



Special Stains Using the Microwave

*Joyce Moore HT/HTL (ASCP) Technical Specialist
Jefferson Regional Medical Center Histo-Path Laboratories
1515 West 42nd Avenue . Pine Bluff, AR 71603
(501) 541-7544*

[A Little About Microwaves](#)

[Masson's Trichrome Stain](#)

[Grocott's Silver-Methenamine Method for Fungus](#)

[Kinyons Carbol Fuschsin Acid-Fast Organisms](#)

[Gordon and Sweet's Silver Impregnation of Reticulum](#)

[Warthin Starry Techniques for Spirochetes](#)

[Alcian Blue Method](#)

[Southgate's Muciarmin Stain](#)

[Periodic Acid Schiff Reaction](#)

A Little About Microwaves

Microwaves travel directly to the solution. In a microwave oven, a magnetron converts ordinary electrical energy into microwaves, which are electromagnetic waves, just like radio and television waves, but have shorter wavelengths and higher frequency. The microwaves enter the oven cavity through a wave guide. A fan-like device called the field stirrer or microwave stirrer helps distribute the microwaves evenly throughout the oven chamber.

Microwaves are waves of energy, not heat. The amount of microwave energy absorbed by a given specimen (or "load") depends on many factors. Among these are the size of the load, its orientation with respect to the waves, and the dielectric and thermal properties of the material.

Depending upon the material, microwaves may be reflected, passed through, or absorbed. For example, metal reflects microwaves. This is why the walls of a microwave oven are generally made of metal, confining the microwaves inside the cavity. The see-through panel in the microwave oven door contains a metal screen. The metal screen reflects the microwaves yet enables the interior of the cavity to be observed because wavelengths of visible light can still pass through. The microwaves cannot penetrate this screen, because the holes in the screen are much smaller than the microwaves.

So-called "microwave transparent" materials such as some glass, pottery, paper and most plastics allow the waves to pass through. When used as containers, these materials do not take up microwave energy, but allow the solutions inside of them to absorb the microwaves. The absorbed microwave energy causes dipolar molecules (such as water) to rotate at the rate of 2.45 billion cycles per second. The interaction between the rotating dipolar molecules, ions and non-moving molecules induces friction, which in turn produces the heat that warms the solution. This is somewhat like the way heat is generated when you rub your hands together. Microwaves heat the solutions from the outside in, just like sources of ordinary heating, but much faster.

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

Energy Absorption: Many characteristics affect the heating rate of a material, such as volume, size, composition and starting temperature.

Volume: The larger the volume of solution the more time is needed to heat it to a given temperature.

Size: The frequency of 2.45 GHz used in all domestic microwave ovens corresponds to a wavelength (in air) of 12.2 cm (about 4.8"). The "antenna property" of a material is best when times the diameter is close to 12.2 cm. Therefore, a cup of espresso is going to be a very good antenna, whereas a 30 μ l droplet on a slide will have poor receiving properties. At the other extreme, if the size of the load is greater than 2 times the penetration depth of the microwaves, the heating will not be homogeneous throughout the material.

Composition: It takes microwaves longer to heat the center of a solid block of material if it contains dielectric molecules. The higher the content of these molecules, the more energy is absorbed, leaving less energy for the center.

Starting temperature: The temperature at which solutions and tissue are placed in the microwave affects the length of heating time. More time is needed to reach a specific temperature for refrigerated materials than for room temperature materials, even though energy absorption in a colder solution is greater than in a warmer solution.

Containers: Metal containers should never be used because they reflect the microwaves and shield the solutions from heating. Any conductive material placed in the microwave cavity can radically change the electromagnetic fields within the oven and may cause sparking (also known as arcing). For most applications, microwave-transparent containers are preferred. Containers should also be selected based on their size and shape, to optimize penetration of the microwaves (see note on antenna properties, above).

