adequate with much less tendency for tissue sections to detach from the slides.

This stain is mainly ordered for kidney biopsies. The Gomori-trichrome method is used for other tissues because it is easier to perform and provides equally satisfactory staining of collagen and muscle.

References:

Masson, P.J.: Some histological methods. Trichrome stainings and their preliminary technique. J. Techn. Methods 12:75-90, 1929.

MASSON'S TRICHROME STAIN FOR GMA

PLASTIC BONE MARROW SECTIONS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

TECHNIQUE: Undecalcified glycol methacrylate embedded bone marrow sections cut at 4 μm.

SOLUTIONS:

Modified Weigert's Iron Hematoxylin

Solution A

Hematoxylin, C.I. 75290 ---------- - 2.0 gm

Alcohol, 90% ---------- 100.0 ml

Solution B

Ferric chloride, FeCl3 . 6H20, 62% aqueous ---------- 4.0 ml

Distilled water ---------- 95.0 ml

Hydrochloric acid, concentrated ---------- 1.0 ml

Working Modified Weigert's Iron Hematoxylin

Equal parts of Solution A and Solution B

Biebrich Scarlet-Acid Fuchsin Solution

Biebrich scarlet, C.I. 26905 ---------- 0.45 gm

Acid fuchsin, C.I. 42685 ---------- 0.05 gm

Acetic acid, glacial ---------- 0.50 ml

Phosphomolybdic-Phosphotungstic Acid Solution

Phosphomolybdic acid ---------- 2.5 gm

Phosphotungstic acid ---------- 2.5 gm

Distilled water ---------- 100.0 ml

Aniline Blue Solution

Aniline blue, C.I. 42755 ---------- 1.0 gm

Distilled water ---------- 40.0 ml

Acetic acid, glacial ---------- 0.8 ml

1% Acetic Acid Solution

Acetic acid, glacial ---------- 1.0 ml

Distilled water ---------- 99.0 ml

Staining Procedure:

1. Place in modified Weigert's iron hematoxylin for 5 minutes.
2. Wash briefly in running tap water and rinse in two changes of distilled water.
3. Decolorize with 0.5% hydrochloric acid in 70% alcohol for 5 seconds.
4. Wash in running tap water for 30 seconds and rinse in two changes of distilled water.
5. Place in biebrich scarlet-acid fuchsin solution for 30 minutes.
6. Rinse in three changes of distilled water.
7. Place in phosphomolybdic-phosphotungstic acid for 10 minutes.
8. Rinse in two changes of distilled water.
9. Place in aniline blue solution for 7 minutes.
10. Rinse in two changes of distilled water.
11. Place in 1% acetic acid for 1 minute.
12. Rinse in three changes of distilled water and air dry.
13. Dip in xylene and mount with synthetic resin.

Staining Results:

Osteoid seams ---------- red

Mineralized bone ---------- blue

Nuclei ---------- dark gray

References:

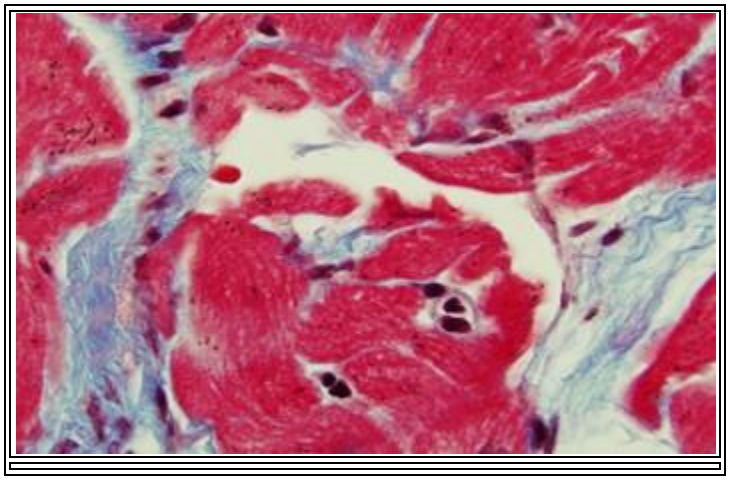
Masson, P.J.: Some histological methods. Trichrome stainings and their preliminary technique. J. Tech. Methods 12:75-90, 1929.

Elbadawi, A.: Hexachrome modification of Movat's stain. Stain Technol. 51:249-253, 1976.

MODIFIED MASSONS TRICHROME STAIN FOR CARDIAC TISSUES

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Modified Weigerts Iron Hematoxylin

Solution A

Hematoxylin, C.I. 75290 ---------- 2.0 gm

90% ethyl alcohol ---------- 100.0 ml

Solution B

Ferric chloride, FeCl3, 6H20, 62% ---------- 4.0 ml

Distilled water ---------- 95.0 ml

Hydrochloric acid, concentrated ---------- 1.0 ml

Working Modified Weigerts Iron Hematoxylin

Equal parts of Solution A and Solution B

Alcoholic Picric Acid Solution

Picric acid, saturated aqueous ---------- 10.0 ml

Alcohol, 95% ---------- 40.0 ml

1% Ponceau S Solution

Ponceau S, C.I. 27195 ---------- 0.5 gm

Distilled water ---------- 50.0 ml

Acetic acid ---------- 0.5 ml

1% Phosphomolybdic Acid Solution

Phosphomolybdic acid ---------- - 1.0 gm

Distilled water ---------- 100.0 ml

Aniline Blue Solution

Aniline blue, C.I. 42755 ---------- -- 1.0 gm

Distilled water ---------- 44.0 ml

Acetic acid ---------- 0.9 ml

1% Acetic Acid Solution

Acetic acid ---------- 1.0 ml

Distilled water ---------- 99.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in modified Weigerts iron hematoxylin for 5 minutes.
3. Wash briefly in running tap water and rinse in two changes of distilled water.
4. Decolorize with 0.5% hydrochloric acid in 70% alcohol for 5 seconds.
5. Wash in running tap water for 1 minute.
6. Rinse in two changes of distilled water.
7. Place in alcoholic picric acid alcohol solution for 3 minutes.
8. Rinse with three changes of distilled water.
9. Place in ponceau S solution in a glass Coplin jar. Place in a microwave oven and microwave at power level 1 (60W) for 3 minutes. Agitate slides for about 15 seconds and allow to set in the warm solution for 2 minutes.
10. Rinse with three changes of distilled water.
11. Place in phosphomolybdic acid solution for 5 minutes.

12. Rinse quickly with two changes of distilled water.

13. Place in aniline blue solution for 2 minute.

14. Place in 1% acetic acid solution for 1 minute.

15. Rinse quickly with two changes of distilled water.

16. Dehydrate in graded alcohols.

17. Clear in three or four changes of xylene.

18. Mount with synthetic resin.

Staining Results:

Nuclei ---------- blue-black

Intercellular fibers ---------- red

Collagen ---------- blue

Cytoplasm ---------- red

Mucin ---------- blue

Keratin ---------- red

Argentaffin granules ---------- black or red

Neuroglia fibers ---------- red

Comment:

References:

Masson, P.J.: Some histological methods. Trichrome stainings and their preliminary technique. J. Tech. Methods 12:75-90, 1929.

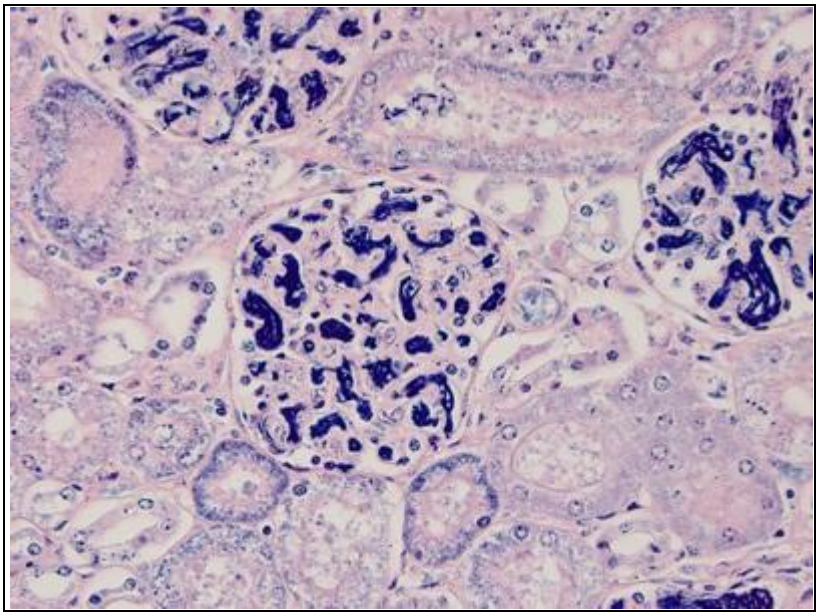
Clark, G.: Staining Procedures, 4th edition, Baltimore, Williams and Wilkins, 1981, pp. 118-120.

MICROWAVE MALLORY'S PHOSPHOTUNGSTIC ACID

HEMATOXYLIN METHOD (PTAH)

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Fibrin deposition in kidney (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.25% Potassium Permanganate Solution

Potassium permanganate ---------- 0.25 gm

Distilled water ---------- 100.00 ml

1% Oxalic Acid Solution

Oxalic acid ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Modified PTAH Solution

Solution A

Dissolve 0.5 gm of hematoxylin (C.I. 75290) in 400 ml of distilled water with the aid of gentle heat. The temperature of the solution should not exceed 60o C. Allow to cool for 20 minutes and add 0.089 gm of potassium permanganate. Allow to stand for one hour.

Solution B

Dissolve 10 gm of phosphotungstic acid in 100 ml of distilled water.

PTAH Staining Solution

Combine solutions A and B and let stand for one hour. Store in an amber bottle away from strong light. The solution is stable for at least several months.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate slides to distilled water.
2. Potassium permanganate solution for 5 minutes.

3. Rinse in three changes of distilled water.

4. Bleach in oxalic acid solution for 2 minutes.

5. Wash in running water for 1 minute and rinse with two changes of distilled water.

6. Mordant in 40 ml of Zenker's solution that contains 5% acetic acid in a glass Coplin jar and microwave at power level 1 (60W) for 1 minute. Transfer to a 37o C oven for 2 ½ hours. An alternate method is to place the slides in Zenker's solution overnight at room temperature.

7. Wash in running water 3 minutes.

8. Two changes of distilled water.

9. Place in 40 ml of PTAH solution in a glass Coplin jar and microwave at power level 1 (60W) for 1 minute. Transfer to a 37o C oven for 3 hours. An alternate method is to place the slides in PTAH solution overnight at room temperature.

10. Transfer slides directly to 95% alcohol in Coplin jars, three changes.

11. Dehydrate with absolute alcohol, four changes.

12. Clear in xylene, three or four changes.

13. Mount with synthetic resin.

Staining Results:

Nuclei ---------- blue

Skeletal and cardiac muscle ---------- blue, with cross striations well defined

Fibrin ---------- blue

Fibroglia and microglia ---------- blue

Collagen ---------- reddish

Erythrocytes ---------- greyish blue

Glial fibers ---------- blue

Myelin ---------- blue

Comment:

Some authors recommend that the tissue sections be treated with potassium permanganate and oxalic acid after they have been mordanted in acidified Zenker's. We have found that this sequence tends to

give less satisfactory results in demonstrating cross striations of muscle than the sequence described in this method.

This stain is most frequently used to demonstrate fibrin. It is occasionally used to demonstrate skeletal muscle cells (cross striations) in mesenchymal tumors, and the degree of skeletal muscle degenerative changes in various myopathies. In the central nervous system it is useful for staining glial fibers.

References:

Mallory, F.B.: Pathological Technique, New York, Hafner, 1968, pp. 76-77.

Meloan, S.N. and Puchtler, H: On the chemistry of phosphotungstic acid-hematein: development of a rapidly ripening PTAH solution. J. Histotechnol. 11:153-157, 1988.

VAN GIESON'S METHOD FOR COLLAGEN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Weigert's Iron Hematoxylin Solution

(see Masson's Trichrome Stain)

Van Gieson's Solution

Acid fuchsin, C.I. 42685, 1% aqueous ---------- 4.0 ml

Picric acid, saturated aqueous ---------- 40.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Weigert's iron hematoxylin solution for 5 minutes.
3. Wash briefly in running tap water and rinse in two changes of distilled water.
4. Van Gieson's solution for three minutes.
5. Dehydrate in three changes of 95% alcohol (Coplin jars) and complete dehydration in three changes of absolute alcohol.
6. Clear in three or four changes of xylene.
7. Mount with synthetic resin.

Staining Results:

Collagen ---------- red

Muscle ---------- yellow

Cornified epithelium ---------- yellow

Nuclei ---------- blue-black

Comment:

The main advantage of the Van Gieson stain is that it provides for a sharp contrast between collagen (red) and everything else (yellow). It is also quite useful as a counterstain in various other procedures, i.e., Verhoeff's and Iron Gallein elastic methods, especially when these are used in tissue such as cirrhotic liver.

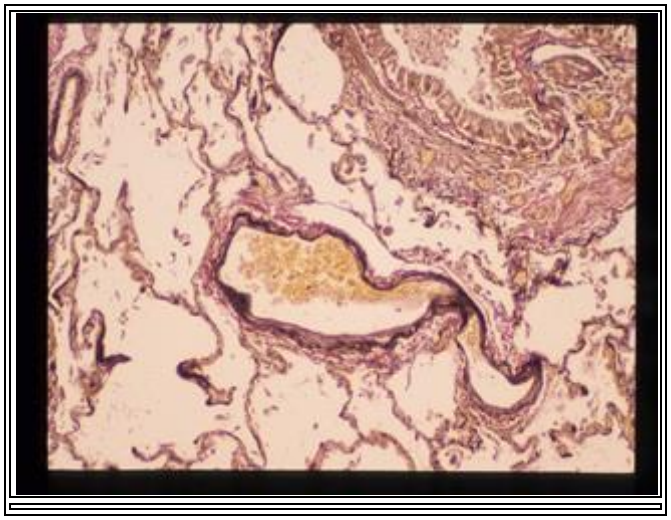
References:

Mallory, F.B.: Pathological Technique, New York, Hafner, 1968, pp. 92-93, 152-153.

VERHOEFF'S ELASTIC STAIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 μm.

SOLUTIONS:

5% Alcoholic Hematoxylin

Hematoxylin crystals, C.I. 75290 ---------- 5.0 gm

Alcohol, 80% ---------- 100.0 ml

10% Ferric Chloride

Ferric chloride ($FeCl3 \cdot 6H20$) ---------- 10.0 gm

Distilled water ---------- 100.0 ml

Lugol's Iodine Solution

Potassium iodide ---------- 4.0 gm

Iodine ---------- 2.0 gm

Distilled water ---------- 100.0 ml

Dissolve the potassium iodide in a little of the distilled water, add the iodine and dissolve. Add the remainder of the distilled water.

Verhoeff's Staining Solution

5% alcohol hematoxylin ---------- 20.0 ml

10% ferric chloride ---------- 10.0 ml

Lugol's iodine ---------- 10.0 ml

Prepare fresh each time.

2% Ferric Chloride

10% Ferric Chloride ($FeCl_3 \cdot 6H_2O$) ---------- 10.0 ml

Distilled water ---------- 40.0 ml

Van Gieson's Stain

Acid Fuchsin, C.I. 42685 ---------- 0.1 gm

Picric acid, saturated aqueous (approximately 1.4%) ---------- 100.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in Verhoeff's staining solution for 10 minutes.
3. Wash briefly in running tap water and rinse in two changes of distilled water.
4. Differentiated in 2% ferric chloride for 15 seconds. Stop the differentiation with two changes of distilled water. Check microscopically for black elastic fiber staining and gray background. Repeat 2% ferric chloride treatment and distilled water rinses as necessary for adequate elastic fiber demonstration. It is better to slightly under differentiate the tissue, because the subsequent Van Gieson counterstain will extract some of the elastic stain. Brain and heart tissue should be less differentiated with ferric chloride.
5. Rinse in two changes of distilled water.
6. Counterstain in Van Gieson's solution for 1 minute.
7. Dehydrate in three changes of 95% alcohol (in Coplin jars) and complete dehydration in four changes of absolute alcohol.
8. Clear in three or four changes of xylene.
9. Mount with synthetic resin.

Staining Results:

Elastic fibers ---------- black

Nuclei ---------- blue to black

Collagen ---------- red

Other tissue elements ---------- yellow

Comment:

This is our most popular stain for elastic tissue. The only problem is that not every technologist will differntiate the slides the same way in Step #4. Therefore, there will be some variation in the quality of the staining results.

References:

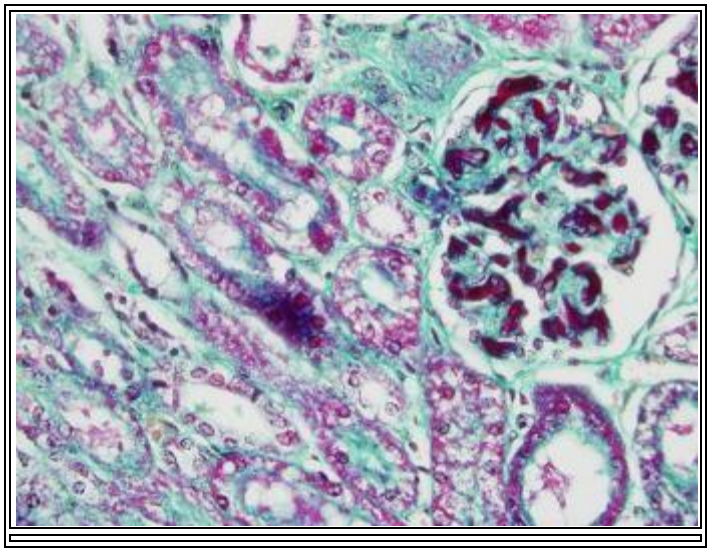
Mallory, F.B.: Pathological Technique, New York, Hafner, 1968, pp. 170-171.

Sheehan, D.C. and Hrapchak, B.B.: Theory and Practice of Histotechnology, St. Louis, The C.V. Mosby Co., 1980, pp. 196-197.

MODIFIED FRAZER-LENDRUM METHOD FOR FIBRIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Zenker's Solution, Modified

Zinc acetate	3.0 gm
Potassium dichromate	7.5 gm
Distilled water	300.0 gm
Acetic acid, glacial	15.0 ml

Dissolve the zinc acetate and potassium dichromate in the distilled water and then add the acetic acid.

Modified Weigert's Iron Hematoxylin

(See Gomori's One Step Trichrome Method)

0.5% Acid Alcohol Solution

(See Truant's Method for Acid Fast Bacilli)

Orange G-Picric Acid Solution

Picric acid, saturated aqueous	100.0 ml
Orange G, C.I. 16230	1.0 gm

1% Acid Fuchsin Solution

Acid fuchsin, C.I. 42685 ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Glacial acetic acid ---------- 1.0 ml

Tartarazine Differentiating

Tartarazine, C.I. 19140 ---------- 1.0 gm

Picric acid, saturated aqueous ---------- 100.0 ml

0.2% Light Green Solution

Light green, SF yellowish, C.I. 42095 ---------- 0.2 gm

Distilled water ---------- 100.0 ml

Glacial acetic acid ---------- 1.0 ml

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Place in Zenker's prepared with zinc acetate overnight at room temperature. An alternate method is to place in 40 ml. of Zenker's solution in a glass Coplin jar and microwave at power level 1 (60W) for 1 minute. Transfer to a 37o C oven for 3 hours.
3. Wash in running water for 3 minutes and rinse in two changes of distilled water.
4. Modified Weigert's iron hematoxylin for 5 minutes.
5. Wash in running water for 1 minute and rinse in distilled water.
6. Place in acid alcohol for 5 seconds.
7. Wash in running water for 1 minute and rinse in distilled water.
8. Orange G-picric acid solution for 5 minutes.
9. Rinse in 3 changes of distilled water.

10. Acid fuchsin solution for 5 minutes.
11. Rinse in 3 changes of distilled water.
12. Tartarazine differentiating solution for 2 minutes.
13. Rinse with 3 changes of distilled water.
14. Light green solution for 15 seconds.
15. Rinse in 3 changes in distilled water.
16. Dehydrate in graded alcohols.
17. Clear in xylene, three or four changes.
18. Mount with synthetic resin.

Staining Results:

Fibrin, keratin, some cytoplasmic granules ---------- red

Erythrocytes ---------- orange

Muscle ---------- may stain red

Collagen ---------- green

Comment:

We have found this staining method to be useful in demonstrating fibrin. More consistent staining of fibrin, and better contrast with background is obtained than with the Mallory PTAH method.

The use of Zenker's prepared with zinc acetate does not produce the precipitate that results when mercuric chloride is used to prepare Zenker's solution. This eliminates the necessity of treating the tissue sections with Lugol's iodine and sodium thiosulfate.

This modified method greatly reduces the amount of picric acid needed to prepare the solutions that require picric acid. The staining results are as good as, if not better, than the original Frazer-Lendrum technique.

References:

Lendrum, A.C., et al.: Studies on the character and staining of fibrin. J. Clin. Path. 15:401-413, 1962.

Luna, L.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Ed., New York, McGraw-Hill, 1968, pp. 81-82.

MODIFIED CARSTAIR'S METHOD FOR FIBRIN AND PLATELETS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin for 48 hours or longer.

TECHNIQUE: Paraffin sections cut at 5 μm.

SOLUTIONS:

Modified Weigert's Iron Hematoxylin

(See Gomori's One Step Trichrome Method)

0.5% Acid Alcohol Solution

(See Truant's Method for Acid Fast Bacilli)

Picro-Orange G Solution

Picric acid, saturated aqueous	100.0 ml
Orange G, C.I. 16230	1.0 gm

Ponceau-Fuchsin Solution

Acid fuchsin, C.I. 42685	0.5 gm
Ponceau 2R (Ponceau Xylidine) C.I. 16150	0.5 gm
Distilled water	99.0 ml
Acetic acid	1.0 ml

*Phosphomolybdic-Phosphotungstic Acid Solution

Phosphomolybdic acid	2.5 gm
Phosphotungstic acid	2.5 gm
Distilled water	100.0 ml

*This solution is the same one used in Masson's trichrome stain.

*Aniline Blue Solution

Aniline blue, C.I. 42755 ----------	-	1.0 gm
Distilled water ----------		40.0 ml
Acetic acid ----------		0.8 ml

*This solution is the same one used in Masson's trichrome stain.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Modified Weigert's iron hematoxylin for 5 minutes.
3. Wash in running water and rinse in distilled water.
4. Place in acid alcohol for 5 seconds.
5. Wash in running water for 1 minute and rinse in distilled water.
6. Picro-orange G solution for 3 minutes.
7. Rinse in three changes of distilled water.
8. Ponceau-fuchsin for 3 minutes.
9. Rinse in three changes of distilled water.
10. Differentiate in phosphomolybdic acid-phosphotungistic acid solution for 3 minutes.
11. Rinse in three changes of distilled water.
12. Aniline blue solution for 2 minutes.
13. Rinse in three changes of distilled water.
14. Dehydrate in graded alcohols and clear in xylene, three or four changes.
15. Mount with synthetic resin.

Staining Results:

Fibrin	clear bright red
Muscle	red
Platelets	leaden blue to navy blue
Collagen	blue
Erythrocytes	yellow or orange

Comment:

This is a difficult stain to evaluate. Results are occasionally erratic and platelet staining tends to be variable.

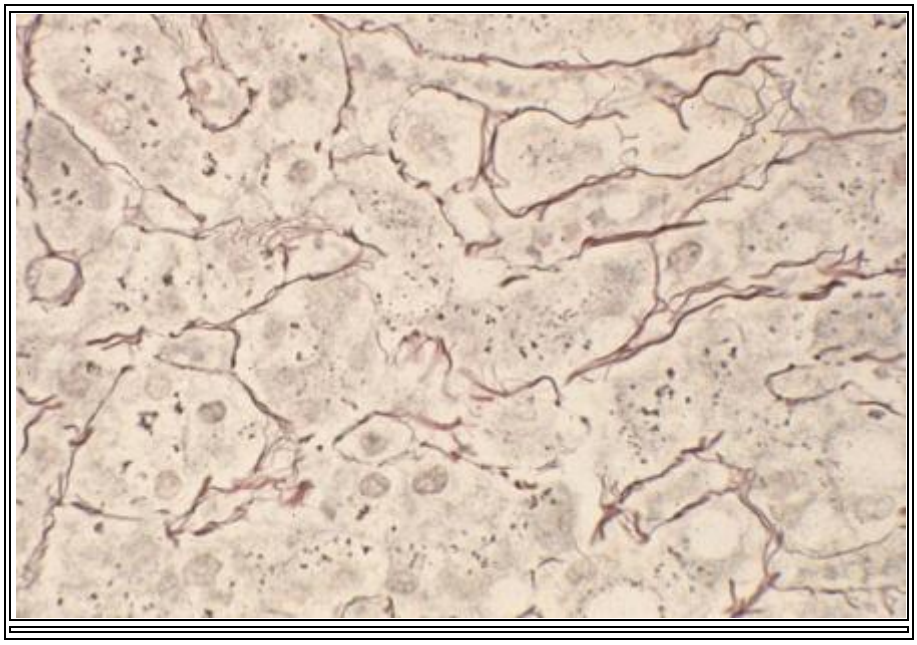
References:

Carstairs, K.C.: The identification of platelets and platelet antigens in histological sections. J. Path. Bact. 90:225, 1965.

MODIFIED GOMORI'S METHOD FOR RETICULUM

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin. Bouin's fixative should not be used.

TECHNIQUE: Paraffin sections cut at 5 μm or undecalcified, plastic embedded, bone marrow biopsies cut at 4 μm.

SOLUTIONS:

Acidified Potassium Permanganate

Potassium permanganate ---------- 0.3 gm

Distilled water ---------- 100.0 ml

Sulfuric acid ---------- 0.2 ml

This solution is good for about two days.

1% Potassium Metabisulfite

Potassium metabisulfite ---------- 1.0 gm

Distilled water ---------- 100.0 ml

10% Silver Nitrate

Silver nitrate ---------- 1.0 gm

Distilled water ---------- 10.0 ml

Store in a refrigerator at 3-6o C.

10% Potassium Hydroxide

Potassium hydroxide ---------- 10.0 gm

Distilled water ---------- 100.0 ml

Ammoniacal Silver Solution

To 10 ml of 10% silver nitrate add 2.5 ml of 10% potassium hydroxide. Then add concentrated ammonium hydroxide, drop by drop with constant shaking, until the precipitate just dissolves. Carefully add 10% silver nitrate, drop by drop, while shaking the container continuously, until the last drop causes the solution to become slightly cloudy. Make up the solution to 50 ml with distilled water. This solution is stable for about 7 days if stored in a refrigerator at 3-6o C.

10% Formalin

Formaldehyde, 38-40% ---------- 10.0 ml

Distilled water ---------- 90.0 ml

0.2% Gold Chloride

Gold chloride ---------- 0.2 gm

Distilled water ---------- 100.0 ml

0.2% Sodium Thiosulfate

Sodium thiosulfate ---------- 2.0 gm

Distilled water ---------- 100.0 ml

0.1% Nuclear Fast Red

(see Pearl's method for iron)

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Oxidize with acidified potassium permanganate for 3 minutes.
3. Rinse with three changes of distilled water.
4. Reduce with 1% potassium metabisulfite for 1 minute.
5. Wash with running tap water for three minutes and rinse with four changes of distilled water.
6. Ammoniacal silver solution for two minutes.
7. Rinse with three changes of distilled water.
8. Reduce in 10% formalin for 1 minute.

9. Wash with running tap water for 1 minute and rinse with two changes of distilled water.

10. Tone in 0.2% gold chloride for 30 seconds.

11. Rinse with two changes of distilled water.

12. Fix in 2% sodium thiosulfate for 1 minute.

13. Wash well with tap water and rinse with two changes of distilled water.

14. Counterstain with 0.1% nuclear fast red for 3 minutes.

15. Rinse with three changes of distilled water.

16. Dehydrate in graded alcohols.

17. Clear in xylene, three or four changes.

18. Mount with synthetic resin.

Staining Results:

Reticulum ---------- black

Nuclei ---------- red

Comment:

When preparing the ammoniacal silver solution it is important to use the smallest amount of ammonium hydroxide necessary to dissolve the precipitate resulting from the addition of potassium hydroxide to the silver nitrate. If too much ammonium hydroxide is used, not only will there be unsatisfactory staining of reticulum but sections are likely to become detached from the slides.

We have observed that silver nitrate crystals stored at room temperature will gradually take on a grayish-violet color. This does not occur when silver nitrate is kept refrigerated, even after several years. Solution made with silver nitrate which has become discolored are usable but from our observations, seem to be less stable than those prepared with non-discolored silver nitrate.

After preparing the ammoniacal silver solution, we place it in the refrigerator. Just prior to use the solution is brought to room temperature and immediately after use it is returned to the refrigerator. On a number of occasions we have observed that reticulum fibers stain better after the ammoniacal silver has aged in the refrigerator for a few days. After a few days the solution begins to take on a pale yellow-brown color which may or may not become progressively darker. This colorization does not appear to adversely affect the quality of the staining results. By using the same ammoniacal silver solution for several days we have been able to greatly reduce our consumption of silver nitrate with considerable cost savings. Extreme care should be exercised in the preparation and use of

ammoniacal silver. Store ammoniacal silver in a refrigerator to avoid the formation of explosive compounds. If ammoniacal is exposed to direct sunlight it will explode.

References:

Churukian, C.J.: Modified Gomori's method for staining reticulum and collagen. Histo-Logic 2:23, 1972.

Churukian, C.J.: Prolonging the shelf-life of solutions containing silver nitrate. Histo-Logic 10:147, 1980.

Gomori, G.: Silver impregnation of reticulum in paraffin sections. Amer. J. Clin. Path. 13:993-1002, 1937.

