

HISTO-LOGIC[®]

No reader should utilize materials and/or undertake procedures discussed in HISTO-LOGIC articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

Editor, Lee G. Luna, D. Lit., H.T. (ASCP)

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Editor's Statement

An apology to the *American Journal of Medical Technology* and Miss Evelyn F. Ballou for failure to obtain permission to publish an update of the article titled: "A Paraffin Technique For Processing Eyes," which originally appeared in Volume 32, No. 5, pages 287/45-291/49, September-October, 1966 issue of the *American Journal of Medical Technology*. The modified article, written by Edna B. Prophet, appeared in Volume VI, No. 1, pages 75-76, January, 1976, issue of *Histo-Logic*.

NSH Symposium/Convention Tentative Program - 1978

Following is a list of papers and workshops planned for presentation at the 1978 Symposium/Convention being held by the National Society for Histotechnology, September 25-29, 1978, Skirvin Plaza Hotel, Oklahoma City, OK. Also provided is an estimate of cost to assist you in requesting funding for attendance. A complete program and registration form will be printed in the April issue of *Histo-Logic* and the June issue of the *Journal of Histotechnology*.

Tentative Cost List

- | | |
|---|---------------|
| 1. Workshops (full day)* | \$ 30.00 each |
| 2. Workshops (half day)* | 20.00 each |
| 3. Scientific Sessions* (Wednesday thru Friday) | 40.00 |
| 4. Banquet | 15.00 |
| 5. Accommodations 26.00 Singles, 31.00 Doubles, 38.00 - 45.00 Jr. Suites, 85.00 and up Suites. | |

*Non NSH members must add \$5.00 for each workshop and the Scientific Sessions.

Workshops - Full Day

1. Introduction to Stain Mechanisms
2. Tissue Identification for Histotechnologists
3. Function of Major Organs and Identification
4. Chemistry — Its Organization, Principles and Application
5. Management for Histotechnologists
6. Special Stains (May be optional 1/2 day)
7. Fluorescence Microscopy
8. Histochemistry**

Workshops - Half Day

1. Cytotechnology
2. Cryotomy
3. Maintenance - Equipment - Basic Instrumentation
4. Embedding Principles and Procedures
5. Plastic Technique
6. Medical Photography
7. Autoradiography
8. Mathematics for Histotechnology
9. Electron Microscopy Techniques
10. The Diagnosis
11. Proper Methods for Submitting Specimens
12. Infectious and Neoplastic Disease Common to Man and Animals
13. Introductory Histotechnology**
14. Human Microscopic Anatomy**

**Highly recommended for Thomas A. Edison examinees.

Scientific Session Papers

1. Techniques for Special Tissues
2. Methods of Cell and Tissue Observation
3. Radioactive Nucleotides in the Histopathology Laboratory
4. Knife Sharpening Revolutionized
5. Rapid Method for Esterase
6. Bone Marrows - How, Why and the Results
7. Demonstration of Three Fiber Types by Myosin ATPase from a Single Preparation
8. Proper Preparation of Lymph Nodes
9. Practical Methodologies in Neuropathology
10. Histotechnology and the Child Maltreatment Syndrome
11. Egg Yolk Embedding for Whole Brain Frozen Sections
12. Multiple Mounting and Staining on 2x2 Glass Slides
13. Vacuum Processing for Small Biopsies
14. Computer in the Histopathology Laboratory
15. Variables in Decalcification
16. Spironolactone Bodies in the Adrenal Gland
17. Photomicrography of Special Stains
18. Ten Easy Steps to an Accredited School of Histotechnology
19. Reconstitution of Dried Tissue Specimens
20. A Modified Method for the Identification of Acetalated and Non Acetalated Sialic Acid
21. A Plastic Embedding Technique
22. Utility of Tissue Enzyme Methods for Research

Gram-Positive and Gram-Negative Bacteria Control

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There are many good histologic techniques for the demonstration of bacteria and all should be controlled with a tissue section containing both Gram-negative and Gram-positive bacteria. Both Gram-negative and Gram-positive bacteria rarely grow together in the same tissue, therefore one type of bacteria will probably predominate in the tissue. Consequently, there is an urgent need for readily available material containing both Gram-negative and Gram-positive bacteria and cellular structures for proper staining and differentiation.