How to check to see if containers are microwave proof. Fill a glass container with approximately 50 ml of water. Place in the microwave next to the container you are testing. Set microwave on maximum power and heat for one minute. If the new container is hot, it is absorbing microwave energy; if it remains at room temperature, it is microwave-proof. Always use thermal mitts or potholders when handling any container with a microwave oven. Even though the container itself may be microwave-transparent, the heated solution inside can still conduct heat to the hands.

Agitation: Agitation promotes even heating throughout a volume of liquid. A good example is the pink ring commonly seen at the top of the fluid in microwave PAS staining procedures. This layer rises to the top due to vaporization of SO₂. Agitation would overcome any large SO₂ difference in the fluid.

Rotators: The microwave stirrer moves ("stirs") the electromagnetic waves with respect to the load; a turntable moves the load with respect to the standing electromagnetic fields within the oven cavity. The theory behind this is that the distribution of microwave energy within the cavity is always imperfect, and the rotator will cause the material to pass through hotter and cooler spots, averaging out the exposure to microwaves.

Covering: All containers used in a microwave oven should be open, vented, or loosely covered to prevent pressurization. Vented containers are advised when working with volatile fluids. They reduce evaporation and allow for more efficient heating. Paper toweling can be used as a light covering to prevent splatter and to absorb moisture. Waxed paper helps to retain heat and moisture.

Standing Time: Heat continues to be conducted from the outside to the center.

High Altitude Adjustments: Solutions may require a slightly longer heating time, as water boils at a lower temperature.

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

Cost: The cost containment factor is a big concern in any laboratory. Using a microwave oven in the lab is a labor saving device. Most solutions used must be discarded. Measure tech time vs. cost of solution.

Safety: Are microwave ovens dangerous? Most researchers agree that high doses of exposure can lead to biological damage due to heat. There is still no clear consensus regarding the danger from low levels of microwave exposure. Microwaves, like radio waves, have much less energy than the amount needed to break even the weakest molecular bond. The potential hazards from microwave energy should, therefore, not be confused with those of ionizing radiation from, say, gamma rays. Microwave ovens can be safely used in a laboratory, provided that certain precautions are scrupulously observed. Here is a list of simple safety dos and don'ts:

- Use microwave-transparent containers whenever possible.
- Always handle containers with potholders or thermal mitts.
- Never cover containers tightly.
- Never operate the microwave without a minimum volume of microwave-absorbing material inside the cavity.
- Never put a conductive material (such as a metal container) inside the microwave.
- Periodically inspect and clean door seals and hinges.
- Periodically use a microwave leakage detector to check for microwave leakage from the door seals.
- Never heat food in a microwave oven used for laboratory procedures.
- Never use toxic chemicals in a microwave oven unless you have a laboratory microwave with a high-volume exhaust (100 cubic feet per minute should be the standard) ventilating the cavity into a fume hood.
- Never use flammable solvents in a kitchen microwave oven; these chemicals (e.g. alcohol concentrations of 50% or higher, xylene) can only be safely heated in a laboratory microwave designed for this purpose, with a temperature feedback system which will prevent overheating.

References

1. Login, G.R., and Dvorak, A. M. (1994). The Microwave Tool Book. A Practical Guide for Microscopists. Boston: Beth Israel Hospital.

Masson's Trichrome Stain

Fixation: Bouin-fixed tissue is preferable. May mordant in Bouin's fluid for one hour at 56 C or overnight at room temperature.

Technique: Cut paraffin sections at 6 microns.

Solutions:

Picric acid, saturated aqueous solution

Weigert's Iron Hematoxylin

Solution A

Hematoxylin 1.0 g
Absolute alcohol 100.0 ml

Solution B

30% aqueous ferric chloride 4.0 ml
Distilled water 95.0 ml
Hydrochloric acid 1.0 ml

Working solution

Equal parts of Solution A and Solution B

Biebrich Scarlet-Acid Fushin

1% aqueous Biebrich scarlet 90.0 ml
1% acid fuchsin solution 10.0 ml
Glacial acetic acid 1.0 ml

Phosphotungstic Acid

Phosphotungstic acid 5.0 g
Distilled water 100.0 ml

Aniline Blue Solution

Aniline Blue 2.5 gm
Acetic acid 2.0 ml Distilled water 100.0 ml

Acetic Water

Glacial acetic acid 1.0 ml
Distilled water 100.0 ml

Staining Procedure:

1. Attach to automatic stainer Xylene.
2. Absolute alcohol.
3. 95% alcohol.
4. Rinse in distilled water.
5. Place 50 ml of saturated picric acid in the microwave for 45 seconds. Place slides on staining rack. Drop hot Bouin's on slides. Let set 3 minutes.
6. Cool and wash in running water for 5 minutes.
7. Rinse in distilled water.
8. Weigert's iron hematoxylin solution for 7 minutes. (Note: this step can also be performed in a laboratory microwave at 80% power for 15 seconds)
9. Rinse in distilled water.

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

10. Biebrich scarlet-acid fuchsin solution for 30 seconds.
11. Rinse in distilled water.
12. Phosphotungstic acid solution for 1-3/4 minutes and rinse.
13. Aniline blue solution for 3 minutes.
14. Rinse in distilled water.
15. 1% acetic water for one minute.
16. 95% alcohol.
17. Absolute alcohol - 2 changes.
18. Xylene - 2 changes
19. Mount with xylene soluble media.

Results:

Nuclei - black
Cytoplasm, keratin, muscle fibers, intercellular fibers - red
Collagen, mucin - blue

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory

References

P.J.: Jour. Technical methods, 12: 75-90, 1929 (AFIP Modification)

Grocott's Silver-Methenamine Method for Fungus

Fixation: 10% Neutral buffered formalin

Technique: Paraffin sections cut at 4 microns

Control: Tissue containing fungus

Solutions: 5% aqueous silver nitrate, with 3% methenamine

10% Chromic Acid Solution

Chromium trioxide 5.0 g

Distilled water 50.0 ml

1% Sodium Bisulfite Solution

Sodium bisulfite 0.5 g

Distilled water 50.0 ml

2% Sodium Thiosulfate

Sodium thiosulfate 2.5 g

Distilled water 50.0 ml

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

Methenamine-Silver Nitrate Solution (Stock)

5% Silver nitrate 5.0 ml

3% Methenamine 100.0 ml

A white precipitate forms but immediately dissolves on shaking. Clear solutions remain usable for months. Store in refrigerator.

Methenamine-Silver Nitrate Solution (working)

Methenamine-silver nitrate solution (stock) 25.0 ml

Distilled water 25.0 ml

5% Borax 2.0 ml

Make this solution fresh each time.

0.1% Gold Chloride Solution

1% Gold Chloride solution 5.0 ml

Distilled water 45.0 ml

This solution may be used until brown precipitate appears and the solution is cloudy.

1% Light Green Solution (stock)

Light green SF yellowish 1.0 g

Distilled water 100.0 ml

Glacial acetic acid 0.2 ml

Thymol 2.0 grains

1% Light Green Solution (working)

Light green, stock 10.0 ml

Distilled water 40.0 ml

Staining Procedure:

1. Attach to automatic stainer
Deparaffinize sections and hydrate to distilled water. Slides previously stained with most other stains may be used by removing coverslips and running through xylene and alcohols to water. Subsequent chromic acid treatment will remove any remaining stain.
2. Oxidize in fresh 10% chromic acid solution for 10 minutes at room temperature. (Note: this step may also be performed in a vented laboratory microwave set at 60 C for 90 seconds).
3. Rinse in distilled water for a few seconds.
4. Rinse until clear in 1% sodium bisulfite to remove any residual chromic acid.
5. Rinse in distilled water 5 times.

6. Place slides in Silver Methenamine Solution and microwave for 45 seconds. Let slides set for 2 minutes. Examine microscopically. Fungi should be dark brown. If staining is not complete microwave again in the hot working solution for 10 seconds. Re-examine slides. Repeat this step until the desired staining intensity is reached.
7. Rinse in 5 changes of distilled water.
8. Tone in 0.1% gold chloride solution until sections are gray-lavender, 1 minute.
9. Rinse in distilled water
10. Remove unreduced silver with 2% thiosulfate (hypo) for 1 minute.
11. Wash thoroughly in tap water.
12. Counterstain with working light green solution for 15 seconds.
13. Dehydrate, clear and mount with xylene soluble media.