LIST OF STAINS FOR CYTOPLASMIC AND NUCLEAR ELEMENTS

MICROWAVE AMMONIACAL SILVER METHOD FOR ARGENTAFFIN AND MELANIN

MICROWAVE SCHMORLS METHOD FOR REDUCING SUBSTANCES

CHURUKIAN-SCHENK ARGYROPHIL METHOD

MICROWAVE CHURUKIAN-SCHENK ARGYROPHIL METHOD

MICROWAVE GIEMSA METHOD FOR PLASTIC BONE MARROW SECTIONS

ALDEHYDE FUCHSIN METHOD FOR HEPATITIS B ANTIGEN

MICROWAVE ORCEIN METHOD FOR HEPATITIS B SURFACE ANTIGEN

TOLUIDINE BLUE METHOD FOR MAST CELLS

LONG ZIEHL-NEELSEN METHOD FOR CEROID

METHYL GREEN PYRONIN Y METHOD FOR DNA AND RNA

ALDEHYDE FUCHSIN METHOD FOR PANCREATIC BETA CELL

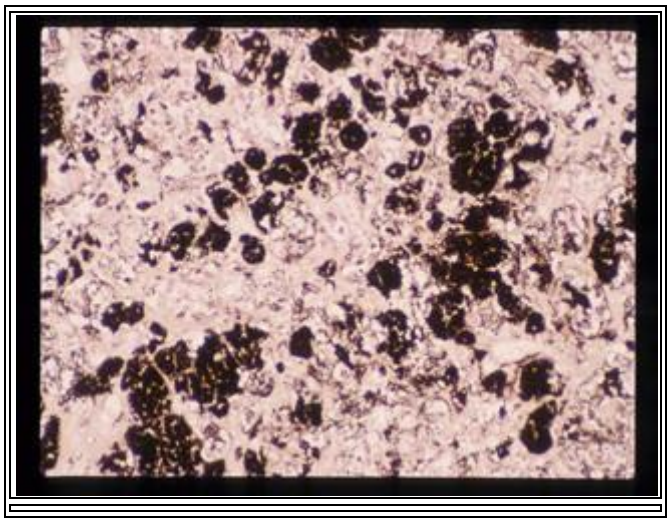
WILSON-EZRIN METHOD FOR PITUITARY

MICROWAVE AMMONIACAL SILVER METHOD

FOR ARGENTAFFIN AND MELANIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Ammoniacal Silver Solution

To 10 ml of 2% silver nitrate (0.2 gm silver nitrate in 10 ml of distilled water) add 5 ml of 0.8% lithium hydroxide, monohydrate (0.04 gm lithium hydroxide in 5 ml of distilled water). Then add concentrated ammonium hydroxide, drop by drop with constant shaking, until the precipitate just dissolves. Make up the solution to 200 ml with distilled water and store in a refrigerator at 3o-6o C. The solution is stable for at least one month.

0.2% Gold Chloride Solution

Gold chloride, 1% aqueous ---------- 20.0 ml

Distilled water ---------- 80.0 ml

2% Sodium Thiosulfate Solution

Sodium thiosulfate ---------- 2.0 gm

Distilled water ---------- 100.0 ml

Nuclear Fast Red (Kernechtrot) Solution

Dissolve 0.1 gm nuclear fast red in 100.0 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, add a few grains of thymol as a preservative.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.

2. Place slides in 40 ml of refrigerated cold ammoniacal silver solution in a plastic Coplin jar and microwave at power level 6 (360W) for 40 seconds. Gently agitate the Coplin jar for about 15 seconds. Microwave again at power level 6 for 40 seconds. Gently agitate the Coplin jar for about 15 seconds. Allow the slides to remain in the hot solution for 2-3 minutes or until the sections appear a medium brown. Check after 2 minutes.

3. Rinse sections with four changes of distilled water.

4. Gold chloride solution for 30 seconds.

5. Rinse with three changes of distilled water.

6. Sodium thiosulfate solution for 30 seconds.

7. Rinse with four changes of distilled water.

8. Nuclear fast red solution for 3 minutes.

9. Rinse with three changes of distilled water.

10. Dehydrate in graded alcohols.
11. Clear in xylene, three or four changes.
12. Mount with synthetic resin.

Staining Results:

Argentaffin cell granules, chromaffin, melanin and other

Silver reducing substances ---------- black

Nuclei ---------- pink

Comment:

This procedure is nonspecific for melanin since chromaffin and argentaffin granules as well as pigments such as lipofuscin and iron are also stained. To demonstrate that the stained material is melanin the melanin bleach procedure must also be done.

For many years we used the Fontana-Masson method to demonstrate argentaffin and melanin using a hot water bath or microwave oven. We found that dilute ammoniacal silver works better than the Fontana-Masson silver solution, requires much less silver nitrate, and has greater stability. The ammoniacal silver solution was originally developed for the demonstration of fungi and has for several years been used for that purpose in the Special Stains Laboratory.

References:

Churukian, C.J. and Schenk, E.A.: Dilute ammoniacal silver as a substitute for methenamine silver to demonstrate pneumocystis carinii and fungi. Lab. Med. 17:87-90, 1986.

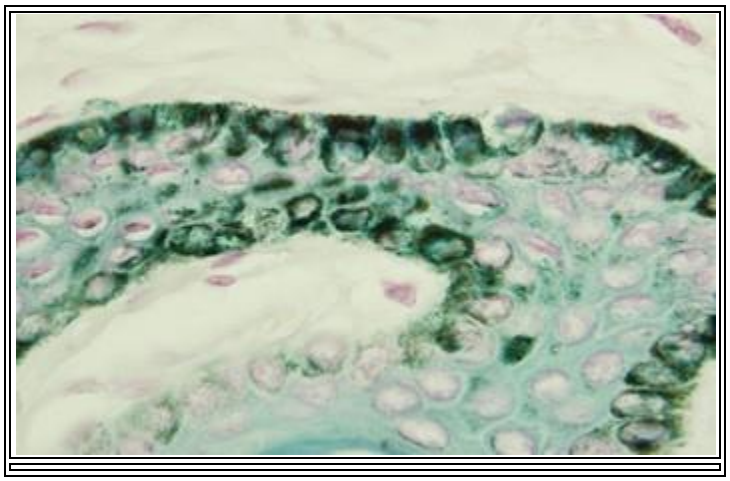
Brinn, N.T.: Rapid metallic histological staining using the microwave oven. J. Histotechnology 6:125-129, 1983.

Masson, P.: Carcinoids and nerve hyperplasia of the appendicular mucosa. Amer. J. Path. 4:181-212, 1928.

MICROWAVE SCHMORL'S METHOD FOR REDUCING SUBSTANCES

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

1% Ferric Chloride

Ferric Chloride ---------- 0.3 gm

Distilled water ---------- 30.0 ml

Prepare fresh.

0.4% Potassium Ferricyanide

Potassium ferricyanide ---------- 0.04 gm

Distilled water ---------- 10.0 ml

Prepare fresh.

Nuclear Fast Red (Kernechtrot) Solution

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, add a few grains of thymol as a preservative.

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Add 10 ml of 0.4% potassium ferricyanide to 30 ml of 1.0% ferric chloride. Pour into a glass Coplin jar, place slides into the solution and microwave at power level one (60W) for 1 ½ minutes. Dip the slides up and down several times and allow them to remain in the warm solution for 30 seconds.
3. Wash in running tap water for 1 minute and rinse in distilled water.
4. Counterstain with nuclear fast red solution for 3 minutes.
5. Rinse with three changes of distilled water.
6. Dehydrate in graded alcohols and clear in xylene, three or four changes.
7. Mount with synthetic resin.

Staining Results:

Argentaffin, chromaffin, lipofuscin and melanin ---------- blue to dark blue

Nuclei ---------- red

Comment:

This procedure will demonstrate the same pigments that stain with the Fontana-Masson silver method which are argentaffin, chromaffin, melanin, and lipofuscin. Performing the stain in a microwave oven produces better staining results than doing the stain at room temperature.

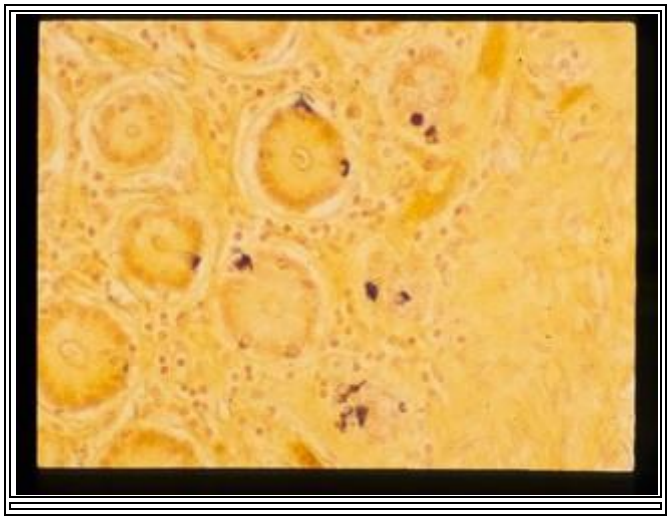
References:

Culling , C.F.A.: Handbook of Histopathological and Histochemical Techniques, 3rd Ed., Butterworths, 1974, p. 386.

CHURUKIAN-SCHENK ARGYROPHIL METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Citric Acid-Sodium Citrate Solution (Stock)

Citric acid, monohydrate ---------- 0.2 gm

Sodium citrate, dihydrate ---------- 0.2 gm

Distilled water ---------- 100.0 ml

The pH of this solution is approximately 3.8. Store in a refrigerator

at 3-6o C.

Citric Acid-Sodium Citrate Solution (Working)

Citric acid-sodium citrate (stock) ---------- 1.5 ml

Distilled water ---------- 150.0 ml

The pH of this solution is approximately 3.8.

0.5% Silver Nitrate Solution

Silver nitrate ---------- 0.2 gm

Citric acid-sodium citrate, working solution ---------- ----- 40.0 ml

Prepare fresh.

Bodian's Developer

Sodium sulfite (anhydrous) ---------- 2.0 mg

Hydroquinone ---------- 0.4 gm

Distilled water ---------- 40.0 ml

0.1% Nuclear Fast Red (Kernechtrot) Solution

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, add a few grains of thymol as a preservative.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to acidulated water pH 3.8.

2. Place sections in 0.5% silver nitrate solution and place in a 43o C water bath for 2 minutes. Transfer to a 58o C water bath for 2 hours. The solution must be freshly prepared.

3. Rinse in five changes of distilled water.

4. Transfer sections to Bodian's developer, which has been previously heated in a water bath to 58o C for 2 minutes.

5. Rinse in five changes of distilled water.

6. Reimpregnate in the same 0.5% silver nitrate solution in the 58o C water bath for 3 minutes.

7. Rinse in five changes of distilled water.

8. Place again in Bodian's developer in the 58o C water bath for 2 minutes.

9. Rinse in five changes of distilled water.

10. Place in the same 0.5% silver nitrate solution in the 58o C water bath for 2 minutes.

11. Rinse in five changes of distilled water.

12. Transfer slides to the Bodian's developer in the 58o C water bath for 2 minutes.

13. Rinse in four changes of distilled water.

14. Counterstain with 0.1% nuclear fast red for 1 minute.

15. Rinse in two changes of distilled water.

16. Dehydrate in graded alcohols.

17. Clear in three or four changes of xylene and mount with synthetic resin.

Staining Results:

Neuroendocrine cell granules (argentaffin and argyrophil) in various organs stain dark brown to black. Nuclei are red and the background a light yellow-orange.

Comment:

This staining method has been found, by Smith and Haggitt, to be a reliable and sensitive silver method for demonstrating secretory granules in carcinoid tumors. They, and others, have found the method to produce superior staining results when compared to the accepted method of Gremelius.

References:

Churukian, C.J. and Schenk, E.A.: A modification of Pascual's argyrophil method. J. Histotechnology 2:102-103, 1979.

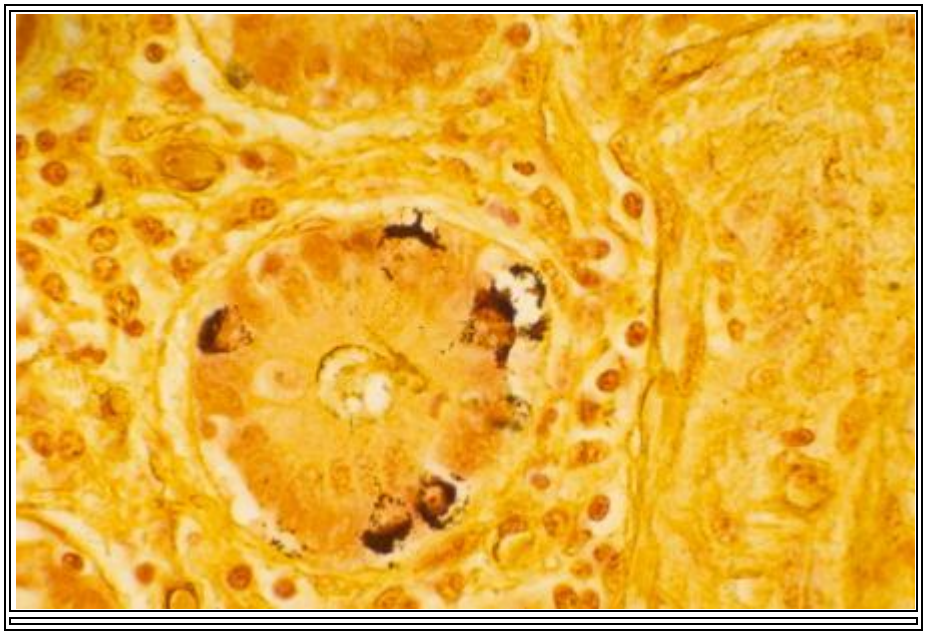
Pascual, J.S.: A new method for easy demonstration of argyrophil cells. Stain Technol. 51:231-235, 1976.

Smith, D.M. and Haggitt, R.C.: A comparative study of generic stains for carcinoid secretory granules. Amer. J. Surg. Pathol. 7:61-68, 1983.

MICROWAVE CHURUKIAN-SCHENK ARGYROPHIL METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 μm.

SOLUTIONS:

Citric Acid – Sodium Citrate Solution (Stock)

Citric acid, monohydrate ---------- 0.2 gm

Sodium citrate, dihydrate ---------- 0.2 gm

Distilled water ---------- 100.0 ml

The pH of this solution is approximately 3.8. Store in a refrigerator at 3-6o C.

Citric Acid – Sodium Citrate Solution (Working)

Citric acid – sodium citrate (stock) ---------- 1.5 ml

Distilled water ---------- 150.0 ml

The pH of this solution is approximately 3.8.

0.5% Silver Nitrate Solution

Silver nitrate ---------- 0.2 gm

Citrate acid – sodium citrate, working solution ---------- ---- 40.0 ml

Prepare fresh.

Bodian's Developer

Sodium sulfite (anhydrous) ---------- 2.0 gm

Hydroquinone ---------- 0.4 gm

Distilled water ---------- 40.0 ml

Prepare fresh.

0.1% Nuclear Fast Red (Kernechtrot Solution)

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, and add a few grains of thymol as a preservative.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to citric acid – sodium citrate working solution.
2. Place slides in 40 ml of 0.5% silver nitrate solution in a glass Coplin jar and microwave at power level 1 (60W) for 5 minutes. Dip the slides up and down several times and allow them to remain in the hot solution (about 80o C) for 10 minutes.
3. Place the slides in distilled water and transfer the Coplin jar containing the silver nitrate solution to a 58o C water bath. Rinse the slides with four changes of distilled water.
4. Transfer slide to the Bodian's developer in a glass Coplin jar which has been previously heated to 58o C in a water bath. Allow the slides to remain in the hot solution for 2 minutes.
5. Rinse in five changes of distilled water.
6. Place the slides in the 0.5% silver nitrate solution in the 58o C water bath for 3 minutes.
7. Rinse in five changes of distilled water.
8. Place the slides in the same Bodian's developer in the 58o C water bath for 2 minutes.
9. Rinse in five changes of distilled water.
10. Place the slides in the same 0.5% silver nitrate solution in the 58o C water bath for 2 minutes.
11. Rinse in five changes of distilled water.
12. Transfer slides to the Bodian's developer in the 58o C water bath for 2 minutes.
13. Rinse in four changes of distilled water.
14. Counterstain with 0.1% nuclear fast red for 1 minute.
15. Rinse in three changes of distilled water.
16. Dehydrate in graded alcohols.

17. Clear in three or four changes in xylene.

18. Mount with synthetic resin.

Staining Results:

Neuroendocrine cell granules (argentaffin and argyrophil) in various organs stain dark brown to black. Nuclei are orange to red the background a light yellow-orange.

Comment:

Argyrophil cells are found in the gastrointestinal mucosa, pancreas, trachea, bronchi, prostate and ovary. Tumors derived from these cells are called apudomas or neuroendocrine tumors. Argyrophil cells, as their names indicates, have an affinity for silver ions but lack the ability to convert the silver ions to the metallic state. This is why a reducing agent is needed in the staining procedure.

In Pascual's method and our modification of Pascual's method, tissue sections are double impregnated with silver nitrate followed by treatment with a reducing solution. In the described staining method a triple treatment with silver nitrate and the reducing solution is necessary in order to obtain optimal staining results.

In our modification of Pascual's argyrophil method, the pH of the distilled water is adjusted to 4.2 with dilute aqueous citric acid. This requires the use of a pH meter and is somewhat time consuming. The described method utilizes a citric acid – sodium citrate buffer with a pH of 3.8 that is easy to prepare. Like most buffer solutions ours is stable when stored in a refrigerator at 3-6o C.

The shelf-life of silver nitrate and hydroquinone can be greatly increased by storing the reagents in a refrigerator at 3-6° C

The use of a microwave oven, plus other modifications, reduces the staining time by more than one hour and thirty minutes from our original method without compromising the quality of the staining results.

References:

Brinn, N.T.: Rapid metallic histologic staining using the microwave oven. J. Histotechnol. 6:125-129, 1983.

Churukian, C.J. and Schenk, E.A.: A modification of Pascual's argyrophil method. J. Histotechnol. 2:102-103, 1979.

Pascual, J.S.: A new method for easy demonstration or argyrophil cells. Stain Technol. 51:231-235, 1976.

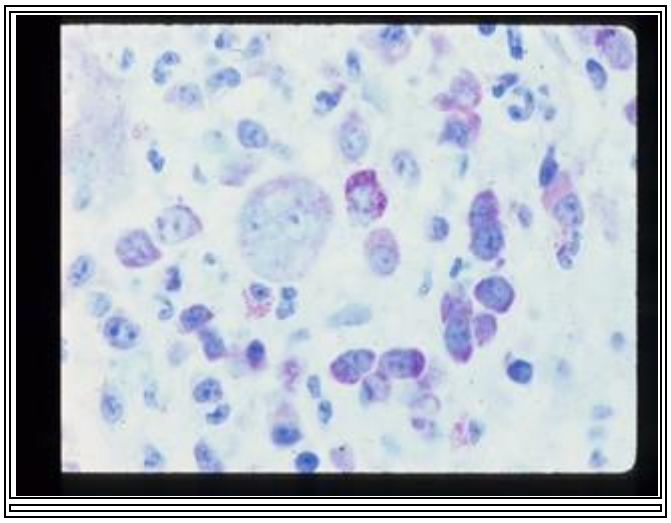
Smith, D.M. and Haggitt, R.C.: A comparative study of generic stains for carcinoid secretory granules. Amer. J. Surg. Pathol. 7:61-68, 1983.

Churukian, C.J.: Microwave modification of Pascual's argyrophil method. Histologic 19:121-123, 1989.

MICROWAVE GIEMSA METHOD FOR PLASTIC BONE MARROW SECTIONS

Click the Section Headings (Blue) to Expand/Collapse Material

⊟Image Examples:



⊟Diagnostic Application:

⊟Material and Solutions:

TECHNIQUE: Undecalcified glycol methacrylate embedded bone marrow sections cut at 3-4 µm.

SOLUTIONS:

0.5% Hydrochloric Acid-Alcohol

Alcohol, 70% ---------- 1000.0 ml

Hydrochloric acid, concentrated ---------- 5.0 ml

10% Triton X-100

Triton X-100 ---------- 10.0 ml

Distilled water ---------- 90.0 ml

Phosphate Buffer, pH 6.6 – Triton X-100

Potassium phosphate, monobasic, KH2PO4 ---------- 6.15 gm

Sodium phosphate, dibasic, Na2HPO4 ---------- 3.85 gm

Distilled water ---------- 1000.00 ml

10% Triton X-100 ---------- 10.00 ml

Store in a refrigerator at 3-5o C.

Giemsa Solution, Stock

Azure A, C.I. 52005 ---------- 0.50 gm

Eosin Y, C.I. 45380 ---------- 0.44 gm

Phloxine B, C.I. 45410 ---------- 0.06 gm

Glycerin ---------- 60.00 ml

Methyl alcohol ---------- 40.00 ml

Pour the glycerol in a beaker and add the dyes. Place on a hot plate stirrer, apply gentle heat and mix for one hour. Do not allow the solution to exceed 55o C. Allow the solution to cool

to 30o C and add the methanol and mix on a stirrer for five

minutes. Store in a tightly stoppered amber bottle. The solution

is stable for at least one year.

Giemsa Solution, Working

Giemsa solution, stock ---------- 4.0 ml

Phosphate buffer, pH 6.6 – Triton X-100 ---------- 40.0 ml

1% Acetic Acid

Staining Procedure:

1. Place in 0.5% hydrochloric acid-alcohol for 10 minutes.
2. Wash well in running tap water.
3. Rinse in two changes of distilled water.
4. Place slides in the working Giemsa solution in a glass Coplin jar and microwave at power level 1 (60 W) for 2 minutes. Allow the slides to remain in the warm solution for 60 minutes.
5. Rinse in distilled water, three dips.
6. Place in 1% acetic acid for 5 seconds.
7. Rinse in distilled water, three dips.
8. Dehydrate in three changes of 95% alcohol and four changes of absolute alcohol, three dips in each.
9. Clear in xylene, three or four changes.
10. Mount with synthetic resin.

Staining Results:

All the colors of the hemopoietic bone marrow are similar to the colors of bone marrow air dried aspirates stained with Giemsa.

Eosinophil granules ---------- round red refractile granules

Erythrocytes, mature ---------- red

Erythrocytes, immature ---------- small purple (azuraphilic) granules

Mast cells ---------- deep purple

Megakaryocyte cytoplasm ---------- pale purple as are the platelets

Megakaryocytes, immature ---------- eosinophilic cytoplasm

Neutrophils, mature ---------- tan to pink

Nuclear chromatin ---------- blue

Plasma cells ---------- purple with the characteristic perinuclear clear region

Comment:

The use of the microwave oven reduces the time required to perform the method. Also, the staining results appear to be better than when the stain is done at room temperature. The final temperature of the solution should be approximately 50o C.

We have found considerable variation in the quality of commercial Giemsa stains in both solution and powder form. The Giemsa formula used in this method compares favorably with the best commercial Giemsa stains and can be used in any Giemsa staining method.

The addition of the detergent Triton X-100 to the staining solution results in more distant staining of the various cell types found in hemopoietic bone marrow. Because morphological details are more distinct microscopical detection and identification of the cells is facilitated. Another advantage gained by the addition of Triton X-100 is that it stabilizes the working Giemsa solution. If the Triton X-100 is omitted a fine precipitate forms in the solution. This precipitate also forms when Triton X-100 is omitted from solutions prepared with commercial Giemsa stains.

The stain may be done at room temperature by staining in the working solution for 2 hours.

References:

Boon, M.E., et al. Microwave-stimulated staining of plastic embedded bone marrow sections with the Romanowsky-Giemsa stain: Improved staining patterns. Stain Technol. 62:257-266, 1987.

Lillie, R.D. and Fullmer, H.M.: Histopathologic Technic and Practical Histochemistry, 4th ed., New York, McGraw-Hill, 1976.

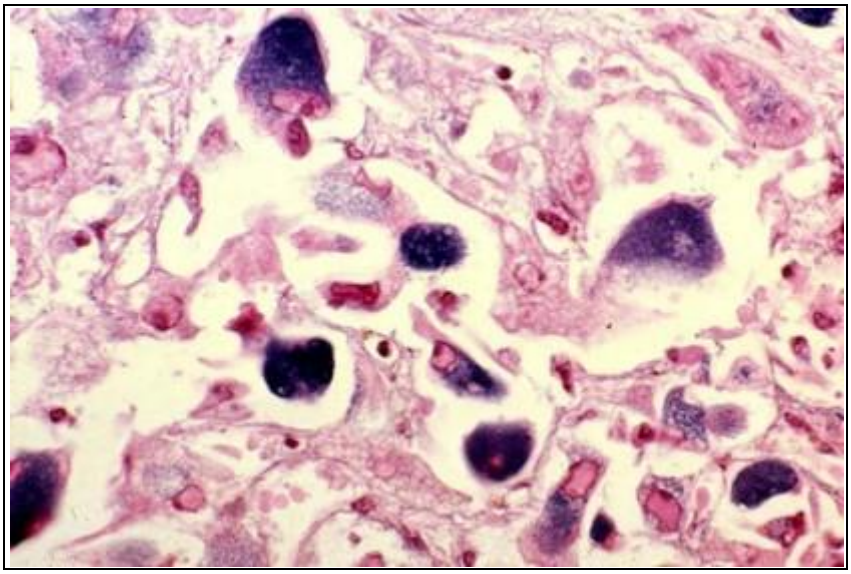
Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 119-120.

Melvin, D.M. and Brooke, M.M.: Triton X-100 in Giemsa staining of blood parasites. Stain Technol. 55:269-275, 1955.

ALDEHYDE FUCHSIN METHOD FOR HEPATITIS B ANTIGEN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Aldehyde fuchsin method for hepatitis-B antigen.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.3% Acidified Potassium Permanganate (Stock)

Potassium permanganate	0.3 gm
Distilled water	100.0 ml
Sulfuric acid, concentrated	0.2 ml

For use dilute with equal parts of distilled water.

1.0% Oxalic acid

Oxalic acid	1.0 gm
Distilled water	100.0 ml

Aldehyde Fuchsin

Pararosanilin, C.I. 42500*	0.5 gm
Alcohol, 70%	96.0 ml
Hydrochloric acid, concentrated	1.0 ml
Paraldehyde or Acetaldehyde	3.0 ml

Let stand for four or five days or until stain is deep purple.

Keeps for six to eight weeks if stored at room temperature.

*Rosanilin, C.I. 42510, and new fuchsin, C.I. 42520, which are closely related to pararosanilin should not be used to prepare

aldehyde fuchsin.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Place in diluted acidified potassium permanganate for 5 minutes.
3. Rinse in two changes of distilled water.
4. Place in 1.0% oxalic acid for 2 minutes.
5. Wash in running tap water for 3 minutes.
6. Rinse in three changes of distilled water.
7. Place in 70% alcohol for 30 seconds.
8. Aldehyde fuchsin for 5 to 60 minutes. The time will depend on the age of the aldehyde fuchsin. Longer staining times may be necessary as the solution slowly deteriorates.
9. Rinse in four changes of distilled water.
10. Dehydrate in graded alcohols.
11. Clear in xylene, three or four changes.
12. Mount with synthetic resin.

Staining Results:

Hepatitis B surface antigen ---------- purple (diffuse cytoplasmic)

Lipofuscin ---------- purple, cytoplasmic granules

Copper binding protein ---------- purple, cytoplasmic granules

Elastic fibers ---------- purple

Comment:

Since hepatocyte and Kupfer cell intracytoplasmic granules (i.e., lipofuscin, copper binding protein) are stained with this procedure, interpretation of the presence of HbsAg has to be based on the finding of diffuse cytoplasmic staining in involved liver cells, which are usually scattered randomly throughout the parenchyma. Lipofuscin granules tend to be perinuclear and present in all liver cells. Copper binding protein granules are usually more coarse than lipofuscin ones, and in cells at the periphery of liver lobules or nodules.

The basic fuchsins rosanilin, C.I. 42510, and new fuchsin, C.I. 42520, are closely related to pararosanilin, C.I. 42500. Only pararosanilin will give satisfactory staining results in this procedure.

The staining time in aldehyde fuchsin will vary considerably depending upon its age. Fresh solution will give adequate staining in 5-10 minutes. Solutions which are over a month old will require 60 or more minutes. We have found that the solution keeps better if stored at room temperature than in a refrigerator.

References:

Deodhar, K.P., et al.: Orcein staining of hepatitis B antigen in paraffin sections of liver biopsies. J. Cin. Path. 28:66-70, 1975.

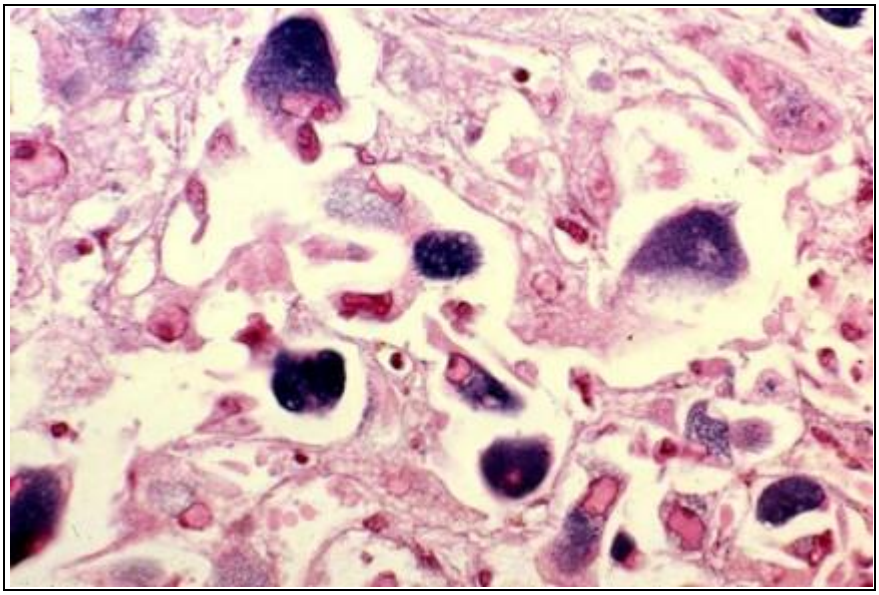
Luna, L.G.: Recommended procedure for demonstrating hepatic B antigen (HBAg) in paraffin sections. Histo-Logic 8:119-120, 1978.

Shikata, T., et al.: Staining methods of Australia antigen in paraffin sections. Jap. J. Exp. Med. 44:25-36, 1974.

MICROWAVE ORCEIN METHOD FOR HEPATITIS B SURFACE ANTIGEN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Hepatits B Surface Antigen

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.3% Acidified Potassium Permanganate (Stock)

Potassium permanganate	0.3 gm
Distilled water	100.0 ml
Sulfuric acid, concentrated	0.2 ml

For use dilute with equal parts of distilled water.