One alternative is to grow Gram-negative and Gram-positive bacteria on separate agar or gelatin blocks. These can be fixed, cut, mounted on slides, and stained. However, agar cultures demonstrate bacterial staining only. The histotechnologist is presented with various types of tissue which must be stained and differentiated as well as demonstrate bacteria. If not properly differentiated, the bacteria in the tissue sections could be masked or completely decolorized. Hence, agar or gelatin blocks would be of limited value because of the lack of tissue to control differentiation.

Instead of using tissue sections in which only Gram-negative or Gram-positive bacteria are present or using bacteria exclusively, a control which accommodates both bacteria and tissue can be made in which each are stained and differentiated simultaneously. Since the bacteria cannot be incubated and grown in the tissue due to necrosis of the tissue, the cultured bacteria must be added to the tissue. A hollow tissue tube with suspending media is the method used.

Required Materials:

1. Two fallopian tubes (unfixed).
2. 0.5 gm of agar.*
3. 10.0 ml of distilled water.
4. Clamps.
5. 10.0% formalin — 200.0 cc.
6. Bacteria, Gram-positive and Gram-negative (*E. coli* and *Staphylococci*) suspended in 3.0 cc of distilled water. This suspension will have a cloudy white color.**

*NOTE: Agar has a melting point of 105° C. Once melted, it will remain liquid until a temperature slightly below 60° C is reached.

**NOTE: Gram-positive (*Staphylococci*) and Gram-negative (*E. coli*) bacteria may be obtained from the Microbiology Department of a hospital or they can be purchased from a pharmaceutical company.

Procedure:

1. Make a 5.0% agar solution in distilled water.
2. Mix the suspension of bacteria with the 5.0% agar solution.
3. Clamp one end of the fallopian tube shut.
4. Place liquid agar and bacteria mixture in hypodermic syringe and inject into the lumen of the fallopian tube.
5. Clamp the open end of the fallopian tube shut.
6. Fix whole fallopian tube in 200.0 cc of 10.0% formalin for 48 hours.
7. The fallopian tube is then cut into pieces suitable for embedding. These pieces are processed and paraffin embedded as usual.
8. Sections are cut at 4 to 6 microns.
9. Sections are now ready to be used as a control.

Remarks:

Both gelatin and agar were tested as a suspending media for the cultured bacteria. Gelatin was found unsuitable because it stains too heavily with basic aniline dyes causing some confusion and occasionally masked the stained bacteria. Agar was then selected and found to stain light blue, but very transparent with basic aniline dyes. This in no way hindered visualization of the bacteria.

The number of bacteria are generally plentiful and easily observed microscopically with this method. The concentration can be easily adjusted by increasing or decreasing the number of bacteria. Two inoculation loops pulled gently over an agar control plate gave an adequate number of bacteria. It is also advisable to have a fresh colony of bacteria (24 hours old). Experience has shown that an older colony of bacteria (over 48 hours) did not stain as well.

The results were excellent with this method. The slide showed not only well differentiated tissue, but also Gram-negative and Gram-positive bacteria in the same area.

References:

1. Lille, R.D.: *Histopathologic Technic and Practical Histochemistry*. McGraw-Hill Book Co., New York, N.Y., 3rd ed., 1965.
2. Falis, B.D. & Ashworth, R.D.: *Textbook of Human Histology*. Little, Brown and Co., Boston, Mass., 1st ed., 1970.
3. Miller, S.E.: *A Textbook of Clinical Pathology*. The Williams and Wilkins Company, Baltimore, Maryland, 5th ed., 1955.
4. Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. McGraw-Hill Book Co., New York, N.Y., 3rd ed., 1968.