Results:

Fungi - sharply delineated black

Mucin - taupe to dark gray

Inner parts of mycelia & hyphae - old rose

Background - pale green

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory

References

Grocott, R.G.: American Journal of Clinical Pathology, 25: pp 975-979, 1955.

Kinyons Carbol Fuchsin Acid-Fast Organisms

for Paraffin Section

Fixation: 10% Neutral Buffer Fromalin

Technique: Paraffin sections cut at 4 microns

Control: Tissue containing acid-fast Bacillus organisms

Solutions:

Kinyoun's Carbol Fuchsin Solution

Basic fuchsin 4.0 g

Phenol crystals, melted 8.0 ml

95% Alcohol 20.0 ml

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

Distilled water 100.0 ml

Mix in order given and filter before using.

Methylene Blue (stock)

Methylene blue 0.7 g

95% alcohol 50.0 ml

Methylene Blue (working)

Methylene blue (stock) 5.0 ml

Tap water 45.0 ml

1% ACID ALCOHOL

HCL concentrated 1.0 ml

70% Alcohol 99.0 ml

Staining Procedure

1. Deparaffinize slide sections to distilled water.
2. Stain slides in Carbol Fuchsin for 10 seconds in microwave (Note: in a laboratory microwave, stain at 60 C for 20 seconds).
3. Wash well in running tap water
4. Differentiate in acid alcohol until tissue is light pink.
5. Wash well in running tap water.
6. Stain in Methylene Blue 1 minute.
7. Rinse in distilled water.
8. Dehydrate, clear and mount in permount.

Results:

Acid fast organisms - red

Background - blue

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory

References

Kinyoun, J.J.: Am. J. Pub. Health, 5, 867, 1915.

Gordon and Sweet's Silver Impregnation of Reticulum

Demonstrates: Reticulum

Fixation: 10% Neutral Buffered Formalin

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

Techniques: Paraffin sections cut at 4 microns

Control: Lymph node

Solutions:

Permanganate Solution

Potassium permanganate 0.5% 0.5 g

Distilled water 50.0 ml

Wilder's Silver Solution

To 5.0 ml of 10% silver nitrate, add 58% ammonium hydroxide drop by drop until the precipitate that forms is just redissolved. Add 5.0 ml of 3% sodium hydroxide. A black precipitate forms. Add 58% ammonium hydroxide again drop by drop until the precipitate is again just dissolved. Bring up to 40.0 ml with distilled water.

This solution will keep several weeks if stored in a dark bottle in the refrigerator. Several hundred milliliters can be made at one time and only used in 50.0 ml quantities and discarded. Dispose of solution when ppt. appears. pH 11-12.

1% Neutral Red

Neutral Red diluent 0.5 g

Distilled water 50.0 ml

2% Ferric Ammonium Sulfate (Iron Alum)

Ferric ammonium sulfate 1.0 g

Distilled water 50.0 ml

1% Oxalic Acid Solution

Oxalic acid 1.0 g

Distilled water 100.0 ml

0.2% Gold Chloride Solution

Stock gold chloride solution (1%) 10.0 ml

Distilled water 40.0 ml

5% Sodium Thiosulfate Solution

Sodium thiosulfate 2.5 g

Distilled water 50.0 ml

Staining Procedure:

1. Deparaffinize sections and hydrate to distilled water.
2. Oxidize in fresh permanganate solution for 2 seconds in microwave.