1% Oxalic Acid Solution

Oxalic acid	1.0 gm
Distilled water	100.0 ml

1% Orcein Solution

Orcein, C.I. (Ed. 1) 1242	1.0 gm
Alcohol, 70%	100.0 ml
Hydrochloric acid, concentrated	1.0 ml

Let solution stand at room temperature for at least one week before use.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Place in diluted acidified potassium permanganate for 5 minutes.
3. Rinse in two changes of distilled water.
4. Place in 1% oxalic acid for 2 minutes.
5. Wash in running tap water for 1 minutes.
6. Rinse in two changes of distilled water.
7. Dip several times in 70% alcohol.
8. Place in 45 ml of orcein solution in a glass Coplin jar and microwave at power level for 1 ½ minutes. Transfer to a 37o oven for 2 hours.
9. Rinse slides in four changes of distilled water.
10. Dehydrate in graded alcohols.
11. Clear in xylene, three or four changes.
12. Mount with synthetic resin.

Staining Results:

Hepatitis B surface antigen (HbsAg) ---------- brown (diffuse cytoplasmic)

Copper binding protein ---------- brown, fine cytoplasmic granules

Elastic fibers ---------- brown

Comment:

Some orcein dyes do not give satisfactory staining results. Dyes which are not satisfactory will either overstain the section or not adequately stain the HbsAg. Dyes certified as orcein by the Biological Stain Commission are tested for their ability to stain HbsAg. The stain may be done at room temperature by staining for 4-8 hours in the orcein solution.

References:

Deodhar, K.P., et al.: Orcein staining of hepatitis B antigen in paraffin sections of liver biopsies. J. Clin. Path. 28:66-70, 1975.

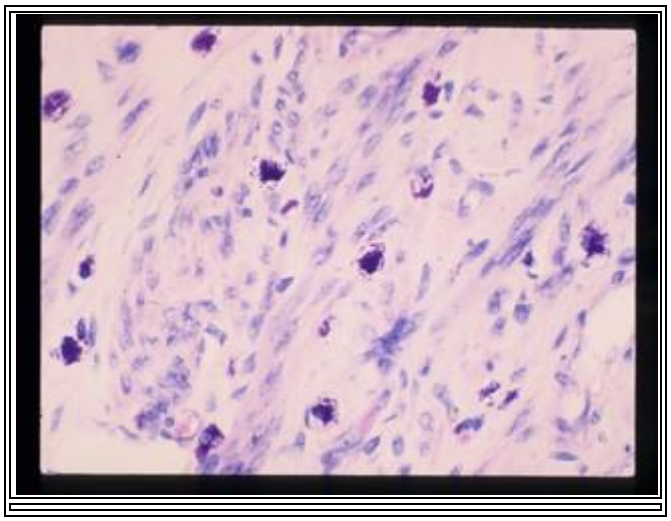
Luna, L.G.: A modified aldehyde fuchsin for HBAg. Histo-Logic 8:119-120, 1978.

Shikata, T., et al.: Staining methods of Australia antigen in paraffin sections. Jap. J. Exp. Med. 44:25-36, 1974.

TOLUIDINE BLUE METHOD FOR MAST CELLS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.5% Potassium Permanganate Solution

Potassium permanganate	0.5 gm
Distilled water	100.0 ml

1% Potassium Metabisulfite Solution

Potassium metabisulfite	1.0 gm
Distilled water	100.0 ml

0.02% Toluidine Blue Solution

Toluidine Blue 0, C.I. 52040	0.02 gm
Distilled water	100.0 ml
Acetic acid, glacial	0.25 ml

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Potassium permanganate solution for 2 minutes.
3. Rinse in two changes of distilled water.

4. Potassium metabisulfite solution for 1 minute.
5. Wash in running tap water for 3 minutes.
6. Rinse in two changes of distilled water.
7. Toluidine Blue solution for 5 minutes.
8. Rinse in three changes of distilled water.
9. Dehydrate in graded alcohols.
10. Clear in xylene, three or four changes.
11. Mount with synthetic resin.

Staining Results:

Mast cell granules ---------- purple (metachromatic)

Acid mucopolysaccharides ---------- red to pink

Nuclei ---------- blue

Comment:

This staining method appears to minimize background staining, which makes it much easier to locate and identify mast cells.

References:

McManus, J.A. and Mowry, R.W.: Staining Methods for Histologic and Histochemical, Paul B. Hoeber, 1960, pp. 132-133.

Churukian, C.J. and Schenk, E.A.: A toluidine blue method for demonstrating mast cells. J. Histotechnology 4:85-86, 1981.

LONG ZIEHL-NEELSEN METHOD FOR CEROID

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Carbol Fuchsin Solution

Basic fuchsin, C.I. 42510 ---------- 0.8 gm

Ethyl alcohol ---------- 14.0 ml
Phenol, liquid, approximately 90% ---------- --- 8.5 ml
Distilled water ---------- 140.0 ml

Dissolve the basic fuchsin in the ethyl alcohol, phenol, and distilled water. Add 3 drops of 25% Tween 20 to 45 ml of this solution and mix well. Filter through Whatman #1 filter paper before use.

25% Tween 20 Solution

Tween 20 (Polysorbate 20) ---------- 25.0 ml

Distilled water ---------- 75.0 ml

0.5% Acid Alcohol

Alcohol, 70 % ---------- 99.5 ml
Hydrochloric acid, concentrated ---------- -- 0.5 ml

Methylene Blue Solution

Methylene blue, C.I. 52015 ---------- - 0.25 gm
Distilled water ---------- 100.00 ml
Acetic acid, glacial ---------- 1.00 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place slides in carbol fuchsin and allow to set overnight.
3. Wash well in running water to remove excess stain.

4. Decolorize with acid alcohol until the sections are clear of excess stain. This may take 30-60 seconds.

5. Wash in running water for 1 minute and rinse in two changes of distilled water.

6. Counterstain with methylene blue solution for 30 seconds.

7. Rinse in three changes of distilled water.
8. Dehydrate in graded alcohols.

9. Clear in xylene, three or four changes.
10. Mount with synthetic resin.

Staining Results:

Ceroid ---------- magenta

Lipofuscin ---------- magenta

Nuclei ---------- blue

Background ---------- pale magenta to pale blue

Comment:

In 1941 Lillie was the first to describe ceroid in cirrhotic livers of animals maintained on inadequate diets. Lillie thought that ceroid was different from lipofuscin because it failed to stain with Schmorls ferric-ferricyanide reaction for reducing substances. In 1985 Pearse states that ceroid is, in fact, a lipofuscin at an early stage of oxidation. Further oxidation would produce lipofuscin proper. Therefore, in order to determine if a pigment is ceroid or lipofuscin Schmorls method should be performed along with the Long Ziehl-Neelsen technique.

References:

Baucroft, J.D., and Gamble, M.: Theory and Practice of Histological Techniques, New York, Churchill-Livingstone, 2002, pp. 256-257.

METHYL GREEN – PYRONIN Y METHOD

FOR DNA AND RNA

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin. Other fixative which may be used are Carnoy's, formol saline and Zenker's.

TECHNIQUE: Paraffin sections cut at 5 μm.

SOLUTIONS:

0.2M Acetic Acid

Acetic acid, glacial ---------- 3.0 ml

Distilled water ---------- 247.0 ml

0.2M Sodium Acetate

Sodium acetate anhydrous ---------- 4.1 gm

Distilled water to make a total of ---------- ----- 250.0 ml

0.2M Acetic Acid-Sodium Acetate, pH 4.2

0.2M acetic acid ---------- 75.0 ml

0.2M sodium acetate ---------- 25.0 ml

Check with pH meter and adjust to pH 4.2 with 0.2M acetic acid

or 0.2M sodium acetate. Store in a refrigerator at 3-6o C.

0.5% Methyl Green Solution

0.2M acetate buffer solution, pH 4.2 ---------- 10.00 ml

Methyl green, C.I. 42585 ---------- 0.05 gm

Methyl Green – Pyronin Y Solution

0.5% methyl green solution ---------- 10.000 ml

Pyronin Y, C.I. 45005 ---------- 0.005 gm

The amount of pyronin Y may need to be increased up to 0.01 gm

with some samples of pyronin Y.

Staining Procedure:

- Use control slides.

1. Deparaffinize and hydrate to distilled water.
2. Place on a staining rack. Flood sections with methyl green – pyronin Y solution. Let stand for 5 minutes.
3. Rinse quickly in distilled water.
4. Blot completely dry with blotting paper.
5. Place in acetone for 10 seconds.
6. Dip several times in equal parts acetone and xylene.
7. Clear in xylene, three or four changes.
8. Mount with synthetic resin.

Staining Results:

DNA ---------- blue to blue-green

RNA ---------- pink to red

Background ---------- pale pink

Comment:

This method produces better staining results than any other methyl green – pyronin Y method which we have tried on formalin-fixed tissues.

The dye content of pyronin Y will vary from sample to sample. Usually the dye content falls between 50% and 90%. This variation in dye content is what sometimes requires increasing the amount of pyronin Y in the methyl green – pyronin Y solution.

Some texts and staining manuals recommend that methyl green be chloroform extracted to remove methyl violet. We have found that good staining results can be achieved with most samples of methyl green without chloroform extraction.

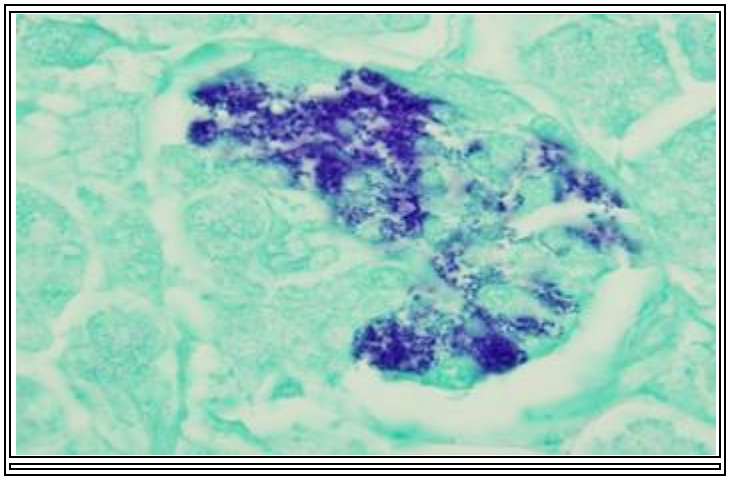
References:

Potvin, C.: A simple, modified methyl green – pyronin Y stain for DNA and RNA in formalin-fixed tissues. Lab. Med. 10:772-774, 1979.

ALDEHYDE FUCHSIN METHOD FOR PANCREATIC BETA CELLS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Lugol's Iodine Solution

(See Verhoeff's Elastic Method)

2% Sodium Thiosulfate Solution

Sodium thiosulfate ---------- 2.0 gm

Distilled water ---------- 100.0 ml

Aldehyde Fuchsin Solution

(See Aldehyde Fuchsin Method for HbsAg)

0.2% Fast Green Solution (Stock)

Fast green FCF, C.I. 42053 ---------- 0.2 gm

Distilled water ---------- 100.0 ml

Acetic acid, glacial ---------- 0.2 ml

Fast Green Solution (Working)

Fast green (stock) ---------- 10.0 ml

Distilled water ---------- 35.0 ml

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water. Omit Steps 2-5 if the aldehyde fuchsin is fresh (5-10 days old).
2. Lugol's iodine solution for 5 minutes.
3. Rinse in two changes of distilled water.
4. Place in 2% sodium thiosulfate for 1 minute.
5. Wash in running tap water and rinse in two changes of distilled water.
6. Place in 70% alcohol for 30 seconds.
7. Aldehyde fuchsin in a covered Coplin jar for 30 to 60 minutes. Longer staining times may be necessary if the solution is old.
8. Rinse in four changes of distilled water.
9. Fast green working solution for 1 minutes.
10. Rinse in three changes of distilled water.
11. Dehydrate in graded alcohols.
12. Clear in three or four changes of xylene.
13. Mount with synthetic resin.

Staining Results:

Beta cells ---------- purple to violet

Elastic tissue ---------- purple to violet

Background ---------- green

Comment:

See COMMENTS under Aldehyde Fuchsin Method for Hepatitis B antigen.

References:

Gomori, G.: Aldehyde fuchsin: A new stain for elastic tissue. Amer. J. Clin. Path. 20:665-666, 1950.

WILSON-EZRIN METHOD FOR PITUITARY

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.5% Periodic Acid Solution

Periodic acid	0.5 gm
Distilled water	100.0 ml

Modified Lillie's Schiff Solution

(see PAS method)

Weigert's Iron Hematoxylin (Modified)

(see Gomori's One Step Trichrome method)

0.5% Acid Alcohol Solution

Alcohol, 70%	1000.0 ml
Hydrochloric acid, concentrated	5.0 ml

1% Orange G Solution

Orange G, C.I. 16230 ----------	1.0 gm
Distilled water ----------	100.0 ml

5% Phosphotungstic Acid Solution

Phosphotungstic acid ----------	5.0 gm
Distilled water ----------	100.0 ml

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in 0.5% periodic acid for 10 minutes.
3. Rinse in four changes of distilled water.
4. Lillie's Schiff solution for 15 minutes.
5. Rinse in three changes of distilled water.
6. Place in 0.3% sodium borate for 15 seconds.
7. Rinse in four changes of distilled water.
8. Modified Weigert's iron hematoxylin for 1 minute.
9. Rinse in two changes of distilled water.
10. Acid alcohol solution for 10 seconds.
11. Wash in running water and rinse in distilled water.
12. Orange G solution for 1 ½ minutes.
13. Place in 5% phosphotungstic acid for 30 seconds.
14. Wash briefly in running water and rinse in two changes of distilled water.

15. Dehydrate in graded alcohols.
16. Clear in three or four changes of xylene.
17. Mount with synthetic resin.

Staining Results:

Beta granules ---------- red

Gamma granules ---------- purple

Acidophils ---------- yellow-brown

Nuclei ---------- black

Comment:

The glandular cells of the adenohypophysis may be broadly classified as chromophilic or chromophobic based on their affinity (or lack thereof) of certain dyes. Chromophilic cells may be subdivided into acidophils or basophils based on the reactions of their specific cell granules with acid or basic dyes. The chromophobe cells are believed to represent either precursor cells for the chromophils, or chromophils in various stages of degranulation. The immunocytochemical procedure is the most specific method for demonstrating the various types of cells found in the pituitary.

References:

Wilson, W.D. and Ezrin, E.: Three types of chromophil cells of the adenohypophysis, demonstrated by a modification of the periodic acid –Schiff technique. Amer. J. Path. 30:891-899, 1954.

LIST OF STAINS FOR PIGMENTS AND METALS

LISONS METHOD FOR HEMOGLOBIN

ALUMINON STAIN FOR ALUMINUM

MICROWAVE RHODANINE COPPER METHOD

PERLS METHOD FOR FERRIC IRON

DAHLS METHOD FOR CALCIUM

VON KOSSAS METHOD FOR CALCIUM

SILVER METHOD FOR MERCURY

HALLS METHOD FOR BILIRUBIN

FORMALIN AND MALARIA PIGMENTS METHOD

LILLIES SULFURIC NILE BLUE METHOD FOR MELANIN AND LIPOFUSCIN

LILLIES FERROUS ION UPTAKE METHOD FOR MELANIN

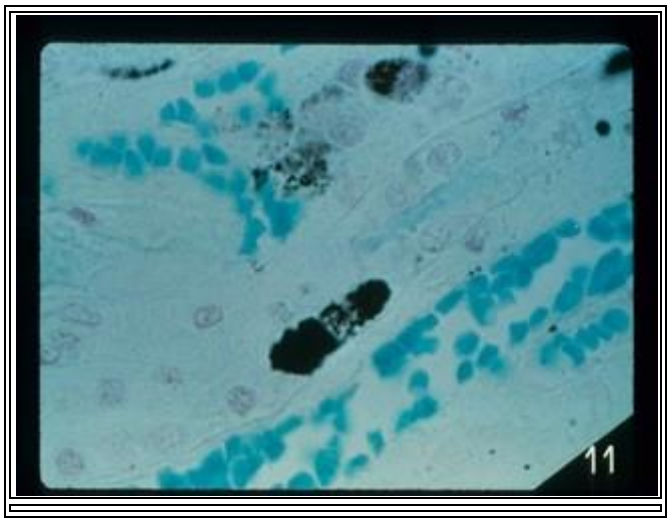
MICROWAVE MELANIN BLEACH METHOD

GOMORIS METHOD FOR URATES

LISON'S METHOD FOR HEMOGLOBIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

Patent Blue V Solution (Stock)

Patent blue V, C.I. 42045 ----------	0.25 gm
Acetic acid, 2% ----------	25.0 ml
Zinc dust, granulated ----------	2.50 gm

Mix well on a magnetic stirrer. The solution will become

a pale greenish-blue. Filter and store in a refrigerator at

3-6o C. The solution is stable for about one week.

Patent Blue V Solution (Working)

Patent blue V solution ----------	10.0 ml
Acetic acid ----------	2.0 ml
Hydrogen peroxide, 3% ----------	1.0 ml

Prepare just before use.

Nuclear Fast Red Solution

(See Pearl's Method for Iron)

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Place slides on a staining rack and flood with freshly prepared patent blue V working solution for 5 minutes.
3. Rinse in three changes of distilled water.
4. Nuclear fast red for 3 minutes.
5. Rinse in three changes of distilled water.
6. Dehydrate in graded alcohols.
7. Clear in three or four changes in xylene.
8. Mount with synthetic resin.

Staining Results:

Hemoglobin ---------- blue to green

Nuclei ---------- red

Comment:

According to Lillie aniline blue (C.I. 42755) may be substituted for patent blue V in this procedure. He states that it will decolorize to a light transparent green with zinc and hot dilute acetic acid and will stain hemoglobin blue. We tried two different samples of aniline blue in this procedure and neither one gave satisfactory staining results.

Lison indicated in this method that the patent blue V stock solution should be boiled until it becomes nearly colorless. We found that the solution becomes nearly colorless without the need to boil it.

References:

Lillie, R.D. and Fullmer, H.M.: Histopathologic Technic and Practical Histochemistry, 4th ed., New York, McGraw-Hill, 1976, pp. 447

ALUMINON STAIN FOR ALUMINUM

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Diagnostic Application:

Material and Solutions:

TECHNIQUE: Undecalcified glycol methacrylate embedded sections cut at 4 μm or paraffin sections cut at 5 μm.

SOLUTIONS:

Buffer Solution, pH 5.2

Ammonium acetate	40.0 gm
Ammonium chloride	28.0 gm
Distilled water	210.0 ml
50% Hydrochloric acid, (6N)	27.0 ml

Adjust to pH 5.2 with concentrated hydrochloric acid or 28% ammonium hydroxide. Store in refrigerator at 3-6o C.

*Aluminon Solution

Aluminon	0.8 gm
Buffer solution, pH 5.2	-- 40.0 ml

Place in a 125 ml flask and place on a hot plate stirrer. Heat to 80-85o C just before use.

*See Step 2 of the Staining Procedure.

1.6M Ammonium Carbonate

Ammonium carbonate	15.4 gm
Distilled water to make a total of	100.0 ml

Store in a refrigerator at 3-6o C.

Differentiating Solution, pH 7.2

Buffer solution, pH 5.2	22.0 ml
1.6M ammonium carbonate	8.0 ml

Fast Green, Stock Solution

Fast green FCF, C.I. 42053	0.2 gm

Distilled water ---------- 100.0 ml

Acetic acid, glacial ---------- 0.2 gm

Fast Green, Working Solution

Fast green, stock solution ---------- 10.0 ml

Distilled water ---------- 35.0 ml

Staining Procedure:

1. Place GMA embedded slides on a hot plate at 60-65o C for 15 minutes. Deparaffinize and hydrate the paraffin sections with distilled water.

2. Heat the aluminon solution in a 125 ml flask to 80-85o C on a hot plate stirrer.

3. Pour the hot aluminon solution in a plastic Coplin jar and place the slides in the solution.

4. Place Coplin jar in a microwave oven and microwave at power level 2 (120 W) for 30 seconds. Dip the slides up and down and allow them to remain in the hot solution for 7 minutes.

5. Rinse in distilled water.

6. Place in differentiating solution for 5 seconds.

7. Rinse quickly in three changes of distilled water.

8. Counterstain with working fast green solution for 3 minutes.

9. Rinse in three changes of distilled water and air dry.

10. Dip in xylene and mount with synthetic resin.

Staining Results:

Aluminum ---------- red

Background ---------- varying shades of green

References:

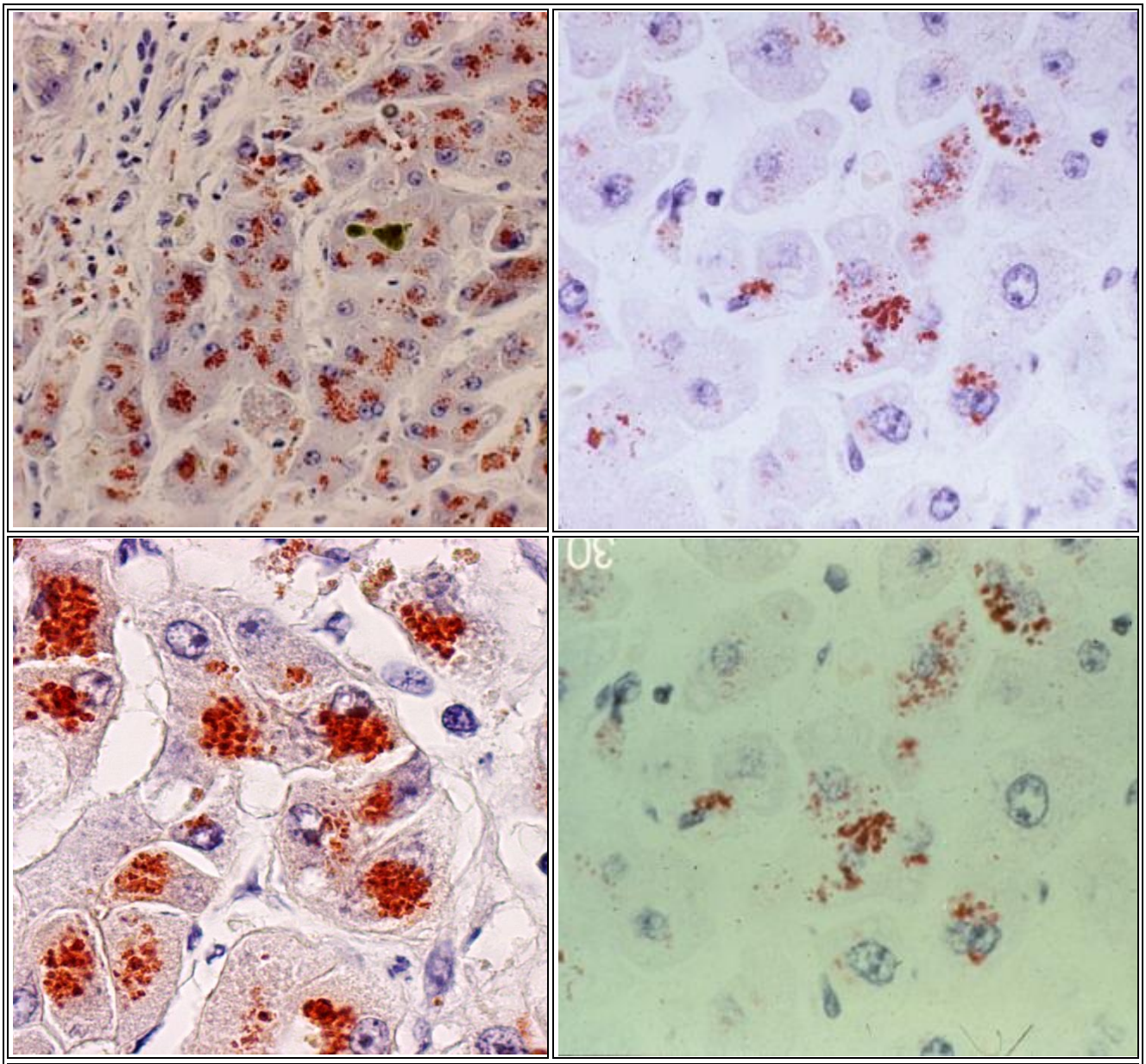
Lillie, R.D.: Histopathologic Technic and Practical Histochemistry, 4th ed., New York, McGraw-Hill, 1976, p. 534.

Ohtsuki, T. et al.: A simplified aluminum stain in paraffin sections of bone from hemodialysis patients. Stain Technol. 64: 55-59, 1989.

MICROWAVE RHODANINE COPPER METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Red-brown cytoplasmic granules are copper deposits in the this liver from a patient with Wilsons diasease [top left]. Note the bile (plug) remains green (left panel). Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Positive stain for copper is strongly indicative of Wilsons disease, although negative stain does not exclude this diagnosis.

See also ____ and _____ stains for copper.

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.4% Rhodanine Solution (prepare fresh)

5 – (p-dimethylaminobenzylidene) rhodanine ---------- 0.024 gm

Absolute alcohol ---------- 6.000 ml

1% Sodium Acetate Solution

*Sodium acetate trihydrate ---------- 1.0 gm

Distilled water ---------- 100.0 ml

*If anhydrous sodium acetate is used, use 0.6 gm.

Rhodanine Solution (Working)

Rhodanine solution (stock) ---------- 5.0 ml

1% sodium acetate ---------- 45.0 ml

Filter the rhodanine solution through Whatman #4 filter paper (70 mm in diameter) into the 1% sodium acetate.

0.4% Sodium Borate

Sodium borate, ($Na_2B_4O_7 \cdot 10 H_2O$) ---------- ----- 0.4 gm

Distilled water ---------- 100.0 ml

Staining Procedure:

- Use positive control slide.

1. Deparaffinize and hydrate to distilled water.

2. Place slides in the working rhodanine solution in a plastic Coplin jar, with the cap loosely applied, and microwave at power level 6 (360W) for 30 seconds. Agitate for about 15 seconds. Return the Coplin jar to the microwave oven and microwave at power level 6 for 35 seconds. Agitate for about 15 seconds and allow the slides to remain in this hot solution (about 80o C) for 5 minutes.

3. Return the Coplin jar to the microwave oven and microwave at power level 6 for 10 seconds. Agitate for about 15 seconds and allow the slides to remain in the hot solution for 5 minutes.

4. Wash with six changes of distilled water.

5. Place in acidified Lillie-Mayer hematoxylin for 10 seconds.

6. Rinse with two changes of distilled water.

7. Blue hematoxylin in 0.4% sodium borate for 15 seconds.

8. Rinse with four changes of distilled water.

9. Mount with Apathy's aqueous mounting media..

Staining Results:

Copper ---------- bright red or rust-red intracytoplasmic granules

Nuclei ---------- blue

Comment:

We have found this stain to be both sensitive and specific and to allow for a semiquantitative evaluation of the amount of copper present (see Irons, et al). This microwave method may not always give as good results as the overnight method which is done in a 37o C oven. A good positive control of this method is any fetal liver of the third trimester fixed in buffered neutral formalin for not longer than 24 hours prior to paraffin processing. Fetal liver of the third trimester contains copper within hepatocytes. Care must be taken not to over counterstain with hematoxylin which will mask the positive staining of the copper.

References:

Wenger, J.B. and Luna, L.G.: A new method for compounding the rhodanine copper stain. Histo-Logic 18:50-51, 1988.

Brinn, N.T. and Goodfellow, B.C.: Metallic histologic impregnation using the microwave oven. Presented at the National Society for Histotechnology Symposium on October 21, 1985 in Crystal City, Virginia.

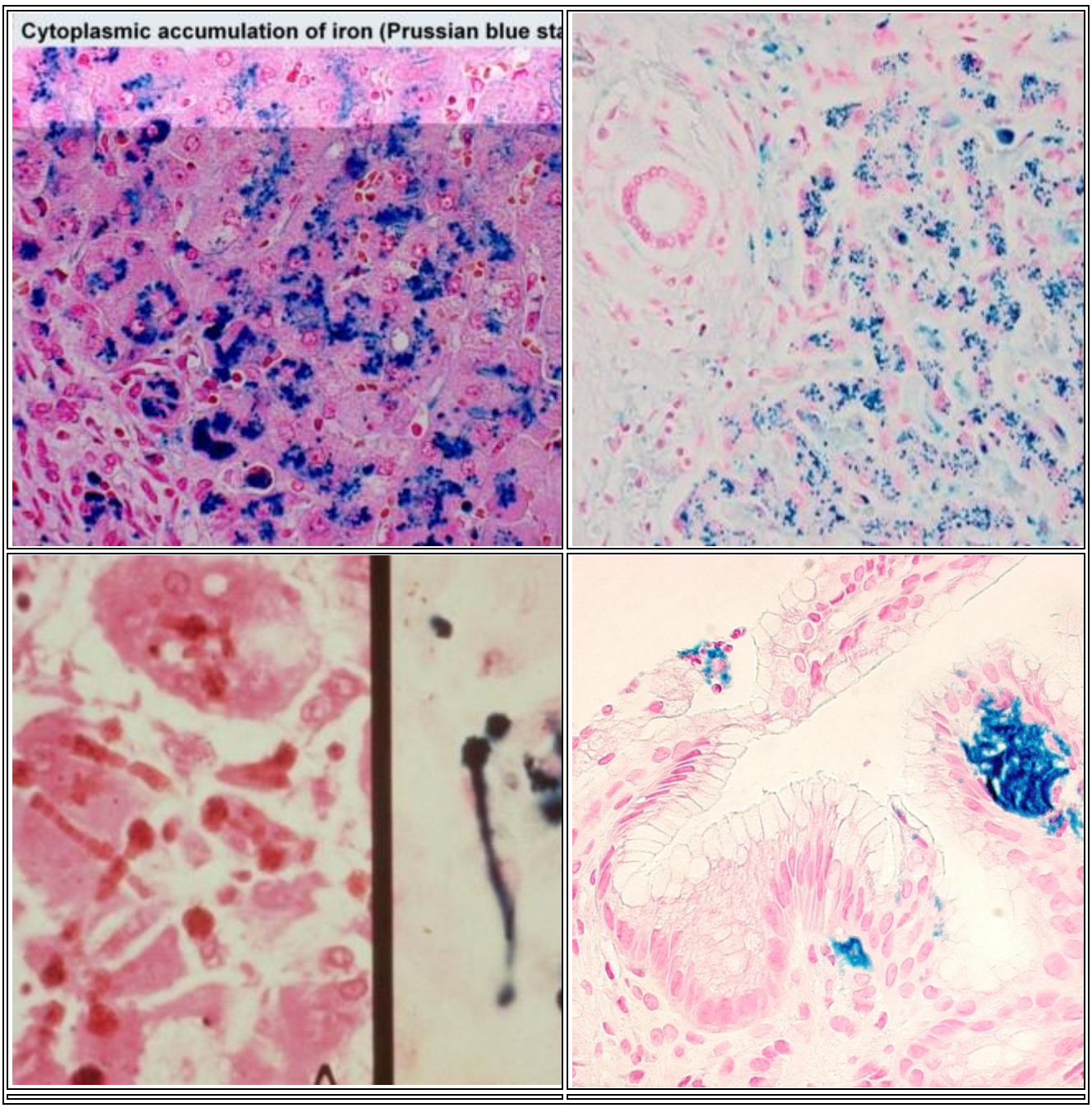
Irons, R.D., Schenk, E.A., and Lee, C.K.: Cytologic methods for copper. Arch. Path. Lab. Med. 101:298-301, 1977.

Lindquist, R.R.: Cytochemical method for the localization of copper. Arch. Path. 87:370-379, 1969.

PERL'S METHOD FOR FERRIC IRON

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Hemochromatosis, esp., liver. excess iron deposition is stained as blue granules

Gastic ulver 2nd to iron overdose:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 μm.

SOLUTIONS:

2% Hydrochloric Acid Solution

Hydrochloric acid, concentrated ---------- 2.0 ml

Distilled water ---------- 98.0 ml

10% Triton X-100

Triton X-100 ---------- 10.0 ml

Distilled water ---------- 90.0 ml

Add a few grains of thymol to prevent the growth of fungi.

1% Potassium Ferrocyanide Solution

Potassium ferrocyanide ---------- 1.0 gm

Distilled water ---------- 98.0 ml

10% Triton X-100 ---------- 2.0 ml

Hydrochloric Acid-Potassium Ferrocyanide Solution

2% hydrochloric acid ---------- 20.0 ml

1% potassium ferrocyanide ---------- 20.0 ml

Prepare just before use and discard after use.

Nuclear Fast Red (Kernechtrot) Solution

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, add a few grains of thymol as a preservative.

Staining Procedure:

- Use positive control slides.

1. Deparaffinize and hydrate to distilled water.
2. Place the slides in the hydrochloric acid-potassium ferrocyanide solution for 30 minutes at room temperature.
3. Rinse with five changes of distilled water.
4. Nuclear fast red solution for 3 minutes.
5. Rinse with three changes of distilled water.
6. Dehydrate in graded alcohols.
7. Clear in xylene, three or four changes.
8. Mount with synthetic resin.

Staining Results:

Ferric iron pigment ---------- bright blue

Nuclei ---------- red

Cytoplasm ---------- pink

Comment:

Hemosiderin is a breakdown product of hemoglobin and is thought to be composed of ferric iron and protein. It may be present in tissues in certain pathologic conditions such as hemochromatosis. This yellow-brown pigment is insoluble in alkalis and water but is soluble in acid even after fixation. Treating a tissue section with 10% sulfuric acid overnight will usually remove this pigment.

The principle of Pearl's Prussian blue reaction is that potassium ferrocyanide will form ferric ferrocyanide (Prussian blue) with reactive ferric salts in an acid solution. Dilute hydrochloric acid liberates loosely bound ferric iron from protein.

For many years we used Gomori's method for demonstrating ferric iron. The potassium ferrocyanide and hydrochloric acid solutions used in this method are much stronger that those used in Pearl's method. Because most texts on histologic technic indicate the use of Pearl's method we decided to do a comparative study of the two methods. The staining results on a variety of tissues, including plastic bone marrow sections, were almost identical in this study. Therefore, we decided to use Pearl's method routinely because it is more cost effective.

Triton X-100 is added to the potassium ferrocyanide solution because it helps to stabilize the hydrochloric acid-potassium ferrocyanide solution. If Triton X-100 is omitted there is a greater tendency for the solution to break down resulting in a fine blue precipitate forming in the Coplin jar and sometimes on the slides and tissue sections.

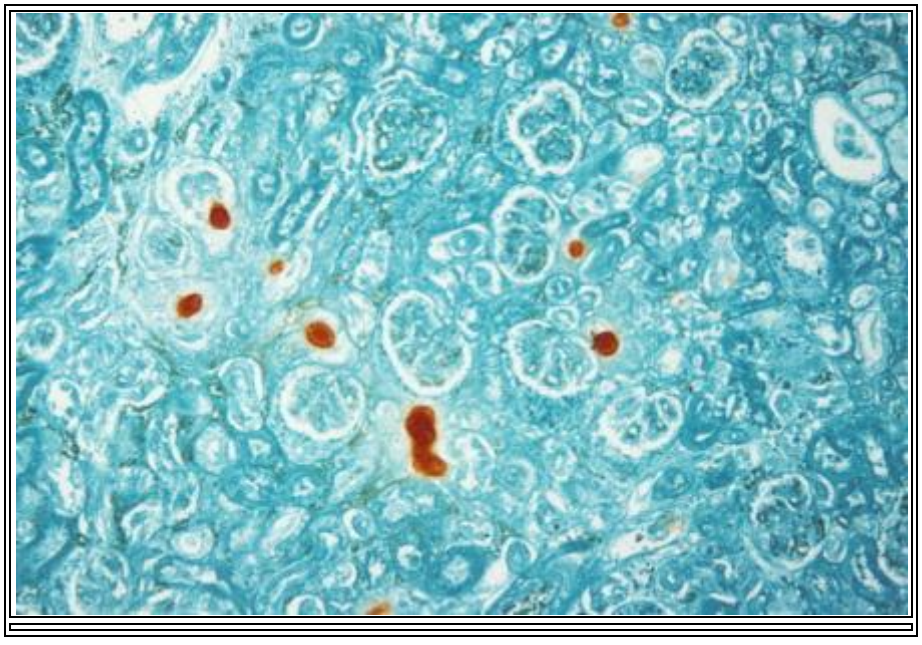
References:

Lillie, R.D.: Histopathologic Technic and Practical Histochemistry, 4th ed., New York, McGraw-Hill, 1976, p. 507.

DAHL'S METHOD FOR CALCIUM

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 95% ethyl alcohol or 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 μm.

SOLUTIONS:

1% Alizarin Red S Solution

Alizarin red S, C.I. 58005 ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Stir dye into the distilled water so that only a few small grains of dye remain undissolved. Add 10% ammonium hydroxide slowly with constant stirring until the pH is 6.3-6.5. Solution is stable for one year.

10% Ammonium Hydroxide Solution

Ammonium hydroxide, 28% ---------- 5.0 ml

Distilled water ---------- 45.0 ml

0.2% Fast Green F.C.F. Solution (stock)

Fast green F.C.F., C.I. 42053 ---------- 0.2 gm

Distilled water ---------- 100.0 ml

Acetic acid ---------- 0.2 ml

Fast Green Solution (working)

Fast green (stock) ---------- 10.0 ml

Distilled water ---------- 35.0 ml

Staining Procedure:

- Use positive control slide.

1. Deparaffinize and hydrate to 95% alcohol.
2. Drain off excess alcohol and allow the sections to dry.
3. Place in alizarin red S solution for 5 minutes.
4. Remove excess stain with distilled water five or six rinses.
5. Counterstain in fast green working solution for 40 seconds.
6. Rinse in three changes of distilled water.
7. Dehydrate in graded alcohols.
8. Clear in xylene, three or four changes.
9. Mount with synthetic resin.

Staining Results:

Calcium salts ---------- intense reddish orange

Background ---------- pale green

Comment:

Some calcium salts are soluble in formalin fixatives. Fixation time should be brief, or ethanol can be used. This method may be slightly more sensitive than the von Kossa stain for calcium.

Some histochemistry textbooks indicate that the alizarin red S solution, used in this method, be adjusted to pH 4.2. We found that calcium will stain equelly well at this pH as it does at pH 6.3-6.5.

Sections must not be hydrated beyond 95% alcohol after being deparaffinized. If the sections are taken to distilled water any calcium salts which may be present will be removed.

Some samples of alizarin red S may cause the staining solution to become a semiliquid gel. If this occurs shake the solution well before use. The solution will become more liquid-like and produce satisfactory staining results.

References:

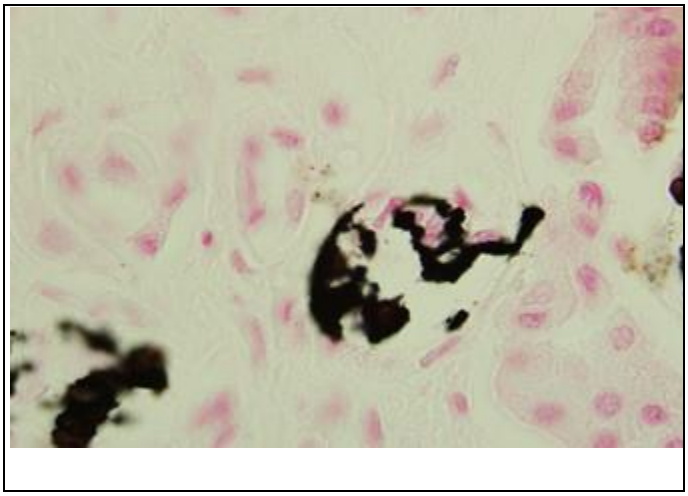
Dahl, L.K.: A simple and sensitive histochemical method for calcium. J. Exp. Med. 95:474-479, 1952.

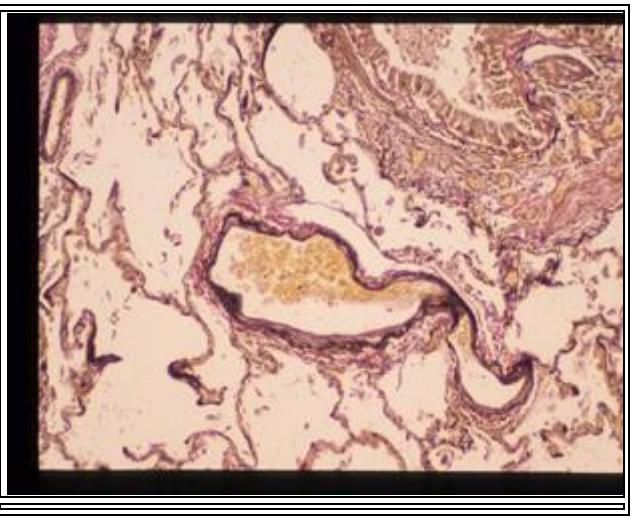
Luna, L.G.: Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts, Maryland, American Histolabs, One., Publications Division, 1992, pp. 327-328

VON KOSSA¡¯S METHOD FOR CALCIUM

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:





Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Cut paraffin sections at 5 µm.

SOLUTIONS:

5% Silver Nitrate Solution

Silver nitrate ---------- 0.5 gm

Distilled water ---------- 10.0 ml

2% Sodium Thiosulfate Solution

Sodium thiosulfate ---------- 2.0 gm

Distilled water ---------- 100.0 ml

Nuclear Fast Red (Kernechtrot) Solution

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, and add a few crystals of thymol as a preservative.

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to to 95% alcohol and air dry the slides.
2. Place slides on a staining rack and flood the sections with 5% silver nitrate. Expose the slides to an ultraviolet lamp for 10 minutes.
3. Rinse with four changes of distilled water.
4. Sodium thiosulfate solution for 1 minute.
5. Rinse with four changes of distilled water.
6. Nuclear fast red solution for 3 minutes.
7. Rinse with three changes of distilled water.
8. Dehydrate through graded alcohols.
9. Clear in xylene, three or four changes.
10. Mount with synthetic resin.

Staining Results:

Calcium salts ---------- black

Nuclei ---------- red

Cytoplasm ---------- light pink

Comment:

Some staining manuals and texts indicate the use of direct sunlight for 30-60 minutes in Step 2. Direct sunlight is not always available and ultraviolet light works just as well in much less time.

References:

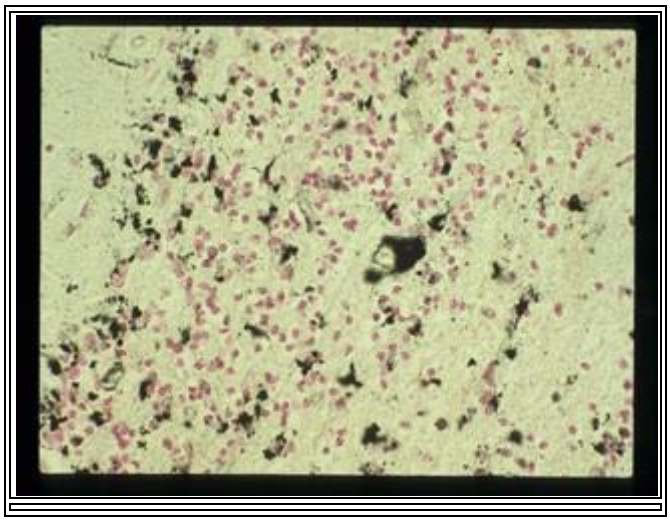
Luna, L.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd edition, New York, McGraw-Hill Book Company, Blakiston Division, 1968, pp. 176-177.

Mallory, F.B.: Pathological Technique, New York, Hafner Publishing Company, 1961, p. 144.

SILVER METHOD FOR MERCURY

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 7 µm.

SOLUTIONS:

Ammonia-Alcohol Solution

Alcohol, 95% ---------- 90.0 ml

Ammonium hydroxide, 28% ---------- 10.0 ml

Glycine-Acetic Acid (Stock Solution)

Glycine ---------- 2.4 gm

Acetic acid, glacial ---------- 0.3 ml

Distilled water ---------- 100.0 ml

The pH of this solution is approximately 3.6. Store in a refrigerator at 3-6o C.

Acetic-Acetic Acid (Working Solution)

Glycine-acetic acid (stock) ---------- 3.0 ml

Distilled water ---------- 300.0 ml

The pH of this solution is approximately 3.6.

2% Silver Nitrate Solution

Silver nitrate ---------- 0.2 gm

Glycine-acetic acid, working solution ---------- 10.0 ml

4% Gelatin Solution

Gelatin ---------- 1.0 gm

Glycine-acetic acid, working solution ---------- 25.0 ml

Dissolve by placing on a magnetic stirrer and apply gentle heat.

0.2% Hydroquinone Solution

Hydroquinone ---------- 0.02 gm

Glycine-acetic acid, working ---------- 10.0 ml

Silver Nitrate – Gelatin – Hydroquinone Solution

Silver nitrate, 2% ---------- 10.0 ml

Gelatin, 4% ---------- 25.0 ml

Hydroquinone, 0.02% ---------- 10.0 ml

Heat the gelatin solution to 60o C on a hot plate. Remove from heat and add the 2% silver nitrate and mix. Then add the 0.02% hydroquinone. Prepare immediately before use.

2% Sodium Thiosulfate

Sodium thiosulfate ---------- 2.0 gm

Distilled water ---------- 100.0 ml

Nuclear Fast Red Solution

(See Pearl's method for Ferric Iron)

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Place in ammonia-alcohol solution, to remove formalin pigment, for 10 minutes.
3. Wash well in running water.
4. Rinse in three changes of distilled water.
5. Place in freshly prepared silver nitrate-gelatin-hydroquinone solution in a plastic Coplin jar and place in a 37o C oven for 18 minutes.
6. Wash well in hot running water.

7. Rinse in two changes of distilled water.
8. Place in 2% sodium thiosulfate for 1 minute.
9. Rinse in four changes of distilled water.
10. Counterstain with nuclear fast red for 3 minutes.
11. Rinse in two changes of distilled water.
12. Dehydrate in graded alcohols.
13. Clear in three or four changes of xylene.
14. Mount with synthetic resin.

Staining Results:

Mercury ---------- black

Calcium ---------- black

Copper ---------- black

Lipofuscin ---------- black

Neuromelanin ---------- black

Nuclei ---------- red

Comment:

This new method for demonstrating mercury is a modification of the Warthin-Starry technique for spirochetes and bacteria. It differs from the original Warthin-Starry procedure in that the tissue sections are not pre-treated with silver nitrate prior to placing them in the silver nitrate-gelatin-hydroquinone solution. Also, the procedure is performed in a dark 37o C oven instead of a water bath or microwave oven. It is important that the silver reaction take place in the dark in order to minimize non-specific staining.

Like most silver methods, this technique is not specific for its desired purpose. Other metals that stain black, besides mercury, are calcium and copper, but not ferric iron. Also, melanin, neuromelanin and lipofuscin stain black.

The type of gelatin used in this method is important in order to obtain satisfactory results. We found that Fisher type A gelatin with 275 bloom (the higher the bloom the stronger the gel) works the best. The presence of gelatin in the silver solution forms an emulsion which is important in that it keeps the silver ions from reacting with the hydroquinone. This prevents the formation of a black metallic silver precipitate on the slides and tissue sections.

References:

Danscher, G. and Moller-Madsen, B.: Silver amplification of mercury sulfide and selenide: a histochemical method for light and electron microscopic localization of mercury in tissue. J. Histochem. Cytochem. 33:219-228, 1985.

Moller-Madsen, B. and Danscher, G.: Localization of mercury in CNS of the rat. Environmental Research 41:29-43, 1986.

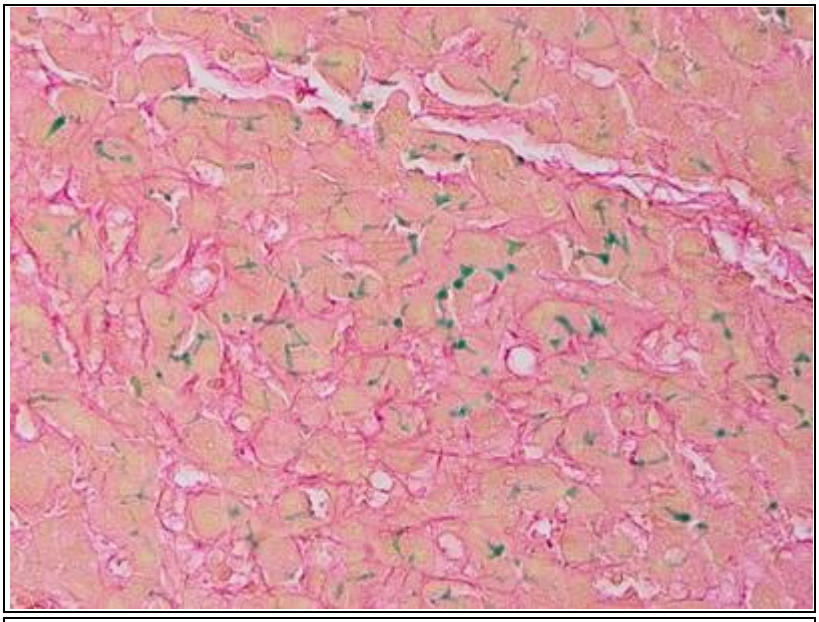
Churukian, C.J. and Schenk, E.A.: A Warthin-Starry method for spirochetes and bacteria using a microwave oven. J. Histotechnol. 11:149-151, 1988.

Churukian, C.J., Rubio, A, and Lapham, L.W.: A simple colloidal silver method (Autometallographic technique) for demonstrating inorganic mercury in brain sections. J. Histotechnol. 23: 337-339, 2000.

HALL'S METHOD FOR BILIRUBIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Bile in canaliculi of the liver (Courtesy of Dr. Zhenhong Qu)

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

25% Trichloroacetic Acid

Trichloroacetic acid ---------- 25.0 gm

Distilled water ---------- 100.0 ml

10% Ferric Chloride Solution

Ferric chloride ---------- 10.0 gm

Distilled water ---------- 100.0 ml

Fouchet's Reagent

25% trichloroacetic acid ---------- 36.0 ml

10% ferric chloride ---------- 4.0 ml

Van Gieson's Stain

1% acid fuchsin, aqueous ---------- 10.0 ml

Picric acid, saturated aqueous ---------- 100.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Stain in Fouchet's reagent for 10 minutes.
3. Wash in running water, then in distilled water.
4. Stain in Van Gieson's stain for 2 minutes.
5. Dehydrate in 95% alcohol, three changes in Coplin jars.
6. Complete dehydration in absolute alcohol, three changes.
7. Clear in xylene, three or four changes.
8. Mount with synthetic resin.

Staining Results:

Biliverdin ---------- green

Collagen ---------- red

Muscle ---------- yellow

Comment:

Bilirubin is oxidized to biliverdin and stains olive drab green to emerald green, depending upon the concentration of bilirubin.

References:

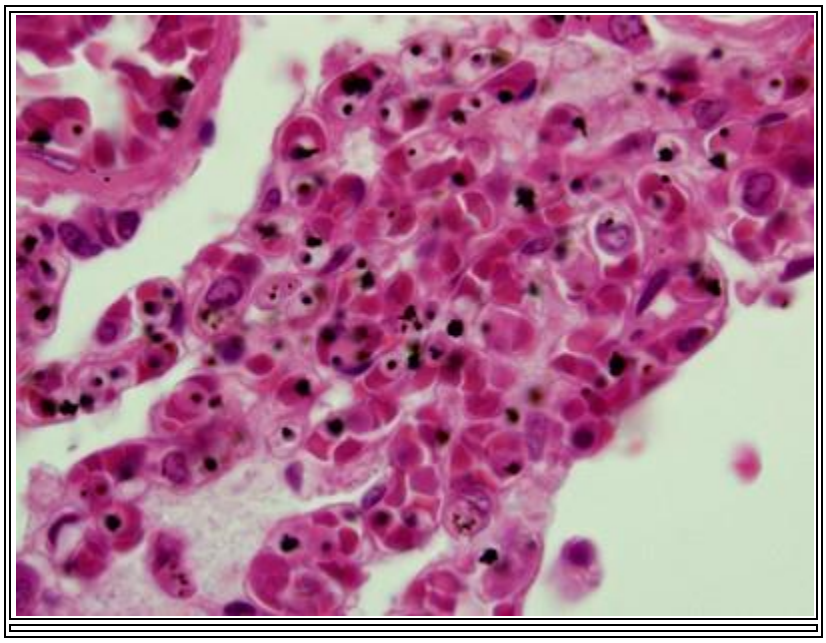
Hall, M.J.: A staining reaction for bilirubin in sections of tissue. Amer. J. Clin. Path. 34:313-316, 1960.

FORMALIN AND MALARIA PIGMENTS

METHOD FOR REMOVING FORMALIN AND MALARIA PIGMENTS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Method:

1. Deparaffinize and hydrate to distilled water.

2. Place in ammonium hydroxide – alcohol for 5-10 minutes. This is prepared by adding 10 ml of concentrated ammonium hydroxide to 90 ml of 95% alcohol.

3. Wash well in running tap water and rinse in distilled water.

4. Stain with 0.1% nuclear fast red for 5 minutes unless otherwise specified.

Comment:

Formalin pigment is also called acid formaldehyde hematein. It may be present in blood-containing tissue, especially if fixation is delayed or if non-buffered formalin is used. It appears as a brown-black deposit in and around blood vessels or where erythrocytes are present. It is birefringent and can be polarized.

Malaria pigment (hemozoin) is found in malarial parasites and may be seen in the liver, spleen, bone marrow, lymph nodes, and brain cappillaries. It is similar to formalin pigment in every respect, and can be differentiated from formalin pigment only by its location and distribution.

References:

Culling, C.F.A.: Handbook of Histopathological and Histochemical Methods, 3rd Ed., London, Butterworths, 1974, pp. 377, 379, 384-385.

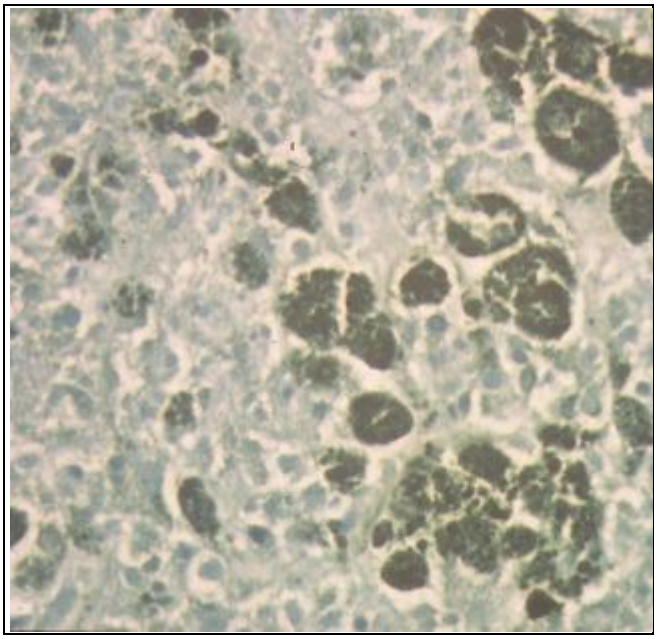
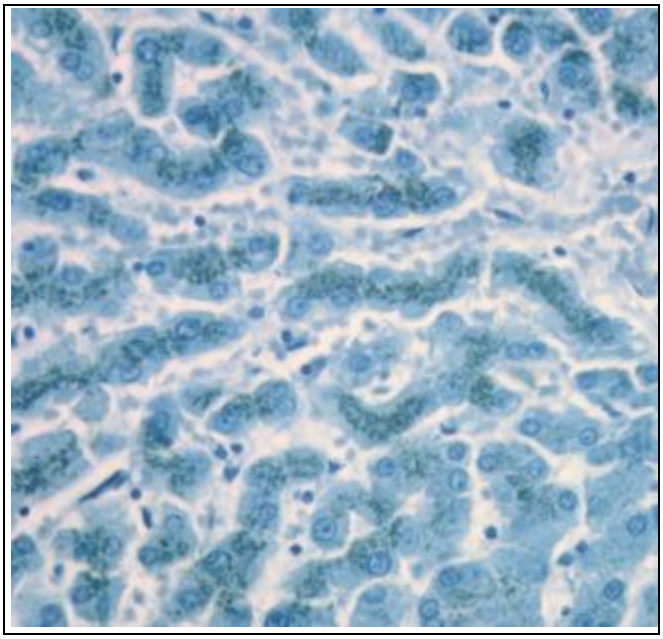
Lillie, R.D. and Fullmer, H.M.: Histopathologic Technic and Practical Histochemistry, 4th Ed., McGraw-Hill, New York, 1976, pp. 488-489.

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Ed., New York, McGraw-Hill, 1968, p. 43.

LILLIE'S SULFURIC NILE BLUE METHOD FOR MELANIN AND LIPOFUSCIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

	
Liver Melanoma	Lipofucsin

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.05% Nile Blue Solution

Nile blue, C.I. 51180	0.05 gm
Distilled water	99.00 ml
Sulfuric acid	1.00 ml

Dissolve the Nile blue in the distilled water and then add the sulfuric acid. The solution is stable for several months.

Staining Procedure:

1. Deparaffinize and hydrate paraffin sections to distilled water.
2. Place slides in the Nile blue solution for 20 minutes.
3. Rinse in four changes of distilled water.
4. Mount with glycerin jelly.

Staining Results:

Comment:

According to Lillie, if the sections are treated with acetone or 1% sulfuric acid after they are stained, lipofuscin will be removed but melanin will remain stained. Therefore, when doing the stain use control slides for both melanin and lipofuscin.

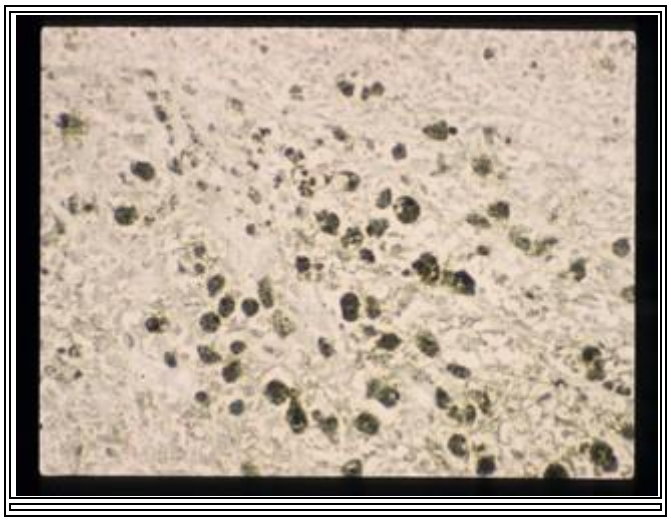
References:

Lillie, R.D.: A Nile blue staining technic for the differentiation of melanin and lipofuscins. Stain Technology 31:151-153, 1956.

LILLIE'S FERROUS ION UPTAKE METHOD FOR MELANIN

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

2.5% Ferrous Sulfate

Ferrous sulfate (Fe S04 . 7H20 ---------- 1.0 gm

Distilled water ---------- 40.0 ml

1% Potassium Ferricyanide - Acetic Acid

Potassium ferricyanide ---------- 0.5 gm

Acetic acid ---------- 0.5 ml

Distilled water ---------- 50.0 ml

0.1% Nuclear Fast Red

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. The solution should be heated to its boiling point. Cool, filter, and add a few grains of thymol as a preservative.

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place slides in 2.5% ferrous sulfate for one hour.
3. Rinse with six changes of distilled water.
4. Place in 1% potassium ferricyanide in 1% acetic acid for 30 minutes.
5. Rinse in four changes of distilled water.
6. Counterstain with nuclear fast red for 3 minutes.
7. Rinse in three changes of distilled water.
8. Dehydrate in graded alcohols.
9. Clear in xylene, three or four changes.
10. Mount with synthetic resin.

Staining Results:

Melanins of skin, eye and pia, and neuromelanin ---------- dark green

Background ---------- faint green

Nuclei ---------- red

Comment:

This method is specific for melanin because it does not stain any of the lipofuscins.