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

3. Rinse in distilled water.
4. Bleach until white in 1% oxalic acid for 30 seconds.
5. Rinse in distilled water, 2 changes.
6. Mordant in fresh 2% iron alum for 5 seconds in microwave.
7. Rinse in distilled water, 2 changes.
8. Impregnate for 5-7 seconds in Wilder's Silver Solution.
9. Rinse 1 dip in distilled water.
10. Reduce in 37-40% formaldehyde for 15 seconds.
11. Rinse in distilled water, 2 changes.
12. Tone in 0.2% gold chloride 1 minute - sections turn gray-lavender.
13. Rinse in distilled water, 2 changes.
14. Remove unreduced silver in 5% sodium thiosulfate for 1 minute.
15. Rinse in distilled water, 2 changes.
16. Counterstain in 1% neutral red for 1 minute.
17. Rinse 10 dips in running tap water.
18. Dehydrate, clear and mount with xylene soluble mounting media.

Results:

Reticulum fibers - black
Nuclei - red
Collagen - yellow or brown

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory

Reference:

American Journal of Pathology, 12, 549, 1936.

Warthin Starry Techniques for Spirochetes

Fixation: Formalin

Technique: Sections cut at 4 microns

Control: Tissue containing Spirochetes

Solutions:

Acidulated Water

Acidulate 1 liter distilled water with 0.1 g citric acid until pH of 3.8-4.4 is reached. A pH of 4.0 is ideal for staining spirochetes. For demonstrating Donovan Bodies of granuloma inguinale, a pH of 3.6 is recommended.

2% Silver Nitrate Solution (developer)

Silver nitrate C.P. crystals 0.5 g

Acidulated water 25.0 ml

1% Silver Nitrate Solution (impregnation)

Silver nitrate C.P. crystals 0.5 g

Acidulated water 50.0 ml

0.5% Hydroquinone Solution

Hydroquinone crystals, photographic quality 0.35 g

Acidulated water 25.0 ml

5% Gelatin Solution

Gelatin 1.5 g

Acidulated water 25.0 ml

Staining Procedure

1. Attach slides to automatic stainer, deparaffinize, hydrate to water.
2. Rinse in distilled water, 2 changes.
3. Place slides in 1% silver nitrate solution for 45 seconds in microwave. Let stand for 1 minute at room temperature. (Note: alternatively, in a laboratory microwave, heat at 80% power, 60 C for 5 minutes, no standing time required).
4. Preheat for 45 seconds in microwave in separate flasks: 2% silver nitrate, 5% gelatin, 0.15% hydroquinone. Preheat empty flask in microwave with these.
5. Mix developer solution in order given: Use warm empty flask:

2% silver nitrate 1.5 ml

5% gelatin 3.75 ml

0.15% hydroquinone 2.0 ml

6. When step 5 is completed, remove slides from silver solution. Do not rinse. Place slides horizontally on a slide rack and cover with developer. Allow sections to develop until they are light yellow to golden brown, approximately 1 minute or less. (Note: developing step can also be carried out in a laboratory microwave at 80% power set for 60 C for 20 seconds).

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

7. Rinse thoroughly in 50 ml tap water which is preheated to approximately 56 C in the microwave at 450W for 45 seconds.
8. Rinse in distilled water.
9. Attach to automatic stainer, dehydrate and clear. Mount in a xylene soluble mounting medium.

Results:

Spirochetes - black

Background - pale yellow to light brown

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory

Alcian Blue Method

Fixation: 10% neutral buffered formalin

Technique: Paraffin sections cut at 4 microns; eye sections at 8 microns. Avoid overheating.

Stains: Acid mucopolysaccharides

Control: Appendix

Solutions:

Eosin solution

3% Acetic Acid Solution

Glacial Acetic Acid 3.0 ml

Distilled water 97.0 ml

Alcian Blue Solution pH 2.5

Alcian blue 8 GS 1.0 g

Acetic acid 3% 97.0 ml

Adjust the pH to 2.5. Filter and add a few crystals of thymol. Note: due to temperature, the acetic acid "buffer" solution can drift in pH; therefore, it is helpful to set the pH at 60 C.

Staining Procedure

1. Deparaffinize sections and hydrate to running tap water using the automatic stainer.
2. Stain in alcian blue solution for 20 seconds in the microwave. (Note: alternatively, in a laboratory microwave, heat at 80% power, 60 C for 45 seconds).
3. Rinse well in distilled water.
4. Place in 2nd Eosin and attach to automatic stainer.
5. Dehydrate, clear and mount in xylene soluble media.