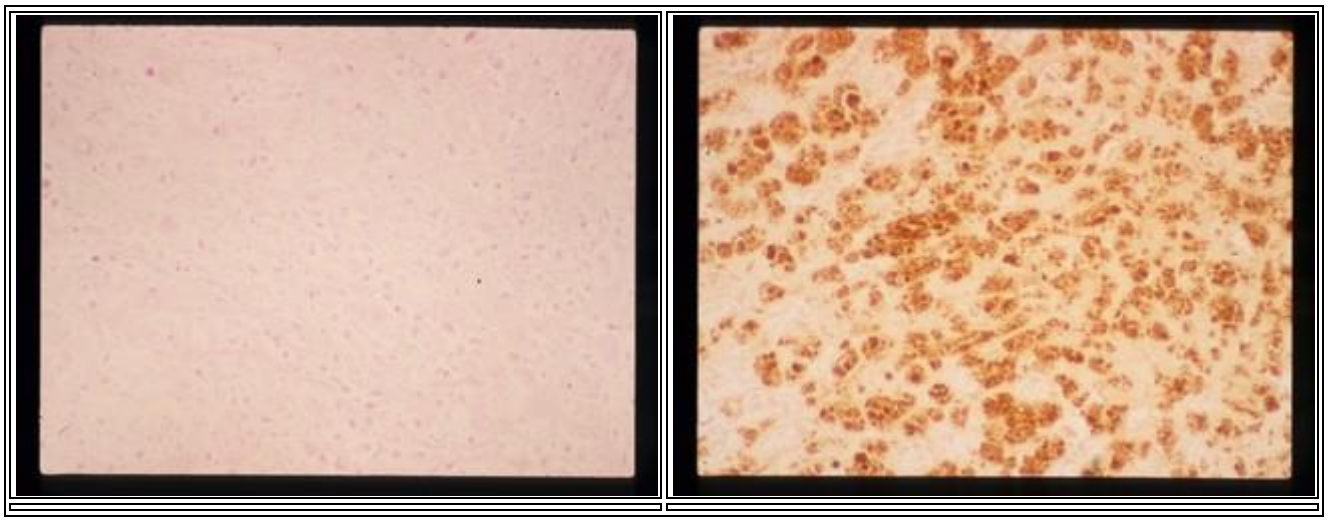
References:

Lillie, R.D. and Fullmer, H.M.: Histopathologic Technic and Practical Histochemistry, 4th Ed., New York, McGraw-Hill, 1976, pp. 526-527.

MICROWAVE MELANIN BLEACH METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

0.5% Potassium Permanganate

Potassium permanganate ---------- 0.5 gm

Distilled water ---------- 100.0 ml

0.25% Potassium Permanganate

0.5% Potassium permanganate ---------- 22.0 ml

Distilled water ---------- 22.0 ml

1% Oxalic Acid

Oxalic acid ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Nuclear Fast Red

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, and add a few grains of thymol as a preservative.

Staining Procedure:

- Use positive control slide.

1. Deparaffinize and hydrate to distilled water.

2. Place in 44 ml of 0.25% potassium permanganate solution in a glass Coplin jar and microwave at power level 1 for 3 minutes. Agitate for about 15 seconds and allow to remain in the hot solution (about 65˚ C) for 2 minutes. An alternate method is to treat the slides with potassium permanganate for 30 minutes at room temperature.

3. Rinse in three changes of distilled water.

4. Place in oxalic acid for 2 minutes.

5. Wash in running tap water for 1 minute and rinse in distilled water.

6. Nuclear fast red solution for 3 minutes.

7. Rinse in three changes of distilled water.

8. Dehydrate in graded alcohols.

9. Clear in xylene, three or four changes.

10. Mount with synthetic resin.

Staining Results:

If the yellow-brown or black granules seen on the H&E stain are no longer visible after the bleaching procedure, but are stained black with the Microwave Ammoniacal Silver Method for silver reducing substances, the pigment may be assumed to be melanin.

Comment:

We routinely do this method on all requests for melanin along with the Microwave Ammoniacal Silver Method. If a suspected malignant melanoma does not stain with the Microwave Ammoniacal Silver Method, we recommend that Schmorls method for reducing substances, the Giemsa or Lillies Nile Blue A be done. These methods may be useful for demonstrating sparse granules in amelanotic melanomas and certain tumors of the brain that may contain melanin such as Schwannomas.

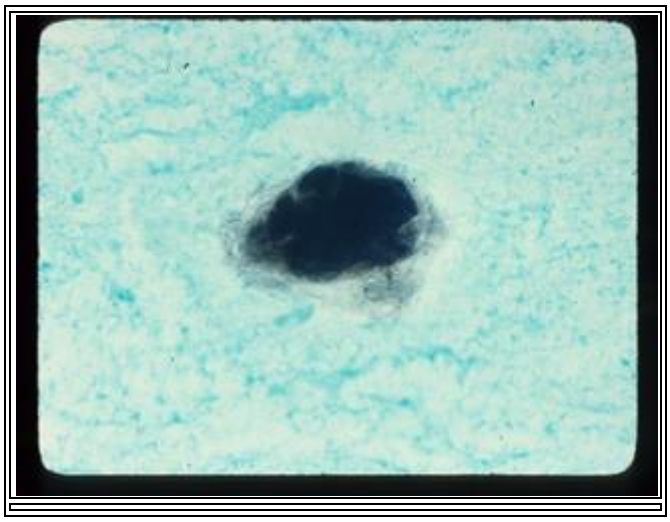
References:

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Ed., New York, McGraw-Hill, 1968, pp. 42, 89.

GOMORI'S METHOD FOR URATES

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 95% or absolute alcohol.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

5% Silver Nitrate Solution

Silver nitrate ---------- 1.0 gm

Distilled water ---------- 20.0 ml

3% Methenamine Solution

Hexamethylenetramine (methenamine) ---------- 12.0 gm

Distilled water ---------- 375.0 ml

Ethylene glycol ---------- 25.0 ml

Methenamine-Silver Nitrate Solution (Stock)

Silver nitrate, 5% solution ---------- 20.0 ml

Methenamine, 3% solution ---------- 400.0 ml

This solution is good for about one month if stored in a refrigerator.

3% Sodium Borate Solution

Sodium borate decahydrate ---------- 3.0 gm

Distilled water ---------- 100.0 ml

Methenamine-Silver Nitrate Solution (Working)

Methenamine-silver nitrate solution (stock) ---------- 20.0 ml

Distilled water ---------- 20.0 ml

Sodium borate, 3% solution ---------- 2.0 ml

Prepare fresh.

2% Sodium Thiosulfate Solution

Sodium thiosulfate ---------- 2.0 gm

Distilled water ---------- 100.0 ml

0.2% Fast Green Solution (Stock)

Fast green FCF, C.I. 42053	0.2 gm
Distilled water	100.0 ml
Acetic acid, glacial	0.2 gm

Fast Green Solution (Working)

Fast green (stock)	10.0 ml
Distilled water	35.0 ml

Staining Procedure:

- Use positive control slide.

1. Deparaffinize and run slides to 95% alcohol. Stand slides on end and allow to thoroughly dry.
2. Place in working methenamine-silver nitrate solution and place in a 37˚ C oven for 2 hours.
3. Rinse in four changes of distilled water.
4. Place in 2% sodium thiosulfate for 1 minute.
5. Rinse in four changes of distilled water.
6. Counterstain with working fast green solution for 30 seconds.
7. Rinse in three changes of distilled water.
8. Dehydrate in graded alcohols.
9. Clear in three or four changes of xylene.
10. Mount with synthetic resin.

Staining Results:

Urates and uric acid ---------- dark brown to black

Melanin and some calcium salts ---------- black

Background ---------- green

Comment:

Uric acid occurs in the form of acid sodium urate. Heavy metal salts of uric acid are insoluble and can be converted into colored compounds as can phosphates and carbonates. Uric acid is an argentaffin substance and therefore can be identified by the above argentaffin method. It can also be polarized which sometimes aids in its identification.

Small amounts of calcium salts are unlikely to give false positive results because they are dissolved by the silver solution. If an additional precaution is required, the section should be treated with 0.5% hydrochloric acid in 70% alcohol for two minutes to remove all traces of calcium salts before treatment with the silver solution.

References:

Sheehan, D.C. and Hrapchak, B.B.: Theory and Practice of Histotechnology, The C.V. Mosby Company, St. Louis, 1980, pp. 225-226.

Culling, C.F.A.: Handbook of Histopathological and Histochemical Techniques, Butterworths, London, 1974, pp. 389-390.

LIST OF STAINS FOR NERVE CELLS AND FIBERS

BODIANS METHOD FOR NERVE FIBERS AND NERVE ENDINGS

LAPHAMS PHLOXINE-FAST GREEN METHOD FOR MYELIN AND GLIAL FIBERS

MICROWAVE LUXOL FAST BLUE - CRESYL VIOLET STAIN FOR MYELIN AND NERVE FIBERS

MICROWAVE LUXOL FAST BLUE - PERIODIC ACID - SCHIFF-CRESYL VIOLET METHOD

ACRIDINE ORANGE METHOD FOR DEMONSTRATING MYENTRIC GANGLION CELLS IN HIRSCHSPRUNGS DISEASE

EINARSONS METHOD FOR NISSL SUBSTANCE

MICROWAVE MODIFICATION OF BIELSCHOWSKYS METHOD

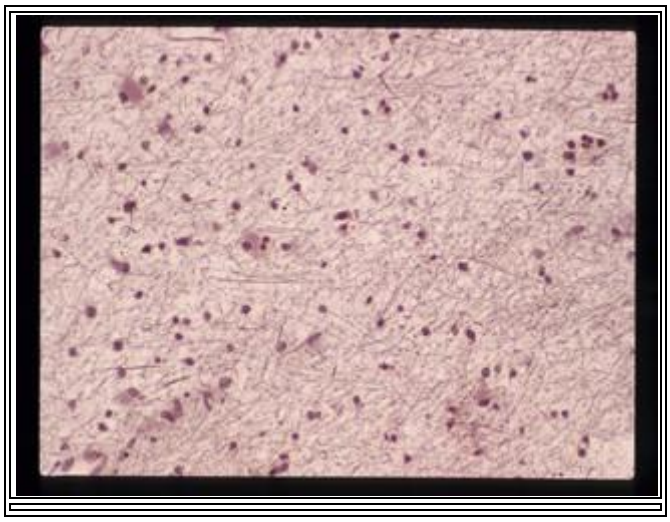
CRESYL VIOLET ACETATE METHOD FOR NISSL SUBSTANCE

THIOFLAVIN S METHOD FOR AMYLOID IN NEUROFIBRILLARY PLAQUES

BODIAN'S METHOD FOR NERVE FIBERS AND NERVE ENDINGS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections at 6-8 µm.

SOLUTIONS:

1% Protargol Solution

Protargol, certified ---------- 0.4 gm

Distilled water ---------- 40.0 ml

Sprinkle the protargol on the surface of the water and allow it to remain undisturbed until it dissolves.

Reducing Solution

Hydroquinone	0.4 gm
Formalin, 37-40%	2.0 ml
Distilled water	40.0 ml

0.2% Gold Chloride Solution

Gold chloride	0.2 gm
Distilled water	100.0 ml

2% Oxalic Acid Solution

Oxalic acid	2.0 gm
Distilled water	100.0 ml

2% Sodium Thiosulfate Solution

Sodium thiosulfate	2.0 gm
Distilled water	100.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.

2. Place slides in protargol solution. Add 4 gm of clean copper shot to 40 ml of solution. Allow to remain in a 55-60° C oven overnight.

3. Rinse in distilled water, three changes.

4. Reducing solution for 10 minutes.

5. Rinse in distilled water, three changes.

6. Tone in gold chloride solution for 10 minutes.

7. Rinse in distilled water, three changes.

8. Develop in oxalic acid solution for 4-6 minutes. Check with a microscope until background is gray and nerve fibers appear clearly.

9. Rinse in distilled water, three changes.

10. Sodium thiosulfate solution for 5 minutes.

11. Rinse in distilled water.

12. Dehydrate in graded alcohols.

13. Clear in xylene, three or four changes.

14. Mount with synthetic resin.

Staining Results:

Nerve fibers ---------- black

Nuclei ---------- black

NOTE:

If desired, counterstains may be used. See Manual of Histologic Staining Method of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 195-196.

Comment:

The glassware should be thoroughly cleaned and rinse with double distilled water.

References:

Luna, L.G.: Further Studies of Bodians Technique. Amer. J. Med. Techn. 30:355-363, 1964.

Mallory, F.B.: Pathological Technique, New York, Hafner, 1968, pp. 228-229.

LAPHAM'S PHLOXINE-FAST GREEN METHOD

FOR MYELIN AND GLIAL FIBERS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 10-12 µm.

SOLUTIONS:

Gallocyanin Solution

Chromium potassium sulfate, CrK(SO4)2 • 12H20) ---------- 5.00 gm

Distilled water ---------- 100.00 ml

Gallocyanin, C.I. 51030 ---------- 0.15 gm

Heat and bring to boiling. Boil for 15-20 minutes. Filter when cool.

0.5% Phloxine Solution

Phloxine B, C.I. 45410 ---------- 0.5 gm

Distilled water ---------- 100.0 ml

The solution is stable for one month.

0.05% Fast Green Solution

Fast green FCF, C.I. 42053	0.05 gm
Distilled water	100.00 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in freshly filtered gallocyanin solution and stain overnight at room temperature.
3. Wash in running tap water for 5 minutes and rinse in two changes of distilled water.
4. Phloxine solution for 5 minutes.
5. Wash in running tap water for 3 minutes.
6. Mordant in 5% aqueous phosphotungstic acid for 1 minute.
7. Wash in running tap water for 3 minutes.
8. Place in 1% acetic acid for 2 minutes.
9. Differentiate in 80% alcohol for 1-2 minutes. Check with a microscope after 1 minute. Myelin should stand out clearly.
10. Place in 1% acetic acid for 1 minute.
11. Fast green solution for 1 minute.
12. Dehydrate in three changes of 95% alcohol (Coplin jars) and three changes of absolute alcohol.
13. Clear in three or four changes of xylene.
14. Mount with synthetic resin.

Staining Results:

Myelin	magenta

Glial fibers ---------- green

Nissl plump astrocytes ---------- green

Nuclei ---------- black or blue black

Nucleoli ---------- usually red

Collagen ---------- green

Neurons ---------- black

Oligodendrocytes ---------- red or black

References:

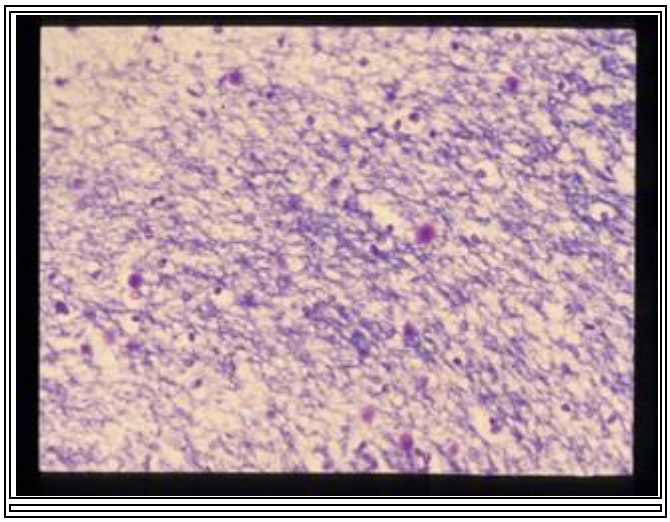
Lapham, L.W., et al.: A New Paraffin Method for the Combined Staining of Myelin and Glial Fibers. J. Neuropath. Exp. Neurol. 23:156-160, 1964

MICROWAVE LUXOL FAST BLUE - CRESYL VIOLET

STAIN FOR MYELIN AND NERVE CELLS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 8-12 µm.

SOLUTIONS:

0.1% Luxol Fast Blue Solution

Luxol fast blue, MBS ---------- 0.1 gm

Alcohol, 95% ---------- 100.0 ml

Dissolve dye in alcohol. Add 0.5 ml of 10% glacial acetic acid

to each 100 ml.

Cresyl Violet Acetate Solution

*Cresyl violet acetate	0.04 gm
Distilled water	40.00 ml
1% oxalic acid	0.20 ml

*Cresyl echt violet may be substituted for cresyl violet acetate.

0.05% Lithium Carbonate Solution

Lithium carbonate	0.05 gm
Distilled water	100.00 ml

70% Alcohol Solution

Alcohol, 100%	70.0 ml
Distilled water	30.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to 95% alcohol.

2. Place in 40 ml of Luxol fast blue solution in a glass Coplin jar and microwave at power level 1 (60W) for 3 minutes. Allow the slides to remain in the hot solution for 30 minutes.

3. Rinse off excess stain in 95% alcohol.

4. Rinse in three changes of distilled water.

5. Begin differentiation by placing in 0.05% lithium carbonate solution for 5 seconds.

6. Continue differentiation in 70% alcohol for 5-10 seconds.

7. Rinse in four changes of distilled water. Check with a microscope and repeat steps 5 and 6 if necessary.

8. Cresyl violet acetate solution for 5 minutes.

9. Rinse in three changes of distilled water.

10. Dehydrate in graded alcohols.

11. Clear in xylene, three or four changes.

12. Mount with synthetic resin.

Staining Results:

Myelin ---------- blue

Cell products ---------- pink to violet

Comment:

The use of the microwave oven greatly reduces the time required to perform the method. The temperature of the solution should be 75-80˚ C after it is microwaved. Another way of performing the stain is by staining overnight in the luxol fast blue solution in a 56-60˚ C oven.

References:

Bajues, E.: Conditioning Factors for Cardiac Necrosis, International Medical Book Corporation, 131: 1963.

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 203-204.

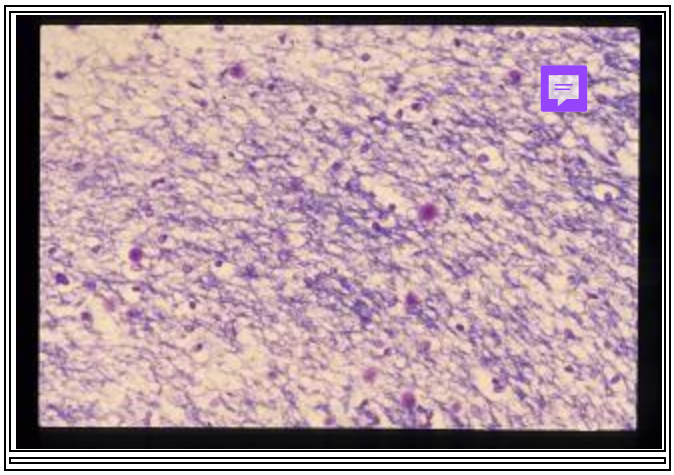
MICROWAVE LUXOL FAST BLUE - PERIODIC ACID -

SCHIFF-CRESYL VIOLET METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 8-12 µm.

SOLUTIONS:

0.1% Luxol Fast Blue Solution

Luxol fast blue, MBS ---------- 0.1 gm

Alcohol, 95% ---------- 100.0 ml

Acetic acid, 10% ---------- 0.5 ml

0.05% Lithium Carbonate Solution

Lithium carbonate ---------- 0.05 gm

Distilled water ---------- 100.00 ml

70% Alcohol Solution

Alcohol, 100% ---------- 70.0 ml

Distilled water ---------- 30.0 ml

0.5% Periodic Acid Solution

Periodic acid ---------- 0.5 gm

Distilled water ---------- 100.0 ml

Modified Lillies Schiff Solution

(see PAS method)

0.3% Sodium Borate

(See PAS method)

Cresyl Violet Acetate Solution

*Cresyl violet acetate ---------- 0.04 gm

Distilled water ---------- 40.00 ml

Oxalic acid, 1% ---------- 0.20 ml

*Cresyl echt violet may be substituted for cresyl violet acetate.

Staining Procedure:

1. Deparaffinize and hydrate to 95% alcohol.

2. Place in 40 ml of luxol fast blue solution in a glass Coplin jar and microwave at power level 1 (60W) for 3 minutes. Allow the slides to remain in the hot solution for 30 minutes.

3. Rinse off excess stain in 95% alcohol.

4. Rinse in three changes of distilled water.

5. Begin differentiation by placing in 0.05% lithium carbonate for 5 seconds.

6. Continue differentiation in 70% alcohol for 5-10 seconds.

7. Rinse in four changes of distilled water. Check under microscope and repeat steps 5 and 6 if necessary.

8. Place in 0.5% periodic acid for 10 minutes.

9. Rinse in four changes of distilled water.

10. Place in modified Lillies Schiff solution for 10 minutes.

11. Rinse in three changes of distilled water.

12. Place in 0.3% sodium borate for 15 seconds and rinse in four changes of distilled water.

13. Cresyl violet acetate solution for 5 minutes.

14. Rinse in three changes of distilled water.

15. Dehydrate in graded alcohols.

16. Clear in three or four changes of xylene.

17. Mount with synthetic resin.

Staining Results:

Myelin ---------- blue

PAS positive elements ---------- rose to red

Capillaries ---------- red

Nuclei ---------- purple-blue

Comment:

The use of the microwave oven greatly reduces the time required to perform the method. The temperature of the solution should be 75-80˚ C. Another way of performing the stain is by staining overnight in the luxol fast blue solution in 56-60˚ C oven.

References:

Garvey, W. et al: Combined modified periodic acid-Schiff and batch staining method. J. Histotechnol. 15:117-120, 1992.

Bajues, E.: Conditioning Factors for Cardiac Necrosis, International Medical Book Corporation, 131:1963.

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., 204.

McManus, J.F.A., and Mowry, R.W.: Staining Methods Histologic and Histochemical, New York, Paul B. Hoeber, 1960, pp. 331-333.

ACRIDINE ORANGE METHOD FOR DEMONSTRATING MYENTERIC GANGLION CELLS IN HIRSCHSPRUNGS DISEASE

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm.

SOLUTIONS:

1% Acetic Acid

Acetic acid ---------- 1.0 gm

Distilled water ---------- 99.0 ml

0.067M Phosphate Buffer, pH 6.0

Potassium phosphate, monobasic ---------- 3.87 gm

Sodium phosphate, dibasic ---------- 0.70 gm

Distilled water to make a total of ---------- 500.00 ml

If necessary, adjust the pH to 6.0 with 1N phosphoric acid or 1N sodium hydroxide.

0.02% Acridine Orange in Phosphate Buffer

Acridine orange, C.I. 46005	0.02 gm
Phosphate buffer, pH 6.0	100.00 ml

0.1M Calcium Chloride

Calcium chloride, anhydrous	1.11 gm
Distilled water to make a total of	100.00 ml

Staining Procedure:

- Use control slides.

1. Deparaffinize and hydrate to distilled water.
2. Place in 1% acetic acid for 1 minute.
3. Place in distilled water for 1 minute.
4. Stain in 0.02% acridine orange in phosphate buffer for 30 seconds.
5. Place in phosphate buffer, pH 6.0 for 1 minute.
6. Differentiate in 0.1M calcium chloride for 45 seconds.
7. Place in phosphate buffer, pH 6.0 for 1 minute.
8. Mount with phosphate buffer, pH 6.0.

Staining Results:

Myenteric Ganglion cells fluoresce an intensive orange-red. Plasma cells also fluoresce orange-red. Smooth muscle and connective tissue will fluoresce a pale green.

References:

Perl, D.P., and Little, B.N.: Acridine orange - nucleic acid fluorescence, its use in routine diagnostic muscle biopsies. Arch. Neurol. 37:641-644, 1980.

Sarnat, H.B., et al.: A fluorochromic stain for nuclei acids to demonstrate submucosal and myenteric neurons in Hirschsprungs disease. Amer. J. Clin. Path. 83:722-725, 1985.

EINARSON'S METHOD FOR NISSL SUBSTANCE

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 6-8 µm.

SOLUTIONS:

Gallocyanin Solution

Gallocyanin, C.I. 51030	0.15 gm
Chromium potassium sulfate	5.00 gm
Distilled water	100.00 ml

Dissolve the chrom alum in warm water, add gallocyanin, and boil gently for 10 minutes. Cool and filter. This solution keeps for one week.

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.

2. Place sections in gallocyanin solution and place in a 37˚ C oven for 6 hours or leave overnight at room temperature.

3. Rinse in two changes of distilled water.

4. Dehydrate in graded alcohols.

5. Clear in three or four changes of xylene.

6. Mount with synthetic resin.

Staining Results:

Nissl substance ---------- blue

References:

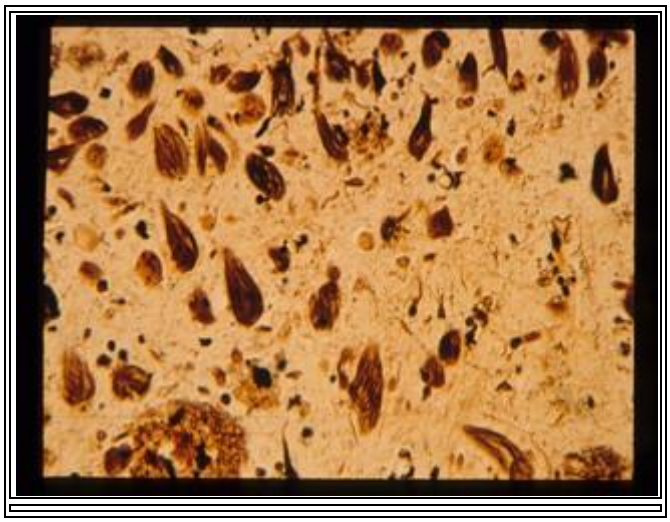
Einarson, L: Amer. J. Path. 88:295-309, 1932.

MICROWAVE MODIFICATION OF

BIELSCHOWSKYS METHOD FOR NERVE FIBERS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 8 µm.

SOLUTIONS:

1.0% Silver Nitrate Solution

Silver nitrate ---------- 0.4 gm

Distilled water ---------- 40.0 ml

Prepare fresh.

5% Silver Nitrate Solution

Silver nitrate ---------- 0.1 gm

Distilled water ---------- 2.0 ml

Store in a refrigerator at 3-6O C.

10% Nitric Acid Solution

Nitric acid, approximately 70% ---------- 1.0 ml

Distilled water ---------- 9.0 ml

Prepare fresh.

Developer Solution

Formaldehyde, 37-40%, reagent grade ---------- 0.4 ml

Distilled water ---------- 4.0 ml

Citric acid ---------- 0.2 gm

10% Nitric acid ---------- 0.1 ml

Prepare fresh.

1% Ammonium Hydroxide Solution

Ammonium hydroxide, 28% ---------- -- 1.0 ml

Distilled water ---------- 99.0 ml

2% Sodium Thiosulfate Solution

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

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.

2. Place slides in 40 ml of 1.0% silver nitrate solution in a plastic Coplin jar and microwave at power level 3 (180W) for 1 minute. Dip the slides up and down several times and allow them to remain in the warm solution (50˚ C) for 15 minutes.

3. Place slides in distilled water.

4. Pour the warm 1% silver nitrate used in step 2 in a 125 ml flask. Add 28% ammonium hydroxide, drop by drop with constant shaking, until the initial precipitate disappears and the solution turns clear. Then add 5% silver nitrate, drop by drop with constant shaking, until the solution becomes slightly cloudy.

5. Pour the ammoniacal silver solution prepared in step 4 into a plastic Coplin jar. Place slides in this solution and microwave at power level 3 (180W) for 1 minute. Dip the slides up and down several times and allow them to remain in the warm solution (60˚ C) for 15 minutes.

6. Place slides in 1% ammonium hydroxide solution for not more than 20 seconds.

7. Add three drops of developer to the ammoniacal silver solution used in step 5. Quickly mix with a glass rod and immediately place the slides in the solution for about 3 minutes or until the tissue sections turn brown. The solution will turn a greyish color and a mirror of silver will form on the sides of the Coplin jar and sometimes on the slides, but not on the tissue sections.

8. Place slides in 1% ammonium hydroxide solution for not more than 15 seconds.

9. Rinse in three changes of distilled water.

10. Wipe off the mirror of silver from both sides of the slides taking care not to damage the tissue sections.

11. Place slides in 2% sodium thiosulfate solution for 30 seconds.

12. Rinse in four changes of distilled water.

13. Dehydrate in graded alcohols.

14. Clear in three or four changes of xylene.

15. Mount with synthetic resin.

Staining Results:

Axones ---------- brown to black (usually sharply linear)

cytoplasmic neurofibrils ---------- brown to black

Neurofibrillary tangles and plaques of Alzheimers disease ---------- dark brown or black

Granulovacuolar bodies ---------- black

Neurons and background ---------- varying shades of brown

Neuromelanin ---------- black

Lipofuscin ---------- brown or black

Comment:

This staining method differs from Bielschowskys method in that it requires much less silver nitrate. In Bielschowskys method at least 8.0 gm of silver nitrate is used as compared to only 0.5 gm of silver nitrate in the described method.

We have found that neurofibrillary tangles and plaques of Alzheimers disease stain better with the described procedure than they do with Bielschowskys method. Our method is also less time consuming requiring only about 40 minutes to perform.

When solutions are heated with microwave irradiation there can be up to 15˚ C difference in temperature between the top and bottom portion of the solutions. Therefore, in order to equalize the temperature of the solutions the slides are dipped up and down in steps 2 and 5 of the staining procedure. This assures uniformity of staining results.

It is essential to use acid cleaned glassware, rinsed in double distilled water.

The shelf-life of silver nitrate can be greatly increased by storing it in a refrigerator at 3-6˚ C.

References:

Mallory, F.B.: Pathological Technique. Hafner Publishing Co., New York, 1968, pp. 158-160.

Ketring, J.L. et al.: Modification of the silver impregnation technique of Bielschowsky for use in glycol methacrylate-embedded brain tissue. Arch. Pathol. Lab. Med. 113:196-198, 1989.

CRESYL VIOLET ACETATE METHOD FOR NISSL SUBSTANCE

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 6-8 µm.

SOLUTIONS:

Cresyl Violet Acetate Solution

Cresyl violet acetate		0.02 gm
Distilled water	----	100.00 ml
Glacial acetic acid		0.25 ml

Staining Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Place slides in cresyl violet acetate solution for 5 minutes.
3. Rinse in three changes of distilled water.
4. Dehydrate in graded alcohols.
5. Clear in three or four changes of xylene.
6. Mount with synthetic resin.

Staining Results:

Nissl substance ---------- purple

Nuclei ---------- purple

Background ---------- clear

Comment:

Some staining methods require differentiation in 95% alcohol until Nissl substance stands out against a clear background. In the described method the differentiation takes place when the slides are dehydrated in graded alcohols.

References:

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 2nd ed., New York, McGraw-Hill Book Co., p,. 160, 1960.

THIOFLAVIN S METHOD FOR AMYLOID IN

NEUROFIBRILLARY PLAQUES

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 6-8 μm.

SOLUTIONS:

1% Thioflavin S

Thioflavin S, C.I. 49010 ---------- - 1.0 gm

Distilled water ---------- 100.0 ml

1% Thioflavin T

Thioflavin T, C.D. 49005 ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Modified Weigert's Iron Hematoxylin

(See Modified Puchtler's Congo Red Method)

0.5% Acid Alcohol Solution

(See Modified Puchter's Congo Red Method)

1% Acetic Acid

Acetic acid ---------- 1.0 ml

Distilled water ---------- 100.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Place in modified Weigert's iron hematoxylin for 15 seconds.
3. Rinse in three changes of distilled water.
4. Place in 0.5% acid alcohol for 5 seconds.
5. Rinse in four changes of distilled water.
6. Stain with 1% thioflavin S for 5 minutes. For all other tissues stain in 1% thioflavin T for 5 minutes.
7. Wash well in running water.
8. Place in 1% acetic acid for 15 minutes.
9. Rinse in three changes of distilled water.
10. Stand slide on end and thoroughly air dry.
11. Dip in xylene and mount with synthetic resin.

Staining Results:

When thioflavin S is viewed with a fluorescence microscope amyloid in neurofibrillary plaques and in blood vessels will fluoresce intense yellow-white. Amyloid in tissues stained with thioflavin T will fluoresce an intense white.

Comment:

Thioflavin S stains amyloid in neurofibrillary plaques in brain much better than thioflavin T. However, there is considerable non-specific background fluorescence. Thioflavin T stains amyloid well in other tissues and there is very little background fluorescence.

Most texts that contain the thioflavin S and thioflavin T methods use alum hematoxylin to quench background fluorescence. We found that Weigert's iron hematoxylin is more effective in quenching background fluorescence.

References:

Schwartz, P.: Amyloid degeneration and tuberculosis in the aged. Gerontologia 18:321-362, 1972.

Vassar, P.S.and Culling, C.F.A.: Fluorescent stains, with special reference to amyloid and connective tissue. Arch. Pathol. 68:487, 1959.

LIST OF STAINS FOR CONNECTIVE TISSUE

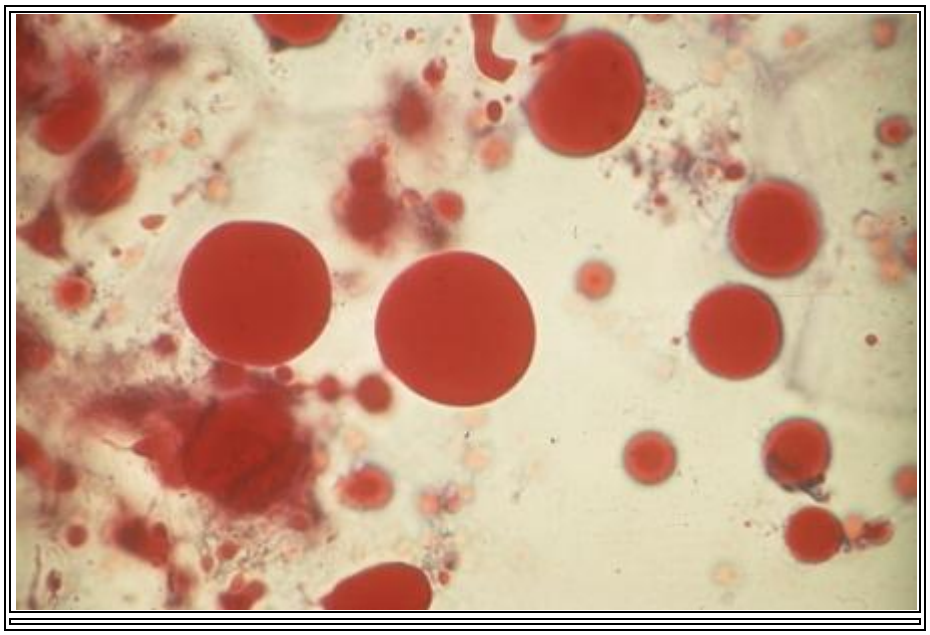
OIL RED O METHOD FOR FATS

SUDAN BLACK B FOR PHOSPHOLIPIDS IN PARAFFIN SECTIONS AND FATS IN FROZEN SECTIONS

OIL RED O METHOD FOR FATS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Unfixed cryostat sections cut at 10 μm or frozen sections of formalin fixed tissue. Touch preparations and material from cytology may be done.

SOLUTIONS:

Oil Red O Stock Solution

Oil red O, C.I. 26125 ---------- 0.5 gm

Isopropyl alcohol, 99% ---------- 100.0 ml

Oil Red O Working Solution

Oil red O stock solution ---------- 60.0 ml

1% aqueous dextrin ---------- 40.0 ml

Allow the solution to set for several days. Filter through Whatman paper #4 before use.

60% Isopropyl Alcohol Solution

Isopropyl alcohol, 99% ---------- 60.0 ml

Distilled water ---------- 40.0 ml

0.3% Sodium Borate

Sodium borate (Na2 B4 O7 10H2O) ---------- -- 0.3 gm

Distilled water ---------- 100.0 ml

Staining Procedure:

1. Mount cryostat-cut sections of clean slides and fix in 10% buffered neutral formalin for 15 minutes. Formalin fixed frozen sections may be floated in water and attached to albuminized slides. Allow to thoroughly dry (one to two hours) to assure paper adherence. Fixation for touch preparations and material from cytology is not necessary.

2. Rinse briefly with distilled water.

3. A few dips in 60% isopropyl alcohol.

4. Oil red O working solution for 20 minutes.

5. A few dips in 60% isopropyl alcohol.

6. Rinse with four changes of distilled water.

7. Counterstain with acidified Lillie-Mayer hematoxylin for 1 minute.

8. Rinse in three changes of distilled water.

9. Blue hematoxylin in 0.3% sodium borate for 15 seconds.

10. Rinse with four changes of distilled water.

11. Mount with glycerin jelly.

Staining Results:

Fats ---------- red

Nuclei ---------- blue

Comment:

In our experience this oil red O-isopropyl alcohol method gives better results than the propylene glycol, alcohol-acetone, or triethylphosphate methods. Oil red O is preferred over Sudan III and Sudan IV for staining of lipids in tissue sections.

According to Lillie the use of 1% aqueous dextrin instead of distilled water, in preparing the oil red O working solution will stabilize and intensify the stain.

References:

Lillie, R.D.: Histopathologic Technic and Practical Histochemistry, 4th ed., New York, McGraw-Hill, 1976, pp. 567-568.

Preece, A.: Manual for Histologic Technicians, 3rd ed., Boston, Little, Brown, 1972, pp. 259-260.

Stotz, H., Schenk, E.A., Churukian, C., and Willis, C.: Oil red O: Comparison of staining quality and chemical components as determined by thin layer chromatography, Stain Technol. 61:187-190, 1986.

SUDAN BLACK B FOR PHOSPHOLIPIDS IN PARAFFIN SECTIONS

AND FATS IN FROZEN SECTIONS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 5 µm or frozen sections cut at 8 µm.

SOLUTIONS:

Sudan Black B Solution

Sudan black B, C.I. 26150 ---------- 1.0 gm

Isopropyl alcohol ---------- 70.0 ml

Place on a magnetic stirrer and allow to mix for 30 minutes.

Dissolve 0.3 gm of dextrin in 30 ml of distilled water and add to

the Sudan black B solution. Mix for 5 minutes and filter through

Whatman #4 filter paper into an amber bottle. Filter again before use.

0.1% Nuclear Fast Red Solution

(see Pearls Method for Iron)

Glycerin Jelly

Gelatin ---------- 10.0 gm

Distilled water ---------- 60.0 ml

Heat until gelatin is dissolved. Add:

Glycerin ---------- 70.0 ml

Phenol, melted ---------- 1.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to 70% alcohol. When staining frozen sections, begin at step 3.

2. Stand slides on end and allow to dry.

3. Place in Sudan black B solution for one to three hours. Check after one hour but first dip in 70% isopropyl alcohol. When staining frozen sections, stain for 20 minutes at room temperature.

4. Rinse thoroughly in two changes of 70% isopropyl alcohol.

5. Wash with six changes of distilled water.

6. Nuclear fast red solution for 3 minutes.

7. Rinse in two changes of distilled water.

8. Mount with glycerin jelly.

Staining Results:

Phospholipids ---------- black or blue-black

Fats ---------- black or blue-black

Erythrocytes ---------- blue-black

Nuclei ---------- pink

Comment:

When paraffin sections are stained with this procedure only undissolved structural lipids (phospholipids) are stained. Fats present in frozen sections stain intensely black or blue-black.

The addition of dextrin to the Sudan black B helps to stabilize the solution and thus extend its shelf-life (six months or longer).

References:

Disbrey, B.D., and Rack, H.H.: Histological Laboratory Methods, E. and S. Livingston, London, 1970, 162-163.

Lison, L. and Dagnelie, J.: Methods for the Staining of Myelin. Bull. Histol. Appl. Physiol. Path. 12:85, 1935.

LIST OF STAINS FOR HISTOCHEMICAL ELEMENTS

ALPHA-NAPHTHYL ACETATE METHOD FOR NONSPECIFIC ESTERASE

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin. Fix smears in absolute methyl alcohol.

TECHNIQUE: Paraffin or plastic sections cut at 5 µm. Smears and touch preparations may be used.

SOLUTIONS:

Phosphate Buffer Solution pH 7.6

Sodium phosphate, dibasic, Na_2HPO_4	3.5 gm
Potassium phosphate, monobasic, KH_2PO_4	0.5 gm
Distilled water to make a total of	400.0 ml

Alpha-Naphthyl Acetate Solution

Alpha-naphthyl acetate	0.04 gm
Ethylene glycol monomethyl ether	2.00 ml

0.3% Sodium Borate

(see PAS Method)

Staining Procedure:

1. Deparaffinize and hydrate sections to distilled water.

2. Mix 1.2 ml of 4% hexazotized pararosanilin and 1.2 ml of 4% sodium nitrite in a 50 ml flask and let stand for one minute. To this add 36 ml of phosphate buffer pH 7.6, mix and add to the alpha-naphthyl acetate solution and mix well. Adjust the pH to 6.1 with 1N hydrochloric acid (range 5.8-6.5 is acceptable). Filter and immediately place slides in this solution and stain for 45 minutes.

3. Wash slides in running tap water for 5 minutes.

4. Rinse in distilled water.

5. Counterstain with acidified Lillie-Mayer or Harris hematoxylin for 1 minute.

6. Rinse in three changes of distilled water.

7. Blue hematoxylin in 0.3% sodium borate for 15 seconds.

8. Rinse in four changes of distilled water.

9. Air dry the slides.

10. Dip in xylene and mount with synthetic resin.

Staining Results:

Nonspecific esterase in the cytoplasm of monocytes,

Histiocytes and megakaryocytes ---------- brown-red

Nuclei ---------- blue

References:

Brown, G.G.: An Introduction to Histotechnology, New York, Appleton-Century-Crofts, 1978, pp. 346-347.

Yam, L.T., Li, C.Y., and Crosby, W.H.: Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol. 55:283-290, 1971.

AMINO ACID NAPHTHYLAMIDASE METHOD

FOR BILE CANALICULI

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: Smears and touch preparations are fixed in ice cold acetone and chloroform 1:1.

TECHNIQUE: Smears of liver aspirates or touch imprints of liver.

SOLUTIONS:

0.1M Acetic Acid

Acetic acid ---------- 2.8 ml

Distilled water to make a total of ---------- 500.0 ml

0.1M Sodium Acetate

Sodium acetate, anhydrous ---------- 4.1 gm

Distilled water to make a total of ---------- 500.0 ml

0.1M Acetic acid-Sodium acetate pH 6.5

0.1M acetic acid ---------- 2.0 ml

0.1M sodium acetate ---------- 198.0 ml

Check with pH meter and adjust to pH 6.5 with either acetic acid

or sodium acetate solution.

0.85% Sodium Chloride

Sodium chloride ---------- 0.85 gm

Distilled water ---------- 100.00 ml

0.02M Potassium Cyanide

Potassium cyanide ---------- 0.65 gm

Distilled water ---------- 100.00 ml

L-alanyl-4-methoxy-2-naphthylamide (AMN) Solution

L-alanine-4-methoxy-2-naphthylamide ---------- 0.02 gm

Distilled water ---------- 2.5 ml

0.3% Sodium Borate

(see PAS Method)

Staining Procedure:

1. Place smears in an ice cold solution of equal parts of acetone and chloroform for 3 minutes.
2. Remove and allow the acetone and chloroform to evaporate form the slides.
3. Incubate the smears in the following solution at 37˚ C for one hour:

0.1M acetic acid - sodium acetate buffer ---------- 25.0 ml

0.85% sodium chloride ---------- 20.0 ml

AMN solution ---------- 2.5 ml

0.02M potassium cyanide ---------- 2.5 ml

Fast red B salt ---------- 25.0 mg

4. Rinse in four changes of distilled water.

5. Counterstain with acidified Lillie-Mayer hematoxylin for 1 minute.

6. Rinse in three changes of distilled water.

7. Blue hematoxylin in 0.3% sodium borate for 15 seconds.

8. Rinse in four changes of distilled water.

9. Mount with glycerine jelly.

Staining Results:

Bile canaliculi appear as a regular network of red bands.

References:

Wasastjerma, C. and Ekelund, P.: The amino acid naphthylamidase reaction of the bile canaliculi in liver smears. Acta Cytologica 18:23-29, 1974.

NAPHTHOL AS - D CHLOROACETATE METHOD FOR

ESTERASE IN PARAFFIN SECTIONS AND SMEARS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:

Pictures will be available soon.

Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin. Avoid fixative containing mercury. For smears and touch preparations fix for two minutes in absolute methanol containing 10% formaldehyde.

TECHNIQUE: Paraffin or plastic sections cut at 5 µm. Smears and touch preparations may be used.

SOLUTIONS:

4% Hexazolized Pararosanilin

Pararosanilin, C.I. 42500 ---------- 1.0 gm

Distilled water ---------- 20.0 ml

Hydrochloric acid, concentrated ---------- 5.0 ml

4% Sodium Nitrite

Sodium nitrite ---------- 0.4 gm

Distilled water ---------- 10.0 ml

Phosphate Buffer, pH 6.5

Sodium phosphate, dibasic, Na2 HPO4 ----------	0.652 gm
Potassium phosphate, monobasic, KH2PO4 ----------	1.348 gm
Distilled water ----------	200.000 ml

Naphthol AS - D Chloroacetate Solution

Naphthol AS - D chloroacetate ----------	0.01 gm
N,N – dimethylformamide ----------	1.00 ml

Prepare shortly before use.

0.3% Sodium Borate

(see PAS Method)

Staining Procedure:

- Use control slide.

1. Deparaffinize and hydrate sections to distilled water.

2. Mix one drop of 4% p-rosanilin in 2N hydrochloric acid and one drop of 4% sodium nitrite in a 50 ml flask and let stand for one minute. To this add 40 ml of phosphate buffer pH 6.5 and mix. Add this solution to the naphthol AS - D chloroacetate solution, mix well, and filter. Immediately place slides in this solution and stain for 45 minutes.

3. Rinse slides in four changes of distilled water.

4. Counterstain with acidified Lillie-Mayer hematoxylin for 1 minute.

5. Rinse in three changes of distilled water.

6. Blue hematoxylin in 0.3% sodium borate for 15 seconds.

7. Rinse in four changes of distilled water.

8. Air dry the slides

9. Dip in xylene and mount with synthetic resin.

Staining Results:

Leukocyte esterase ---------- red

Mast cells ---------- red

Nuclei ---------- blue

Comment:

The staining of esterase is inhibited to varying degrees by mercury, acid solutions, heat and iodine. Poor or false negative results occur when:

1. Slides are overheated when drying.

2. Mercury bichloride crystals are removed from tissue with an iodine solution.

3. Tissues are fixed in an acid fixative such as Zenker-formalin, Zenkers with acetic acid or Bouins fluid.

References:

Brown, G.G.: An Introduction to Histotechnology, New York, Appleton-Century-Crofts, 1978, pp. 345-346.

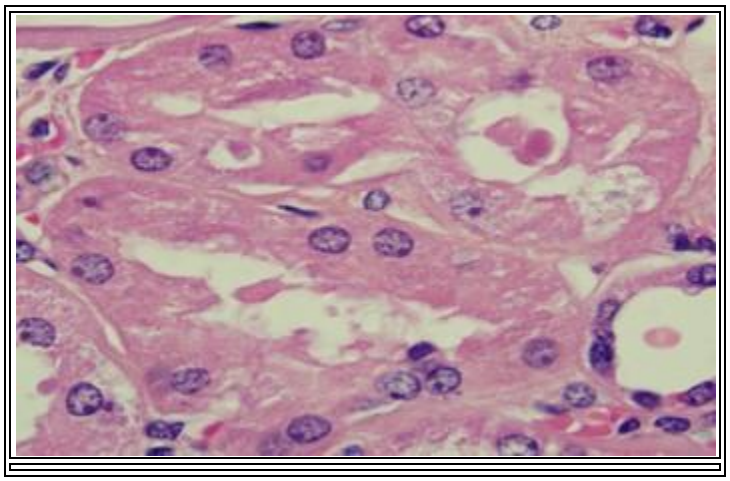
Leder, L.D.: The selective enzymocytochemical demonstration of neutrophil myeloid cells and tissue mast cells in paraffin sections. Klinische Wochenchrift, 42:553, 1964.

LIST OF MISCELLANEOUS METHODS AND INFORMATION

HEMATOXYLIN STAINING METHOD

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections cut at 4-6 µm.

SOLUTIONS:

Modified Lillie-Mayer Hematoxylin

Hematoxylin, C.I. 75290 ---------- 5.0 gm

Ammonium alum (aluminum ammonium sulfate) ---------- 50.0 gm

Distilled water ---------- 800.0 ml

Glycerol ---------- 200.0 ml

Sodium iodate ---------- 0.3 gm

Dissolve the hematoxylin and ammonium alum in the distilled water. Then add the glycerol and sodium iodate. Before use add 3 ml of acetic acid per 100 ml of solution. This increases the precision and selectivity of the nuclear stain. Filter before use.

0.3% Sodium Borate

(See PAS Method)

1% Alcoholic Eosin Solution (Stock)

Eosin Y, C.I. 45380	1.0 gm
Distilled water	20.0 ml

Dissolve and add:

Alcohol, 95%	80.0 ml

Eosin Solution (Working)

Eosin stock solution	100.0 ml
Alcohol, 80%	300.0 ml

Just before use add 1 ml of acetic acid to each 100 ml of stain.

Eosin-Phloxine Solution

Stock Eosin

Eosin Y, C.I. 45380 ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Stock Phloxine

Phloxine B, C.I. 45410 ---------- 1.0 gm

Distilled water ---------- 100.0 ml

Working Eosin-Phloxine Solution

Stock eosin ---------- 100.0 ml

Stock phloxine ---------- 10.0 ml

Alcohol, 95% ---------- 780.0 ml

Acetic acid, glacial ---------- 8.0 ml

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Modified Lillie-Mayer hematoxylin for 1 to 3 minutes.
3. Blue hematoxylin in 0.3% sodium borate for 15 seconds.
4. Rinse in four changes of distilled water.
5. Place in 95% alcohol for 30 seconds.
6. Counterstain with working alcoholic eosin or working eosin-phloxine for 30 seconds to 2 minutes. The time is determined by the age of the solutions and the depth of the counterstain desired.
7. Dehydrate in graded alcohols.
8. Clear in three or four changes of xylene.
9. Mount with synthetic resin.

Staining Results:

Nuclei ---------- blue

Cytoplasm ---------- various shades of pink which identifies different tissue components

Erythrocytes ---------- shades of pink to red

Alcoholic hyalin ---------- pink if the eosin-phloxine counterstain is used

References:

Lillie, R.D., and Fullmer, H.M.: Histopathologic Technique and Practical Histochemistry, 4th ed., New York, McGraw-Hill, 1976, pp. 205-208.

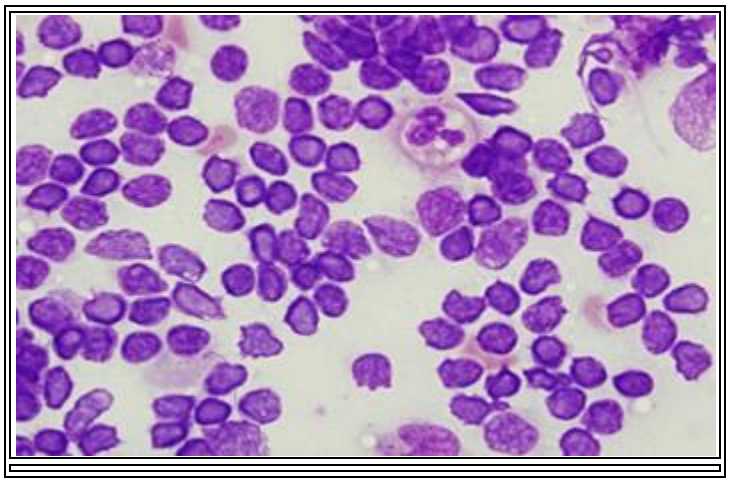
Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 34-35.

Strong - Fast™

METHOD FOR SMEARS AND TOUCH PREPERATIONS

Click the Section Headings (Blue) to Expand/Collapse Material

Image Examples:



Diagnostic Application:

Material and Solutions:

TECHNIQUE: Unfixed smears and touch preparations.

SOLUTIONS:

Fixative Solution

Methyl alcohol	500.000 ml
Toluidine blue O, C.I. 5204	0.002 gm

Phosphate Buffer, pH 6.5

Sodium phosphate, dibasic	1.63 gm

Potassium phosphate, monobasic ---------- 3.37 gm

Distilled water ---------- 500.00 ml

Phosphate Buffer, pH 6.7

Sodium phosphate, dibasic ---------- 2.21 gm

Potassium phosphate, monobasic ---------- 2.79 gm

Distilled water ---------- 500.00 ml

10% Triton X-100

Triton X-100 ---------- 10.0 ml

Distilled water ---------- 90.0 ml

Add a few grains of thymol to prevent the growth of fungi.

Solution I (Eosin Y)

Eosin Y, C.I. 45380 ---------- 0.625 gm

Phosphate buffer, pH 6.5 ---------- 500.000 ml

Sodium azide ---------- 0.050 gm

Solution II (Azure A-Azure B -Methylene Blue)

Azure A, C.I. 52005 ---------- 0.250 gm

Azure B, C.I. 52010 ---------- 0.125 gm

Methylene blue, C.I. 52015 ---------- 0.250 gm

Phosphate buffer, pH 6.7 ---------- 495.000 ml

10% Triton X-100 ---------- 5.000 ml

Staining Procedure:

1. Place slides in fixative solution for 30 seconds.

2. Rinse in three changes of distilled water.

3. Place in Solution I (eosin Y) for 20 seconds.

4. Rinse in three changes of distilled water.

5. Place in Solution II (azure A-azure B-methylene blue) for 40 seconds. If a more intense nuclear stain is desired, stain for 1 minute.

6. Rinse quickly in three changes of distilled water.

7. Blot slides or stand on end and allow to dry.

8. Dip in xylene and mount with synthetic mounting media.

Staining Results:

Nuclei ---------- bluish purple

Cytoplasm ---------- pink, blue or sky blue, depending on the cell type

Erythrocytes ---------- pink

Bacteria ---------- blue

Comment:

The staining results with this method are about the same as those obtained with the Giemsa or Wright-Giemsa method.

The sodium azide in the eosin Y and azure A, azure B and methylene blue solutions helps to retard the growth of microorganisms and the 10% Triton X-100 in the azure A-methylene blue solution gives it greater stability. These solutions have a shelf-life of about one year when stored at room temperature.

References:

Diff-Quick stain for the rapid, differential staining of hematological smears yielding qualitative results similar to Wright-Giemsa stain. Baxter Healthcare Corporation, Dade Division, Miami, Florida 33153-0672, 1989.

FIXATIVES

10% Buffered Neutral Formalin

Formalin, 37-40% ---------- 100.0 ml

Distilled water ---------- 900.0 ml

Sodium phosphate, monobasic NaH2PO4•H2O) ---------- 4.0 gm

Sodium phosphate, dibasic Na2HPO4 ---------- 6.5 gm

The best overall fixative for routine use.

10% Formalin-Sodium Acetate

Formalin, 37-40% ---------- 100.0 ml

Tap water ---------- 900.0 ml

Sodium acetate NaC2H3O2•3H20 ---------- 20.0 gm

Formalin with sodium acetate is an excellent fixative and medium in which to store gross blocks of tissue.

10% Formalin-Alcohol

Formalin, 37-40% ---------- 10.0 ml

Alcohol, absolute ---------- 90.0 ml

This fixative is recommended for the preservation of glycogen.

10% Formalin-Calcium

Formalin, 37-40% ---------- 10.0 ml

Distilled water ---------- 90.0 ml

Calcium chloride, anhydrous ---------- 1.0 gm

This fixative is especially useful when studies on lipids are to be performed.

Zenkers Solution, Modified

Distilled water ---------- 300.0 ml

Zinc acetate ---------- 3.0 gm

Potassium dichromate ---------- -- 7.5 gm

Acetic acid ---------- 15.0 ml

Bouins Solution

Picric acid, saturated aqueous ---------- -- 750.0 ml

Formalin, 37-40% ---------- 250.0 ml

Acetic acid, glacial ---------- 50.0 ml

Carnoys Solution

Alcohol, absolute ---------- 60.0 ml

Chloroform ---------- 30.0 ml

Acetic acid, glacial ---------- 10.0 ml

One of the best penetrating and quick acting fixatives known.

It gives excellent nuclear fixation with preservation of Nissl substance, plasma cells, and glycogen, but it hemolyzes red blood cells.

Lillies B-5 Fixative, Modified

Zinc chloride	6.0 gm
Sodium acetate, anhydrous	2.5 gm
Distilled water	180.0 ml
Formaldehyde, concentrated	20.0 ml

Add the formaldehyde just before use.

Zinc Formalin

Formalin, 37%	10.0 ml
Distilled water	100.0 ml
Zinc chloride	1.0 gm

References:

Lillie, R.D., and Fullmer, H.M.: Histopathologic Technic and Practical Histochemistry, New York, McGraw-Hill, 1976, pp. 45-62.

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill, 1968, pp. 3-5.

Herman, G.E. et al: Zinc formalin for automated tissue processing. J. Histotechnol. 11:85-89, 1988.

Barszcz, C.A.: Use of zinc chloride in Zenker type fixatives. Histo-Logic 6:87, 1976.

REMOVING STAINS FROM HANDS AND CLOTHING

Some stains can be removed form the hands by using *Erado-Sol. Dilute Chlorox (or similar bleach) and Erado-Sol are useful for removing stains from white clothing. Care should be exercised in removing stains from colored clothing.

To remove the following stains use the solutions indicated.

1. Alcian blue - to remove from the hands use the acid alcohol solution. For cleaning glassware try using dilute Chlorox or acid alcohol. If the stain resists, use them alternatively.

2. Auramine-rhodamine - acid alcohol.

3. Basic fuchsin - Erado-Sol.

4. Carmine - acid alcohol.

5. Chromic acid - concentrated aqueous sodium thiosulfate containing a few drops of sulfuric acid.

6. Crystal violet - Erado-Sol or Chlorox.

7. Eosin Y - saturated aqueous lithium carbonate.

8. Fast green and similar acid dyes - saturated aqueous lithium carbonate.

9. Hematoxylin (alum and iron) - Erado-Sol or acid alcohol.

10. Iodine - 5% sodium thiosulfate.

11. Methylene blue - acid alcohol, Erado-Sol or Chlorox.

12. Nuclear fast red - acid alcohol.

13. Picric acid - saturated aqueous lithium carbonate.

14. Potassium permanganate - 5% oxalic acid.

15. Schiffs solution - Erado-Sol or Chlorox.

16. Silver deposits - Lugols iodine followed by 5% sodium thiosulfate.

17. Sudan stains (Oil red O, Sudan black B) - acetone.

18. Thioflavin T - acid alcohol.

*American Scientific Products

CLEANING SOLUTIONS FOR GLASSWARE

Acid Dichromate Solution

Potassium dichromate ---------- 36.0 gm

Distilled water ---------- 1800.0 ml

Sulfuric acid ---------- 200.0 ml

Add the potassium dichromate to the distilled water and then add the sulfuric acid.

Acid Alcohol Solution

Alcohol, absolute ---------- 500.0 ml

Distilled water ---------- 500.0 ml

Hydrochloric acid, concentrated ---------- 10.0 ml

Nitric acid, concentrated ---------- 10.0 ml

Add the distilled water to the alcohol and then add the acids.

To remove the following stains from glassware, use the solutions indicated:

1. Alcian blue - acid alcohol or Ausome (Ausome Products, Inc., 6370 Altura Blvd., Buena Park, CA 90620, 1-800-482-2875).

2. Auramine-rhodamine - acid alcohol.

3. Basic fuchsin - acid dichromate.

4. Carmine - acid alcohol.

5. Crystal violet - acid dichromate.

6. Hematoxylin - acid dichromate.

7. Methylene blue - acid dichromate.

8. Nuclear fast red - acid alcohol.

9. Silver deposits - acid dichromate.

10. Sudan stains (Oil red O, Sudan black B) - acetone.

11. Thioflavin T - acid alcohol.

12. For other stains - acid dichromate.

REMOVING MERCURY PIGMENT FROM ZENKERS FIXED TISSUE

1. Deparaffinize and hydrate to distilled water.
2. Place in Lugols iodine for 5 minutes.
3. Rinse in two changes of distilled water.
4. Place in 2% sodium thiosulfate for 1 minute.
5. Wash in running tap water and rinse in two changes of distilled water.
6. Stain as requested.

REMOVING PICRIC ACID FROM SECTIONS FIXED IN BOUINS SOLUTION

1. Deparaffinize and take to 70% alcohol.
2. Place in 0.5% hydrochloric acid in 70% alcohol until yellow color is removed.
3. Wash well in running water and rinse in two changes of distilled water.
4. Stain as desired.