Energy Beam Sciences
29B Kripes Road, East Granby, Connecticut 06026-9669
Phone: 800-992-9037 or 860-653-0411 Fax: 860-653-0422
E-mail: ebs@ebosciences.com

Results:

Acid mucopolysaccharides - blue
Nuclei - pink

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory

Reference:

Lev, R. and Spicer, S.S. J. Histochem. Cytochem. 12: 309, 1964. Williams & Wilkins Co.

Southgate's Muciarmin Stain

Demonstrates: Acid mucosubstances

Fixation: 10% Neutral buffered formalin

Technique: Paraffin sections cut at 4 microns

Control: Appendix

Solutions:

Muci-10 plus product of American Histology Co., Tartrazine Solution

Working Muciarmin Solution

The working solutions are 1:4 dilutions of the stock and the diluent is distilled water. Stronger or weaker dilutions may be needed, depending on the strength of the stock solution.

Staining Procedure:

1. Attach to automatic stainer.
Deparaffinize sections, hydrate and run slides through Harris Hematoxylin, acid alcohol, blueing solution and water. Remove from automatic stainer.
2. Stain with tartrazine, 1 minute.
3. Stain in diluted mucicarmine for 40 seconds in microwave and let stand for 3 minutes at room temperature. (Note: alternatively, in a laboratory microwave, drop 2 ml mucicarmine on the section, and microwave the slide at 100% power for 1 minute; place slide in staining jar containing 50 ml of diluted mucicarmine and microwave at 80% power at 60 C for 10 minutes; standing time not required). Check under microscope.
4. Stain is complete, drain slides.
5. Rinse quickly in distilled water.
6. Attach to automatic stainer and dehydrate and clear. Mount in xylene soluble media.

Results:

Mucin - rose to red
Nuclei - blue to black
Capsule of Cryptococcus - deep rose to red
Other tissue elements - yellow

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory

Periodic Acid Schiff Reaction

Fixation: Formalin

Technique: Cut paraffin sections at 4 microns

Control: Tissue containing PAS positive material

Solutions:

0.5% Periodic Acid Solution

Periodic acid crystals 0.5 g

Distilled water 100.0 ml

Schiff's Reagent

Pour approximately 40 ml of Schiff solution in coplin jar. Store in refrigerator. Discard when solution becomes pink.

0.5% Diastase solution light green

If staining for glycogen, use liver with glycogen in it for a control.

Staining Procedure:

1. Deparaffinize and hydrate sections to distilled water with the aid of the automatic stainer.
2. Rinse in distilled water.
3. If PAS reaction with digestion is desired, place sections in coplin jar in 0.5% diastase for 45 seconds in microwave. Parallel slides should be placed in distilled water in coplin jar and microwaved for 45 seconds. Rinse slides in running tap water. Rinse in distilled water.
4. Periodic acid solution for 10 seconds in microwave.
5. Rinse in distilled water, 2 changes.
6. Place in Schiff's reagent for and microwave for 30 seconds (Note: in a laboratory microwave, use 100% power at 30 C for 30 seconds). In a laboratory microwave, stir solution while heating with an air bubble agitator. Otherwise, remove from microwave and agitate the solution by hand to equilibrate the solution temperature. This will allow for more even staining. Let stand in stirred solution for 30 seconds to 5 minutes after removing from microwave, until sections have assumed a pinkish color. Check with microscope for correct staining.

7. Wash in running water for 5 minutes.
8. Attach to automatic stainer. Stain in Hematoxylin solution for 15 seconds.
9. Rinse in water.
10. Differentiate in acid alcohol.
11. Rinse in water.
12. Blue in running tap water.
13. Rinse in water.
14. Dehydrate, clear. Mount with xylene soluble media.

Results:

Glycogen - rose to purplish red

Mucopolysaccharides, fungi - rose to purplish red

Background - blue

Adapted for the Jefferson Regional Medical Center Histo-Path Laboratory