RESTAINING SLIDES

Slides which have been already stained may be restained after going through the following steps:

1. Remove cover glass by soaking the slides in xylene until the cover glass slips off.
2. Hydrate to distilled water.
3. Place in 0.5% hydrochloric acid in 70% alcohol for 1-3 minutes.
4. Wash in running tap water and rinse in two changes of distilled water.
5. Stain as requested.

REMOVAL OF COVER GLASSES

There are various reasons why it may become necessary to remove cover glasses. You should keep in mind that the older the slide, the longer it will take to remove the cover glass.

1. Place slide in xylene in a Coplin jar until the cover glass becomes detached from the slide. If it does not appear that the cover glass will detach place the Coplin jar in a 60˚ C oven. Check occasionally until the cover glass detaches.

2. Hydrate to distilled water.

3. Stain as desired.

WHERE TO ORDER CHEMICALS AND DYES

1. Where to order chemicals:

    1. Aldrich Chemical Co.
    2. Fisher Scientific.
    3. Gallard-Schlesinger.
    4. Pfaltz and Bauer.
    5. Polysciences.
    6. Sigma.
    7. VWR Scientific.

2. Where to order dyes:

    1. Aldrich Chemical Co.
    2. Mallinckrodt Baker Inc.
    3. Fisher Scientific.
    4. Pfaltz and Bauer.
    5. Polysciences.
    6. EMD Chemical.
    7. Sigma Chemical Co.
    8. Poly Scientific.
    9. VWR Scientific. They are distributors for J.T. Baker and EMD Chemical.

SOURCES OF CONTROLS FOR SPECIAL STAINS

Entity	Source
alkaline phosphatase	normal rat kidney
amyloid	autopsy, amyloidosis
argentaffin granules	small intestine
argyrophil granules	small intestine
axons	cerebrum, cerebellum, spinal cord
basement membrane	normal kidney
bile	liver, jaundice
calcium carbonate	rat bone and calcified lesions
calcium oxalate	normal bone
chromaffin	adrenal medulla with chromate fixative
cholesterol	normal adrenal gland
collagen	normal skin, lung, kidney

copper	premature baby liver or liver of Wilsons disease; formalin fixed for not longer than 48 hours
cross striations	normal skeletal and cardiac muscle
dendrites	cerebrum, cerebellum, spinal cord
DNA-RNA	normal lymph node
elastica	normal lung, intestine, aorta
fibrin	clot - less than 72 hours old
fat - lipoids	adipose (breast), fatty liver
glycogen	normal liver; fix in alcoholic formalin
hepatitis B surface antigen (HBsAg)	liver, viral hepatitis
hemoglobin	kidney, hemoglobinuria
iron	liver, hemochromatosis
keratin	skin, palm of hand, or sole of foot, keratinizing tumor
leukocyte esterase	skin, large intestine, uterus

mast cells	skin, large intestine, uterus
melanin	normal skin, malignant melanoma
mucin	normal small intestine and submaxillary glands
mucopolysaccharides	normal skin, umbilical cord
myelin	cerebrum, cerebellum, spinal cord
Nissl substance	cerebrum, cerebellum, spinal cord
nonspecific esterase of monocytes, histiocytes and megakaryocytes	lung containing histiocytes
phospholipids	normal cerebrum and spinal cord
plasma cells	lymph node
reticulum	normal liver, lymph node, intestine
uric acid	gouty joint articulations

Controls for bacteria and microorganisms usually may be obtained from positive autopsy and surgical cases. If you are unable to obtain positive material, controls are available from most local and state public health services.

DYES CERTIFIED BY THE BIOLOGICAL STAIN COMMISSION

Acid fuchsin, C.I. 42685

Alcian blue 8 GX, C.I. 74240

Alizarin red S, C.I. 58005

Aniline blue WS, C.I. 42755

Auramine O, C.I. 41000

Azocarmine G, C.I. 50085

Azure A, C.I. 52005

Azure B, C.I. 52010

Azure C, C.I. 52002

Basic fuchsin, C.I. 42510

Bismark Brown Y, C.I. 21000

Brilliant cresyl blue, C.I. 51010

Brilliant green, C.I. 42040

Carmine, C.I. 75470

Chlorazol black E, C.I. 30235

Congo red, C.I. 22120

*Crystal violet acetate

Crystal violet, C.I. 42555

*Darrow red

Eosin B, C.I. 45400

Eosin Y, C.I. 45380

Erythrosin, C.I. 45430

Ethyl eosin, C.I. 45386

Fast green F C F, C.I. 42053

Martius yellow, C.I. 10315

Methyl green, C.I. 42585

Methyl orange, C.I. 13025

Methyl violet 2B, C.I. 42535

Methylene blue, C.I. 52015

*Methylene blue thiocyanate

Methylene violet, C.I. 52041

Neutral red, C.I. 50040

*Nigrosin

Nile blue A, C.I. 51180

Oil Red O, C.I. 26125

Orange G, C.I. 16230

Orange II, C.I. 15510

*Orcein

Pararosanilin, C.I. 42500

Phloxine B, C.I. 45410

*Protargol S

Pyronin B, C.I. 45010

Pyronin Y, C.I. 45005

Resazurin

Rose Bengal, C.I. 45435

Safranin O, C.I. 50240

Sudan III, C.I. 26100

Sudan IV, C.I. 26105

*Fluorescein Isothiocyanate Sudan black B, C.I. 26150

*Giemsa Stain *Tetrachrome stain (MacNeal)

Hematoxylin, C.I. 75290 Thionin, C.I. 52000

Indigo carmine, C.I. 73015 Toluidine blue O, C.I. 52040

Janus green B, C.I. 11050 *Wrights stain

*Jenners stain

Light green SF, C.I. 42095

Malachite green, C.I. 42000

*Does not have a C.I. number.

SPECIAL STAINS WHICH REQUIRE POSITIVE CONTROLS

1.ACID FAST BACTERIA

a. Fite’s
b. Truant’s
c. Ziehl-Neelsen

The above also require negative controls.

2.AMYLOID

a. Congo red
b. Crystal violet
c. Thioflavin T

3.ARGENTAFFIN

a. Churukian’s ammoniacal silver
b. Schmorl’s

4.ARGYROPHIL

a. Churukian-Schenk

5.BACTERIA

a. Brown-Brenn

b. Cresyl violet for helicobacter pylori

c. Steiner’s (spirochetes)

d. Warthin-Starry

6.BILE

a. Hall’s

7.CALCIUM

a. Dahal’s Alizarin red S

b. Von Kossa

8.CENTRAL NERVOUS SYSTEM

a. Bielschowsky

b. Bodian

c. Luxol fast blue

9.CHROMAFFIN

a. Churukian’s ammoniacal silver

b. Schmorl’s

10. COPPER

a. Rhodanine

11. DNA-RNA

a. Methyl green-pyronin Y

12. ENZYMES

a. Naphthol AS-D chloroacetate esterase
b. Non-specific esterase

13. FIBRIN

a. Carstair’s
b. Frazer-Lendrum
c. PTAH

14. FUNGI

a. Churukian’s ammoniacal silver
b. Gomori-Grocott (GMS)
c. PAS

15. GLYCOGEN

a. Best’s Carmine
b. PAS with and without diastase digestion

16. HEMOGLOBIN

a. Ralph’s

17. HEPATITIS B SURFACE ANTIGEN

a. Aldehyde fuchsin

b. Orcein

18. IRON (FERRIC)

a. Pearl’s

19. KERATIN

a. Kreyberg’s

20. MAST CELLS

a. Giemsa

b. Toluidine blue O

21. MELANIN

a. Churukian’s ammoniacal silver

b. Melanin bleach

c. Lillies

22. MUCOPOLYSACCHARIDES

a. Alcian blue

b. Colloidal iron

c. Kreyberg’s

d. Southgate’s mucicarmine

23. MUSCLE STRIATIONS

a. PTAH

24. NAPHTHOL AS-D CAE

a) Uterus

25. NERVE FIBERS AND MYELIN

a. Bielschowsky

b. Bodian

c. Luxol fast blue

d. Luxol fast blue – PAS

26. NEUTRAL FATS AND LIPIDS

a. Oil red O

b. Sudan black B

27. NISSL SUBSTANCE

a. Cresyl Violet Acetate

b. Einarson’s

28. NOCARDIA

a. Brown-Brenn

b. Fite’s

c. Gomori-Grocott (GMS)

d. Warthin-Starry

29. PANCREATIC ISLET CELLS

a. Aldehyde fuchsin

30. PITUITARY CELLS

a. Wilson-Ezrin

31. PNEUMOCYSTIS CARINII

a. Churukian’s ammoniacal silver

b. Gomori-Grocott (GMS)

32. RETICULUM

a. Gomoris reticulum

33. SPIROCHETES

a) Steiner’s

STABILITY OF SPECIAL STAINING SOLUTIONS

General Guidelines Concerning Solutions

1. Check stock solutions regularly for signs of deterioration.

2. All solutions should be dated and initialed by the person who prepared the solution.

3. Use caution to prevent cross solution contamination.

4. Use distilled or double distilled water to prepare solution.

5. Use reagent grade chemicals.

6. Use dyes which are certified by the Biological Stain Commission.

7. Keep solutions in well stoppered bottles at all times.

8. Refrigerated solutions should be poured into the staining jar, and the bottled solution returned to the refrigerator before it warms to room temperature.

9. Return all staining solutions to shelves soon after use to prevent mix-up.

10. Always use clean glassware.

11. Make sure that balance, pH meter, etc., are in proper working order.

12. The following chemicals should be kept refrigerated:

    1) All buffer solutions

    2) Hydroquinone

    3) Paraldehyde keeps well in the freezing compartment. It will freeze to a solid, but can be easily melted at room temperature.

    4) Phosphomolybdic acid

    5) Phosphotungstic acid

    6) Protargol

    7) Silver nitrate and all solutions containing silver nitrate

    8) Gold chloride

    9) Tartaric acid

10) Trypsin

11) Gomori's trichrome solution

13. All stain and chemical solutions are labeled for the temperature at which they should be stored. These are: Store at room temperature, store at 3°-6° C, and store at -14° to -18° Celsius. All the dyes and most of the chemicals are stored at room temperature.

The following information indicates the stability of the staining solutions used in most of the staining procedures in this manual. The shelf-life indicated for the various solutions is based upon our many years of experience with the staining methods in which these solutions are used. There may be some variation of the shelf-life indicated because of the temperature in the room or refrigerator in which they are stored and because of the quality of the distilled water, chemicals and the dyes with which they have been prepared. Some of the solutions may be used several weeks or months longer than what is indicated.

An asterisk preceding the solution indicates it is used for one day and then discarded.

<u>Ziehl-Neelsen (AFB)</u>

1. Carbol fuchsin -------- 6 months
2. *Acid alcohol -------- 6 months
3. Methylene blue -------- 6 months

<u>Fite (AFB)</u>

1. Mineral oil - xylene -------- 1 year
2. Carbol fuchsin -------- 6 months
3. *Acid alcohol-------- 6 months
4. Methylene blue -------- 6 months

<u>Truant (AFB)</u>

1. Auramine - rhodamine -------- 6 months
2. *Acid alcohol -------- 6 months

3. Eriochrome black T -------- 6 months

Brown-Brenn (bacteria)

1. Hucker-Conn crystal violet -------- 6 months
2. *Grams iodine -------- 2 months
3. *Ethyl alcohol - acetone-------- 6 months
4. *Basic fuchsin, stock and working solutions -------- 3 months
5. *Picric acid - acetone -------- 6 months
6. *Acetone - xylene -------- 6 months

Gram (bacteria)

1. Hucker-Conn crystal violet -------- 6 months
2. *Grams iodine -------- 2 months
3. *Basic fuchsin, stock and working solutions---------- 3 months

Wolbachs Giemsa

1. Acid alcohol -------- 6 months
2. Giemsa, stock -------- 1 year
3. 10% Triton X-100 -------- 6 months
4. *Giemsa, working
5. 1% Acetic acid-------- 6 months

Gomori-Grocott (GMS)

1. Chromic acid-potassium dichromate-sulfuric acid-------- 1 year
2. Sodium borate -------- 6 months
3. Methenamine - silver nitrate, stock (refrigerate) -------- 1 month
4. *Methenamine - silver nitrate, working

5. 10% Triton X-100-------- 6 months

6. *Sodium bisulfite -------- 2 months

7. Gold chloride -------- 6 months

Keep gold chloride in an amber bottle. Gold chloride in a Coplin jar should be kept in a dark place until ready for use because it is sensitive to light.

8. Sodium thiosulfate -------- 6 months

9. Fast green, stock -------- 6 months

10. Fast green, working -------- 2 weeks

Churukian's Ammoniacal Silver (fungi and pneumocystis carinii)

1. Periodic acid -------- 6 months

2. Ammoniacal silver (refrigerate) -------- 1 month

3. Gold chloride -------- 6 months

4. Sodium thiosulfite -------- 6 months

5. Fast green, stock -------- 6 months

6. Fast green, working -------- 2 weeks

Warthin-Starry (bacteria)

1. Acetic acid-glycine, stock (refrigerate) -------- 6 months

2. *Acetic acid-glycine, working

3. *Silver nitrate

4. *Gelatin

5. *Hydroquinone

6. *Silver nitrate - gelatin - hydroquinone

7. Tartarazine-------- 6 months

Steiner (spirochetes)

1. Uranyl nitrate-copper nitrate ----------1 months
2. *Silver nitrate
3. Gum mastic -------- 6 months
4. *Hydroquinone
5. *Reducing solution

Alcian Blue (acid mucopolysaccharides)

1. Acetic acid -------- 6 months
2. Alcian blue -------- 6 months
3. Nuclear fast red -------- 4 months

Alcian Blue - PAS (acid and neutral mucopolysaccharides)

1. Acetic acid -------- 6 months
2. Alcian blue -------- 6 months
3. Periodic acid -------- 6 months
4. Modified Lillies Schiff solution (refrigerate) -------- 1 year

Kreyberg (acid mucopolysaccharides)

1. Alcian blue -------- 6 months
2. Ammonia - alcohol -------- 2 months
3. Weigerts hematoxylin, stock -------- 6 months
4. Weigerts ferric chloride - hydrochloric acid -------- 6 months
5. Weigerts working -------- 1 week
6. Acid alcohol -------- 6 months
7. Erythrosin B -------- 2 months
8. Metanil yellow -------- 6 months

PAS and PAS with Diastase (glycogen)

1. Periodic acid -------- 6 months
2. Modified Lillies Schiff solution (refrigerate)-------- 1 year
3. *Diastase
4. Lillie-Mayer hematoxylin -------- 1 year

Southgates Mucicarmine (acid mucopolysaccharides)

1. Weigerts hematoxylin, stock -------- 6 months
2. Weigerts ferric chloride - hydrochloric acid -------- 6 months
3. Weigerts working -------- 1 week
4. Mucicarmine, stock (refrigerate)-------- 4 months
5. Mucicarmine, working ---------- 7 days
6. Tartarazine -------- 6 months

Periodic Acid - Thionin - KOH - PAS (PAT)

1. *Periodic acid
2. *Thionin
3. *Potassium hydroxide
4. Modified Lillies Schiff solution (refrigerate) -------- 1 year

Best Carmine (glycogen)

1. Weigerts hematoxylin, stock -------- 6 months
2. Weigerts ferric chloride - hydrochloric acid -------- 6 months
3. Weigerts working -------- 1 week
4. Carmine, stock (refrigerate) -------- 4 months
5. *Carmine, working solution -------- 3 days

6. *Differentiating solution

Puchtlers Congo Red (amyloid)

1. Weigerts hematoxylin, stock -------- 6 months
2. Weigerts ferric chloride - hydrochloric acid -------- 6 months
3. Weigerts working-------- 1 week
4. 1% Sodium hydroxide -------- 1 year
5. Congo Red -------- 2 months

Lieb Crystal Violet (amyloid)

1. Crystal violet, stock -------- 1 year
2. Crystal violet, working -------- 6 months
3. Apathys mounting media -------- 10 years

Jones (basement membranes)

1. Periodic acid -------- 6 months
2. Methenamine - silver nitrate, stock (refrigerate) -------- 1 month
3. Sodium borate -------- 6 months
4. *Methenamine - silver nitrate, working
5. Gold chloride (keep in the dark) -------- 6 months
6. Sodium thiosulfate -------- 6 months
7. Nuclear fast red -------- 4 months

Alcian Blue - PAS (kidney)

1. Acetic acid -------- 6 months
2. Alcian blue -------- 6 months
3. Periodic acid -------- 6 months

4. Modified Lillies Schiff solution ---------- 1 year

Gomori Trichrome

1. Bouins -------- 6 months
2. Weigerts hematoxylin, stock -------- 6 months
3. Weigerts ferric chloride - hydrochloric acid -------- 6 months
4. Weigerts working -------- 1 week
5. Trichrome (refrigerate) ---------- 6 months
6. Acetic acid -------- 6 months

Masson Trichrome

1. Bouins -------- 6 months
2. Weigerts hematoxylin, stock -------- 6 months
3. Weigerts ferric chloride - hydrochloric acid -------- 6 months
4. Weigerts working -------- 1 week
5. Biebrich scarlet - acid fuchsin -------- 2 months
6. Phosphomolybdic - phosphotungstic acid -------- 6 months
7. Aniline blue -------- 2 months
8. Acetic acid -------- 6 months

Modified Massons (Cardiac Tissues)

1. Alcoholic picric acid -------- 6 months
2. Ponceau S -------- 6 months
3. Phosphomolybdic acid -------- 6 months
4. Aniline blue -------- 2 months
5. Acetic acid -------- 6 months

Mallory (PTAH)

1. *Potassium permanganate -------- 1 week
2. *Oxalic acid -------- 6 months
3. Zenkers, with acetic acid -------- 6 months
4. Lugols iodine -------- 6 months
5. *Sodium thiosulfate -------- 6 months
6. Phosphotungstic acid hematoxylin (PTAH) -------- 1 year

The PTAH solution is generally considered to have a long shelf-life. However, the quality of the staining results should be carefully checked for any signs of deterioration.

Van Gieson (collagen)

1. Weigerts hematoxylin, stock -------- 6 months
2. Weigerts ferric chloride - hydrochloric acid -------- 6 months
3. Weigerts working -------- 1 week
4. Acid fuchsin -------- 6 months
5. Picric acid -------- 6 months

Verhoeff (elastic)

1. Hematoxylin, 5% alcoholic -------- 1 year
2. Ferric chloride, 10% -------- 6 months
3. Lugols iodine -------- 6 months
4. *Verhoeffs, working
5. Acid fuchsin -------- 6 months
6. Picric acid -------- 6 months

Frazer-Lendrum (fibrin)

1. Weigerts iron hematoxylin, working -------- 1 week
2. Orange G - Picric acid-------- 1 year
3. Acid fuchsin -------- 6 months
4. Tartarazine, differentiating -------- 1 year
5. Light green -------- 1 year

Carstairs (fibrin and platelets)

1. Weigerts iron hematoxylin, working -------- 1 week
2. Picro-orange G -------- 1 year
3. Ponceau-fuchsin -------- 6 months
4. Aniline blue -------- 1 year

Gomori (reticulum)

1. *Acidified potassium permanganate -------- 7 days
2. *Potassium metabisulfite -------- 2 months
3. Ammoniacal silver (refrigerate) -------- 10 days
4. *Formalin -------- 6 months
5. Gold chloride (keep in the dark) -------- 6 months
6. Sodium thiosulfate -------- 6 months
7. Nuclear fast red -------- 4 months

Microwave Ammoniacal Silver (argentaffin, melanin)

1. *Ammoniacal silver -------- 1 month
2. Gold chloride (keep in the dark) -------- 6 months
3. Sodium thiosulfate -------- 6 months
4. Nuclear fast red -------- 4 months

Schmorl (reducing substance)

1. *Ferric chloride
2. *Potassium ferricyanide
3. Nuclear fast red -------- 4 months

Churukian-Schenk (argyrophil cells)

1. Citric acid - Sodium citrate, stock -------- 1 year
2. *Citric acid-sodium citrate, working
3. *Silver nitrate
4. *Bodians developer
5. Nuclear fast red -------- 4 months

Giemsa (plastic bone marrow and smears)

1. Hydrochloric acid-alcohol -------- 6 months
2. Triton X-100 -------- 6 months
3. Phosphate buffer, pH 6.6 (refrigerate) -------- 1 year
4. Giemsa, stock -------- 1 year
5. *Giemsa, working

Orcein (HBsAg)

1. *Acidified potassium permanganate
2. *Oxalic acid -------- 6 months
3. Orcein -------- 6 months

Aldehyde Fuchsin (HBsAg)

1. *Acidified potassium permanganate
2. *Oxalic acid -------- 6 months

3. Aldehyde fuchsin -------- 2 months

Toluidine Blue (mast cells)

1. *Potassium permanganate -------- 1 month
2. Potassium metabisulfite -------- 2 months
3. Toluidine blue -------- 6 months

Dahal (calcium)

1. *Alizarin red S -------- 1 year
2. Fast green, stock -------- 6 months
3. Fast green, working -------- 2 weeks

Rhodanine (copper)

1. *Rhodanine, stock
2. *Rhodanine, working
3. Lillie-Mayer hematoxylin -------- 1 year
4. *Sodium borate -------- 6 months

Pearl (iron)

1. Hydrochloric acid -------- 3 months
2. Potassium ferrocyanide -------- 3 months
3. Triton X-100-------- 6 months
4. *Hydrochloric acid - potassium ferrocyanide
5. Nuclear fast red -------- 4 months

Feulgen (DNA)

1. 5N hydrochloric acid -------- 1 year

2. Modified Lillies Schiff solution (refrigerate) -------- 1 year

3. *Fast green

Methyl Green - Pyronin (DNA-RNA)

1. Buffer solutions -------- 1 year

2. Methyl green -------- 6 months

3. Pyronin Y -------- 6 months

Aldehyde Fuchsin (pancreatic beta cells)

1. Lugols iodine -------- 6 months

2. Sodium thiosulfate -------- 6 months

3. Aldehyde fuchsin -------- 2 months

4. Fast green, stock -------- 2 months

5. Fast green, working -------- 2 weeks

Wilson-Ezrin (pituitary)

1. Periodic acid-------- 2 months

2. Modified Lillies Schiff solution (refrigerate) -------- 1 year

3. Weigerts hematoxylin, stock -------- 6 months

4. Weigerts ferric chloride - hydrochloric acid -------- 6 months

5. Weigerts working -------- 1 week

6. Acid alcohol -------- 6 months

7. Orange G -------- 6 months

8. *Phosphotungstic acid -------- 6 months

Hall (bilirubin)

1. Trichloroacetic acid -------- 6 months

2. Ferric chloride -------- 6 months

3. *Fouchets

4. Acid fuchsin -------- 6 months

5. Picric acid -------- 6 months

Lison (hemoglobin)

1. Patent Blue V (stock) -------- 1 week

2. *Patent Blue V (working)

3. Nuclear fast red -------- 4 months

Lillies Nile Blue (melanin and lipofuscin)

1. Nile blue -------- 6 months

Melanin Bleach

1. *Potassium permanganate -------- 1 week

2. Oxalic acid -------- 6 months

3. Nuclear fast red -------- 4 months

Gomori (urates)

1. Methenamine - silver nitrate, stock -------- 1 month

2. Boric acid -------- 6 months

3. Sodium borate -------- 6 months

4. *Methenamine - silver nitrate - buffer

5. Gold chloride (keep in the dark) -------- 6 months

6. Sodium thiosulfate -------- 6 months

7. Fast green, stock -------- 6 months

8. Fast green, working -------- 2 weeks

Bodian (nerve fibers)

1. *Protargol-------- 48 hours
2. *Reducing
3. Gold chloride (keep in the dark) -------- 6 months
4. *Oxalic acid -------- 6 months
5. Sodium thiosulfate-------- 6 months

Cresyl Violet Acetate (nissl substance)

1. Acetic acid -------- 6 months
2. *Cresyl violet acetate

Cresyl Violet (helicobacter pylori)

1. 0.2M Acetate Buffer, pH 4.2 ---------- 1 year
2. Crystal violet acetate solution ---------- 6 months

Einarson (nissl substance)

1. Gallocyanin -------- 1 week

Lapham (myelin and glial fibers)

1. Gallocyanin -------- 1 week
2. Phloxine -------- 1 month
3. Fast green-------- 2 months
4. Acetic acid-------- 6 months

Luxol Fast Blue - Cresyl Violet acetate (myelin and nerve cells)

1. Luxol fast blue -------- 1 year

2. Cresyl violet acetate, stock -------- 1 month

3. *Cresyl violet acetate, working

4. Lithium carbonate -------- 1 year

Thioflavin S (amyloid in neurofibrillary plaques) and Thioflavin T

1. Lillie-Mayer hematoxylin -------- 1 year

2. *Thioflavin S -------- 1 week

3. Thioflavin T ---------- 6 months

4. Acetic acid -------- 6 months

Naphthol AS-D Chloroacetate (leukocyte esterase)

1. Pararosanilin, hexazotized (refrigerate) -------- 1 year

2. Sodium nitrite (refrigerate)-------- 1 year

3. Buffer solutions (refrigerate) -------- 1 year

4. *Naphthol AS-D chloroacetate

5. *Buffered staining solution-------- 1 hour

6. Lillie-Mayer hematoxylin -------- 1 year

Oil Red O (fats)

1. Oil red O, stock -------- 1 year

2. Oil red O, working -------- 1 year

3. Lillie-Mayer hematoxylin -------- 1 year

4. Glycerin-jelly -------- 10 years

Sudan Black B (phospholipids)

1. Sudan black B -------- 1 year

2. Nuclear fast red -------- 4 months

3. Glycerin-jelly -------- 10 years

Hematoxylin and Eosin (H&E)

1. Harris hematoxylin -------- 6 months
2. Lillie-Mayer hematoxylin -------- 1 year
3. Alcoholic eosin, stock -------- 1 year
4. Alcoholic eosin, working -------- 6 months

Strong-Fast (smears and touch preparations)

1. Fixative -------- 1 year
2. Phosphate buffer solutions -------- 1 year
3. 10% Triton X-100 -------- 6 months
4. Solution I (eosin Y) -------- 1 year
5. Solution II (azure A-methylene blue) -------- 1 year

DYE SOLUTIONS

The following information indicates the stability of staining solutions used in several commonly used staining methods. There may be some variation of the shelf-life indicated because of variations of the temperature in the room or refrigerator in which they are stored and because of the quality of the distilled water, chemicals and the dyes with which they have been prepared.

Acid fuchsin, Frazer-Lendrum -------- 1 year

Acid fuchsin-picric acid (Verhoeff)-------- 1 month

Alcian blue 1% (Mowry) -------- 6 months

Aldehyde fuchsin (Gomori) -------- 2 months

Aldehyde thionin (Shikata) -------- 1 month

Alizarin red S, 1% (Dahal) -------- 1 year

Aniline blue (Masson) -------- 2 months

Auramine O-rhodamine B (Truant) -------- 6 months

Basic fuchsin, 0.5% (Brown-Brenn)-------- 3 months

Biebrich scarlet-acid fuchsin (Masson) -------- 2 months

Carbol-fuchsin (Ziehl-Neelsen) -------- 6 months

Congo red (Eastwood) -------- 6 months

Cresyl violet acetate ----------6 months

Crystal violet, Hucker-Conn (Gram) -------- 6 months

Crystal violet (Lieb) -------- 1 year

Diff-Quick fixative solution -------- 1 year

Diff-Quick solution I (eosin Y) -------- 1 year

Diff-Quick solution II (azure A-methylene blue) -------- 1 year

Eosin Y, alcoholic, stock -------- 1 year

Eosin Y, alcoholic, working -------- 6 months

Eriochrome black T (Truant) -------- 6 months

Erythrosin B, 1% (Kreyberg) -------- 2 months

Fast green, stock (counterstain) -------- 4 months

Fast green, working (counterstain) -------- 2 weeks

Gallocyanin (Einarson) -------- 1 week

Giemsa, stock (Wolbach) -------- 1 year

Giemsa, working (Wolbach) -------- 1 day

Gomoris trichrome, refrigerate -------- 6 months

Hematoxylin (Harris) -------- 6 months

Hematoxylin (Lillie-Mayer) -------- 1 year

Hematoxylin (Mallory PTAH) -------- 1 year

Hematoxylin, 5% alcoholic (Verheoff) -------- 6 months

Hematoxylin (Weigert) -------- 1 week

Light green (Frazer-Lendrum) -------- 1 year

Luxol fast blue (myelin) -------- 1 year

Metanil yellow (Kreyberg) -------- 6 months

Methyl green (DNA) -------- 6 months

Methylene blue (Ziehl-Neelsen) -------- 6 months

Mucicarmine (Southgate), refrigerate-------- 4 months

Mucicarmine (Southgate), working solution ---------- 1 week

Nile blue ---------- 6 months

Nuclear fast red (counterstain) -------- 4 months

Oil red O, stock -------- 1 year +

Oil red O, working -------- 1 year

Orange G (Wilson-Ezrin) -------- 6 months

Orange G-picric acid (Frazer-Lendrum) -------- 1 year

Orcein (Shikata) -------- 6 months

Picric acid-acetone (Brown-Brenn) -------- 6 months

Picric acid, saturated aqueous -------- 1 year

Ponceau S ---------- 6 months

Pyronin Y (RNA) -------- 6 months

Schiffs solution (modified Lillie) -------- 1 year

Sudan black B, alcoholic -------- 1 year

Tartarazine, Southgate -------- 6 months

Thioflavin S (amyloid) -------- 1 week

Thioflavin T (Vassar-Culling)-------- 3 months

Toluidine blue (mast cells) -------- 6 months

Verhoeffs elastic -------- 2 hours

Chemical and Other Solutions

Acetic acid, 1% (Vassar-Culling) -------- 6 months

Acetic acid, 3% (Mowry) -------- 6 months

Acetone-xylene (Brown-Brenn) -------- 6 months

Acid alcohol -------- 6 months

Ammonia-alcohol (Kreyburg)-------- 3 months

Ammoniacal silver (Churukian), refrigerate -------- 1 month

Ammoniacal silver (Fontana-Masson), refrigerate -------- 1 month

Ammoniacal silver (Gomori), refrigerate -------- 1 week

Bouins -------- 6 months

Buffer solutions, refrigerate -------- months to years

Chromic acid-potassium dichromate-sulfuric acid -------- 6 months

Diastase of malt (PAS-D) -------- 1 day

Ethyl alcohol-acetone (Brown-Brenn)-------- 6 months

Ferric chloride, 10% (Verhoeff) -------- 6 months

Formalin, 10% -------- 6 months

Formalin, 10% (Gomoris reticulum) -------- 6 months

Fouchets (Hall) -------- 1 day

Gold chloride, 0.2% (keep in the dark) -------- 6 months

Gold chloride, 1% (keep in the dark)-------- 1 year

Gum mastic, 2.5% (Steiner), refrigerate-------- 6 months

Grams iodine -------- 2 months

Hydrochloric acid, 2% (Pearl)-------- 3 months

Hydrochloric acid, 1N (PAS) -------- 1 year

Hydrochloric acid, 5N (Feulgen) -------- 1 year

Lithium carbonate, saturated aqueous -------- 6 months

Lugols iodine -------- 6 months

Methenamine-silver nitrate, stock (GMS), refrigerate -------- 1 month

Mineral oil-xylene (Fite) -------- 1 year

Oxalic acid, 1% and 2%---------- 6 months

Periodic acid, 0.5% (PAS) -------- 6 months

Periodic acid, 4% (MAS) -------- 6 months

Phosphoric acid, 1% ---------- 6 months

Phosphotungstic acid, 5% -------- 6 months

Potassium ferrocyanide, 1% (Pearl) -------- 3 months

Potassium metabisulfite, 2% -------- 2 months

Potassium permanganate, 0.25% -------- 1 week

Potassium permanganate, 0.3%, acidified -------- 1 week

Potassium permanganate, 0.5% -------- 1 month

Sodium bisulfite, 1% (GMS) -------- 2 months

Sodium borate, 3% (GMS) -------- 6 months

Sodium thiosulfate, 2% -------- 6 months

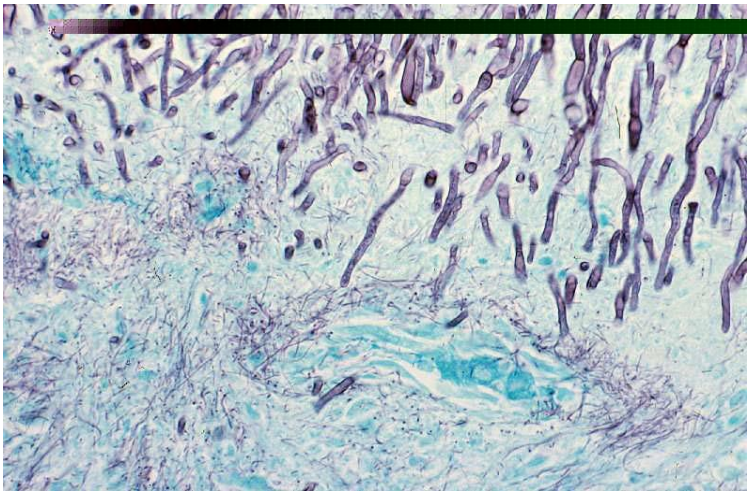
Sodium thiosulfate, 5% -------- 6 months

Trichloroacetic acid, 25% (Hall) -------- 6 months

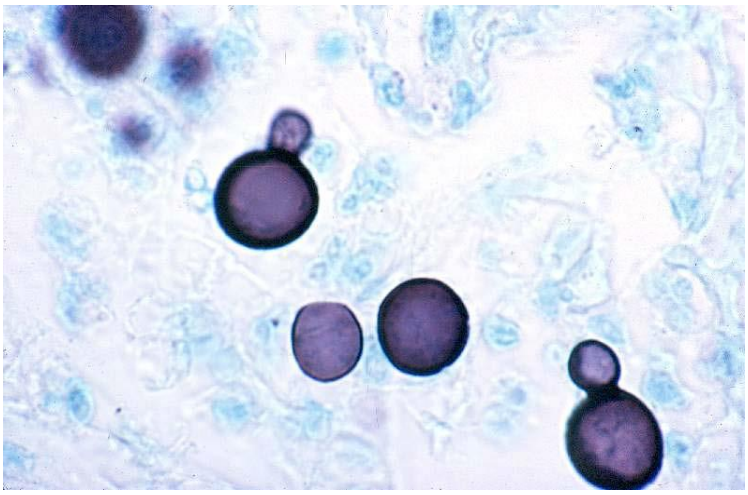
Triton X-100, 10% -------- 6 months

Uranyl nitrate-copper nitrate (Steiner) -------- 1 months

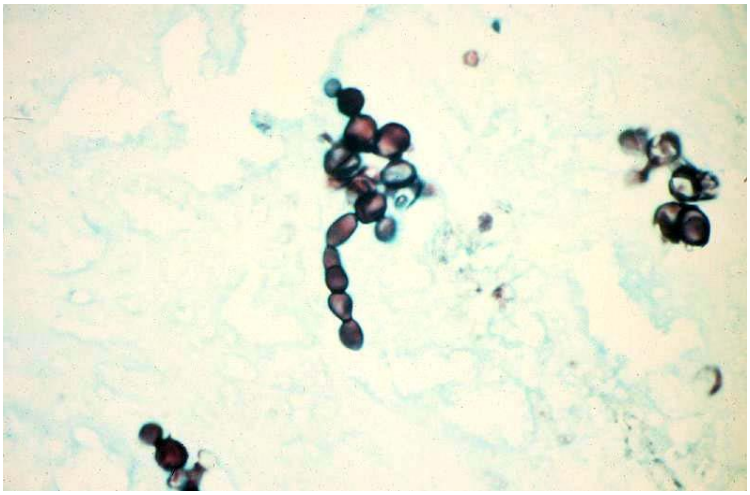
Zenkers with acetic acid -------- 1 year



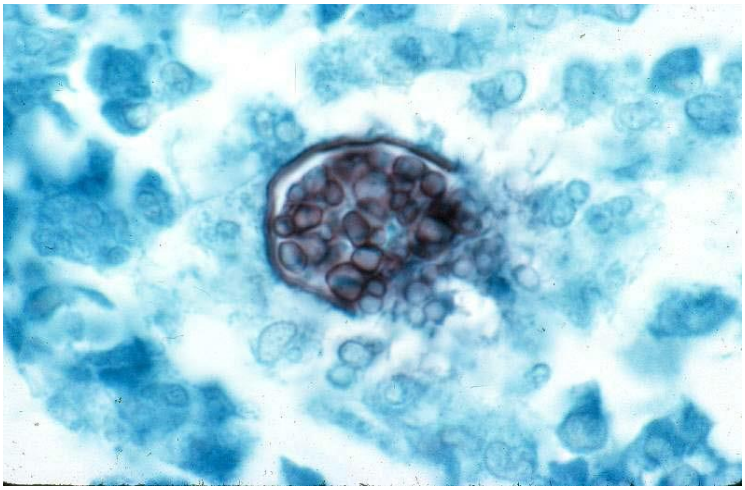
Above: C. Albican



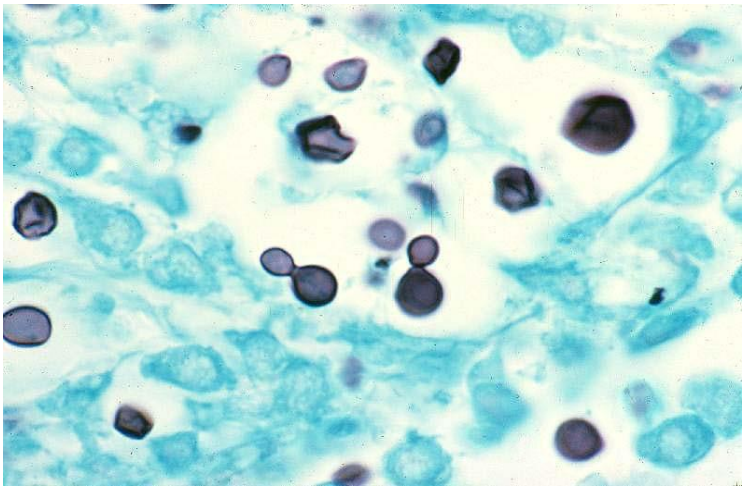
Above: Blastomycosis



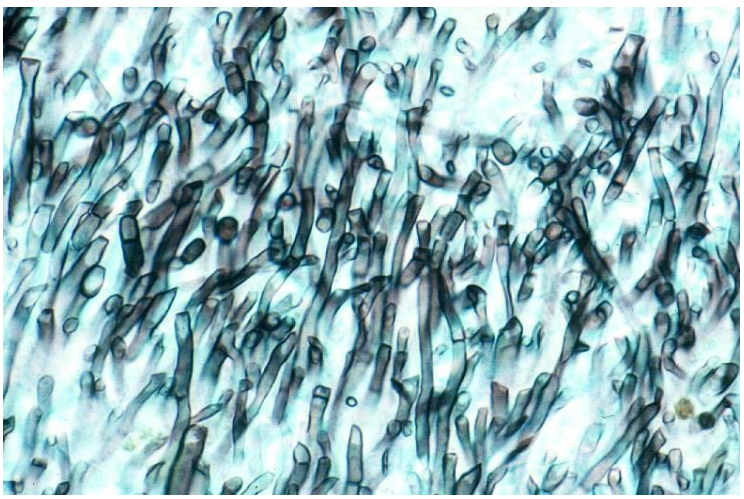
Above: Fungus



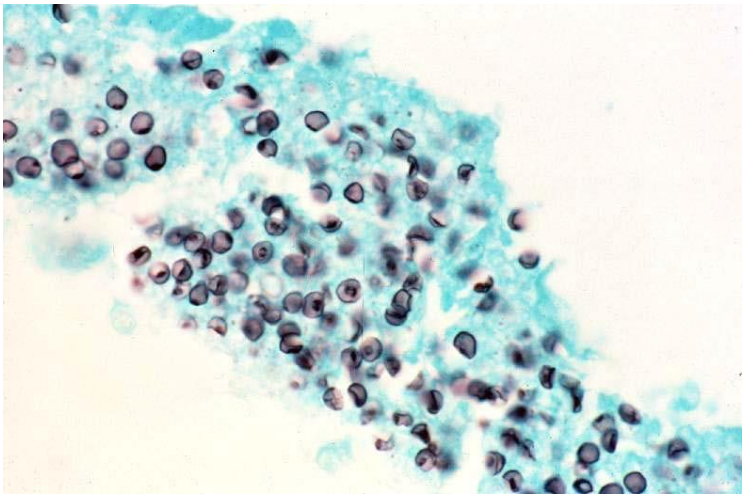
Above: Coccidiomycosis



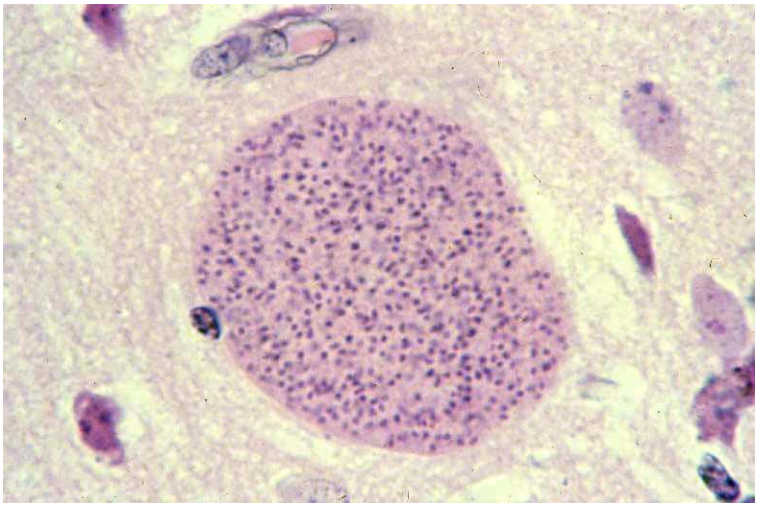
Above: Cryptococcus



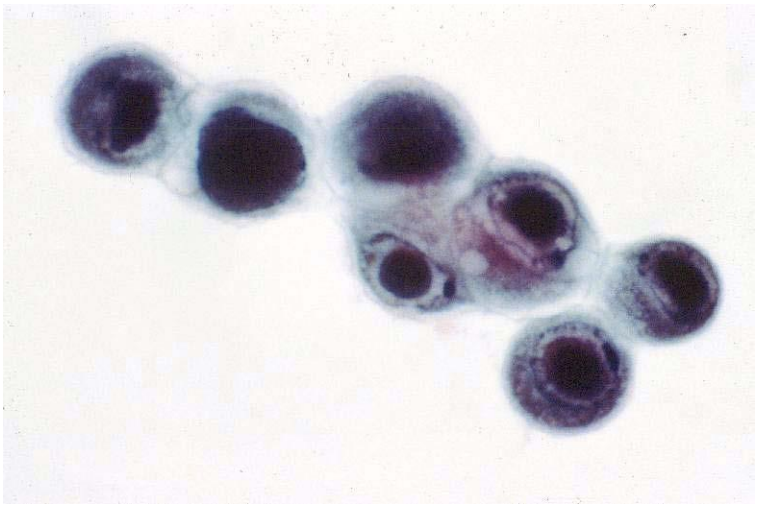
Above: Trichosporan Berguli



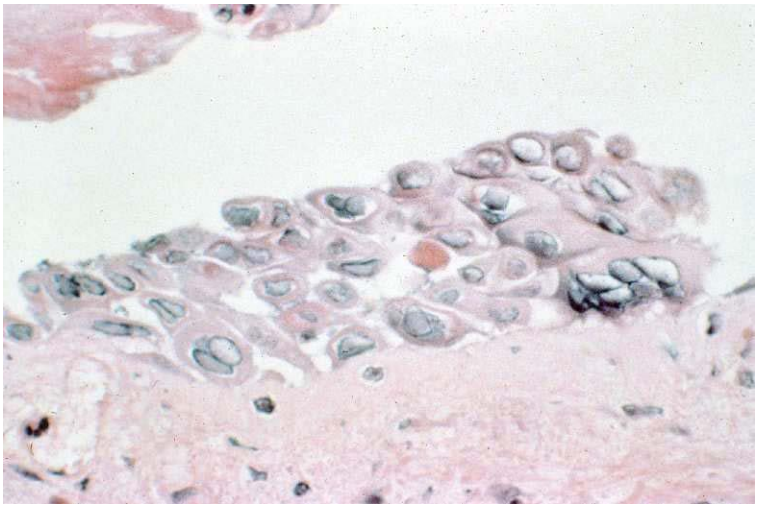
Above: Pneumocystis carinii



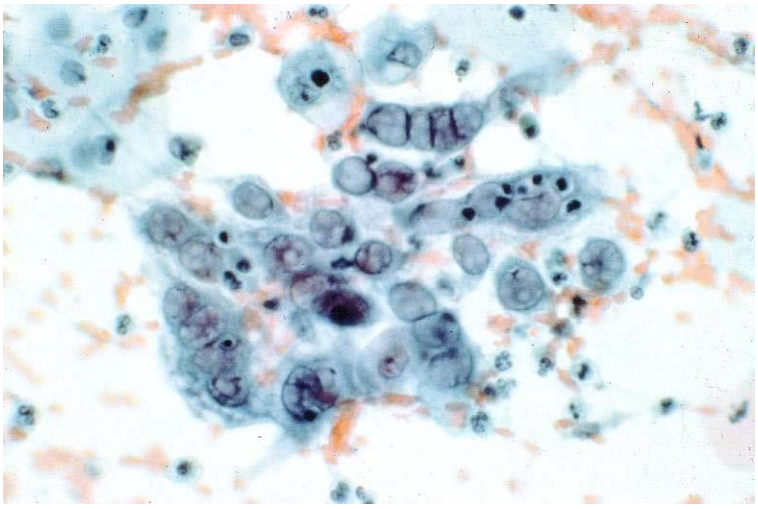
Above: Toxoplasmosis Gondii



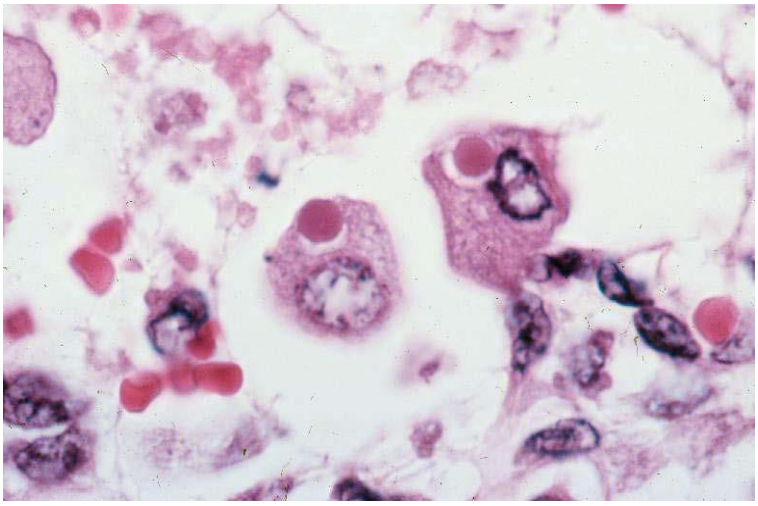
Above: Cytomegalovirus (CMU)



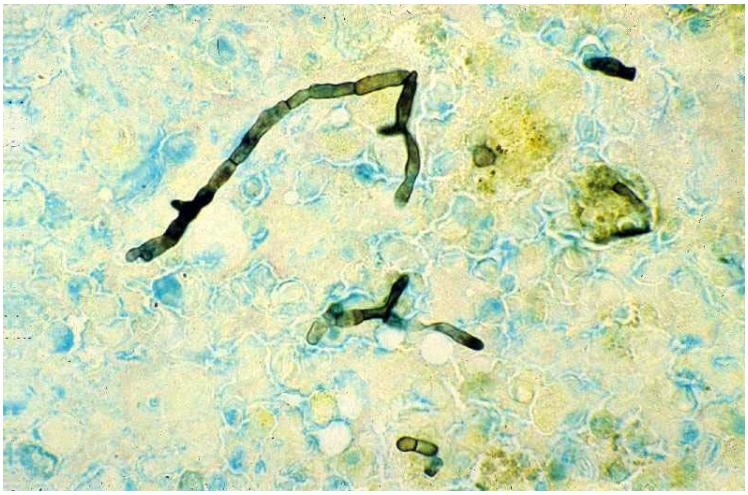
Above: Herpes Virus



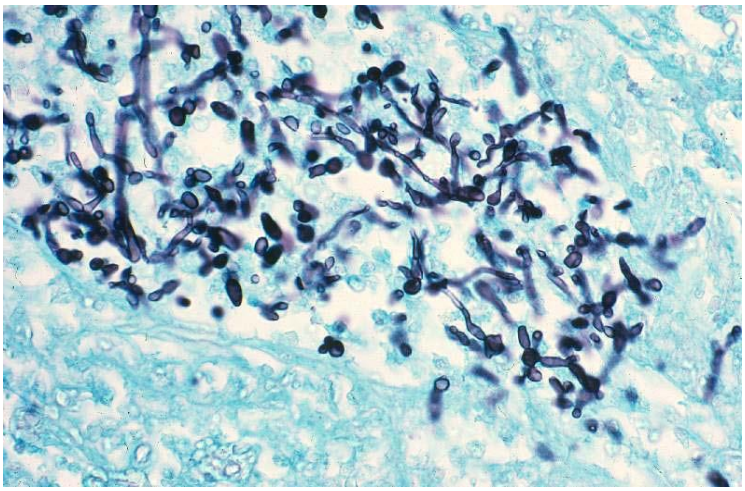
Above: Herpes Virus



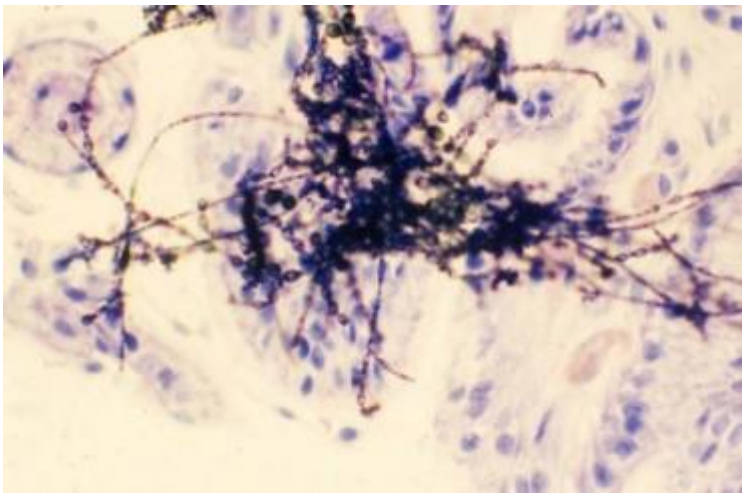
Above: German Measles Inclusion Body



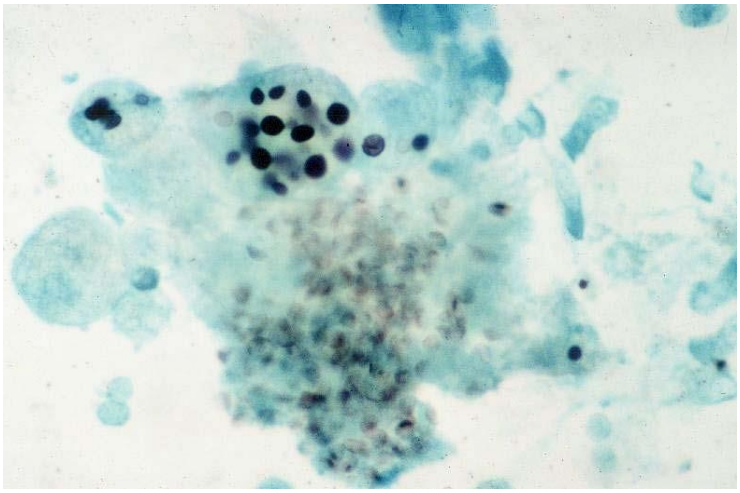
Above: Apergillus



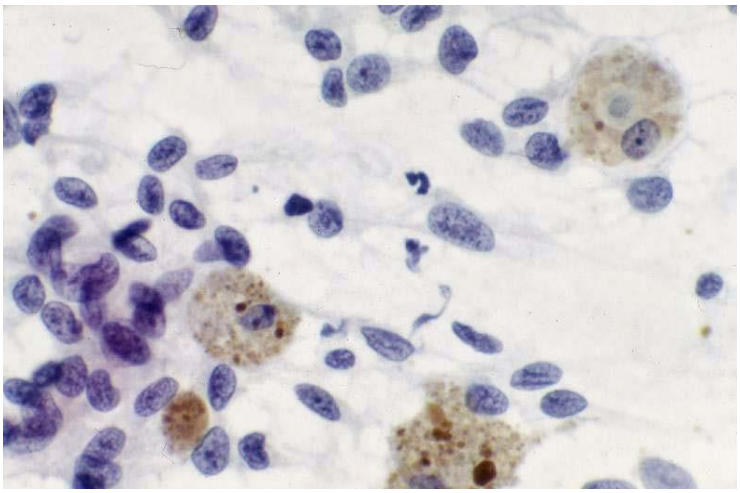
Above: C. Albican



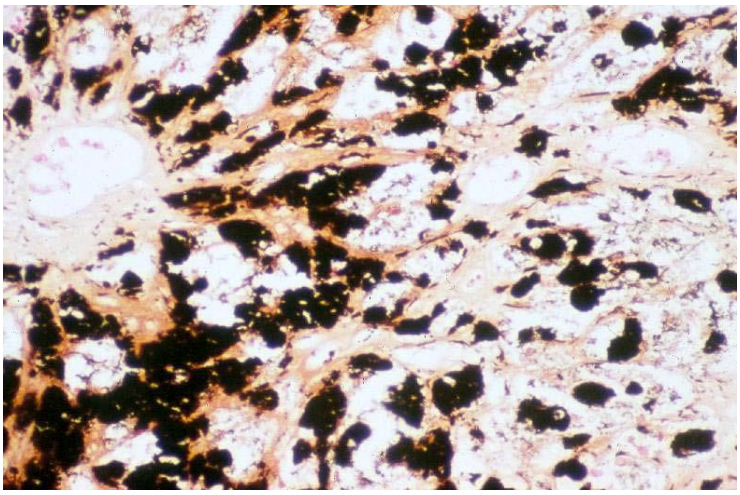
Above: Fungus



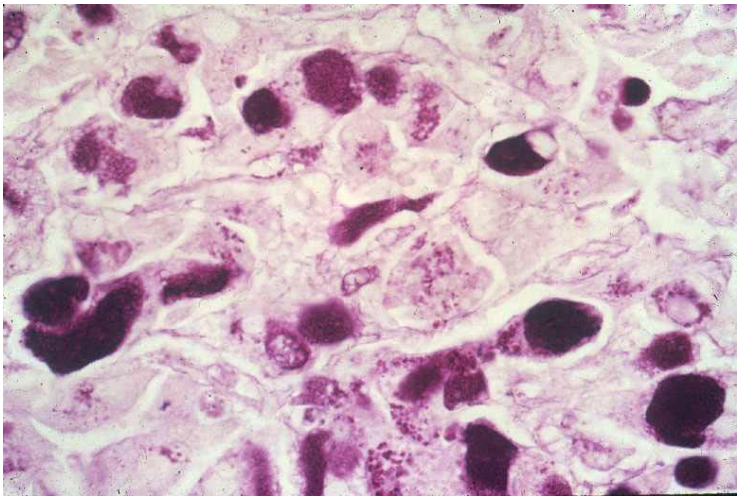
Above: Cryptococcus



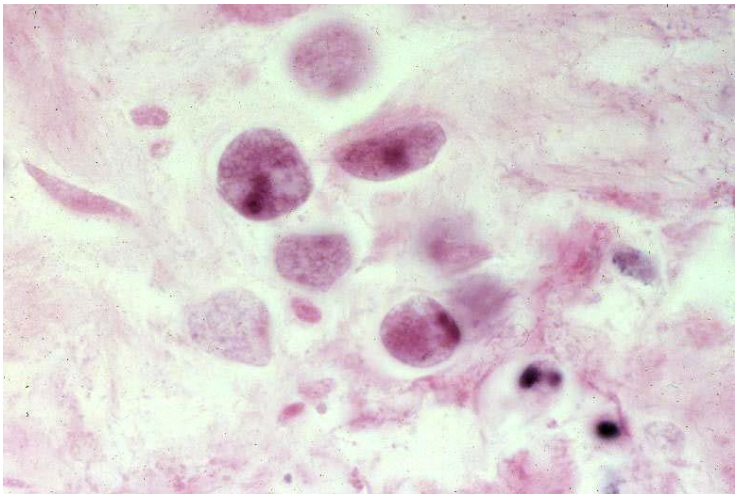
Above: Melanoma-melanoma



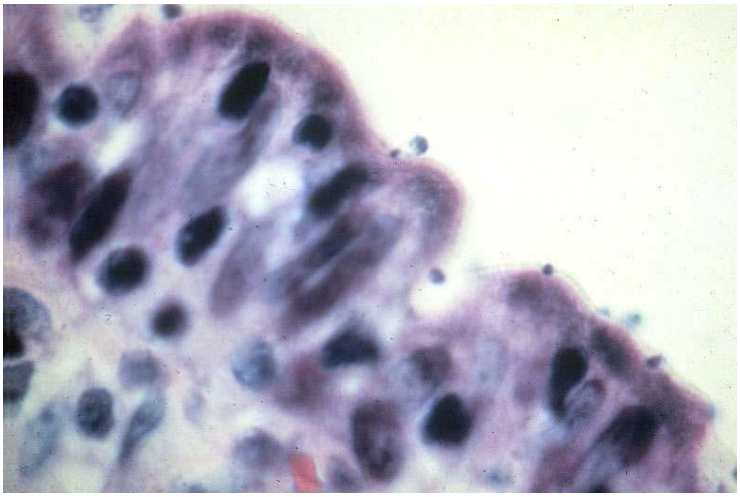
Above: Hepatitis-B



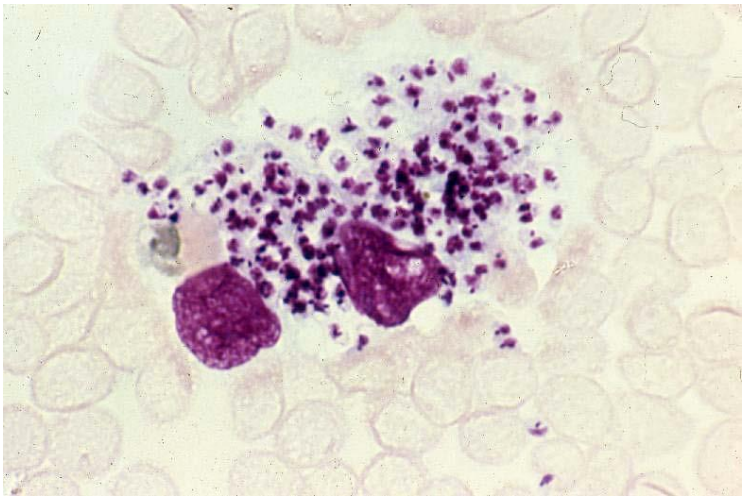
Above: Hepatitis-B



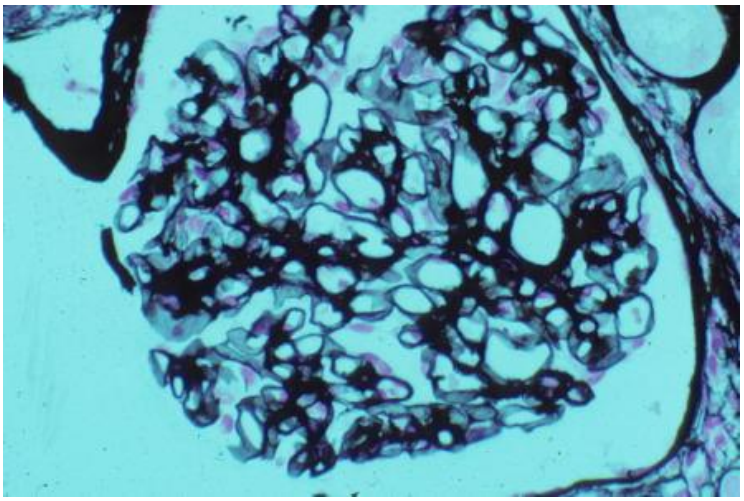
Above: Melanin



Above: Crytosporidium



Above: Leishmania



Above: GMS for Glomerulus of Kidney