Helpful Hints for Taking the H.T. Practical Exam

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During the past few years our students taking the H.T. practical portion of the Registry Examination have overcome several pitfalls. It is hoped that by printing the following tips other examinees may be helped to prepare for taking, and passing, the H.T. Practical Exam.

Editor's Note:

The following suggestions are the personal views of the author and are not to be misconstrued as suggestions from, or approved, by the ASCP Board of Registry.

1. Carefully clean all slides and coverslips with an alcohol-acetone solution.
2. Cut at least 10 slides per block, then stain one by hand (H&E) to see if it stains well. Don't use the automatic stainer for your test slide.
3. Use egg albumin coated slides for specimens of brain and bone.
4. Label blocks and slides from the same tissue, i.e.: appendix 1, appendix 2. This is especially important with appendix and muscle tissue to insure you have matched the correct slide with its corresponding block.
5. When cutting skin, cut through the epidermis last, as it will be more scratchy and drag through the rest of the section.
6. Don't cut an entire block on the same spot of the knife. Keep moving the knife. Cut no more than two ribbons on a particular area of the knife.
7. Consistently mount sections in the non-frosted center part of the slide. Don't mount diagonally. Each slide in a set should be mounted in the same manner.
8. It can be helpful to keep track and label what order slides were cut on a particular piece of tissue and make a notation of every time the knife was moved.
9. Be sure you have a fresh, clean water bath when cutting and wipe surface often.
10. Use a cooler water bath for brain specimens to prevent too much separation of section.
11. Use a second, hotter water bath to flatten out specimens of appendix and bone. Lay ribbon on your regular water bath, then mount section on slide, lay it out on hotter bath and pick up again quickly to remove ripples.
12. Remove all formalin pigment in sections before staining. Especially watch your fungus sections for residue pigment.
13. Be sure your glassware for all silver solution is thoroughly acid-cleaned to avoid any precipitate.
14. Make certain aniline blue solution is working for Masson's trichrome. The stain gets weak and dull after excessive use.
15. Use fresh solutions whenever possible.
16. Try to run two slides together on stains that tend to loosen sections; i.e., argentaffin, LFB.
17. Use a clean jar of mounting media and use it only for your practical slides.
18. After your slides are coverslipped and well dried (2 days), you can scrape excess mounting media from around the coverslip with a razor blade.
19. Type all labels and be sure they are placed on straight. Neatness is very important and can reflect the quality of your work.

Staining of Bacteria in Tissue Sections: A Reliable Gram Stain Method

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Solutions:

| 1% Crystal Violet | |
|----------------------|----------|
| Crystal violet..... | 1.0 gm |
| Distilled water..... | 100.0 ml |

| Gram's Iodine | |
|-----------------------|----------|
| Iodine..... | 1.0 gm |
| Potassium iodide..... | 2.0 gm |
| Distilled water..... | 300.0 ml |

| 0.25% Basic Fuchsin (stock) | |
|-----------------------------|----------|
| Basic fuchsin..... | 0.25 gm |
| Distilled water..... | 100.0 ml |

Filter solution through No. 4 Whatman paper.

| Basic Fuchsin (Working) | |
|---|--------|
| (For formalin, gluteraldehyde, and Bouin's fixed tissue.) | |
| Basic fuchsin (stock)..... | 1.0 ml |

| | |
|--|-----------|
| Distilled water..... | 25.0 ml |
| Basic Fuchsin (Working) | |
| (For Formal-Zenker's fixed tissue.) | |
| Basic fuchsin (stock)..... | 5.0 ml |
| Distilled water..... | 25.0 ml |
| Gallego's Solution | |
| Distilled water..... | 50.0 ml |
| Formaldehyde, concentrated (37-40%)..... | 1.0 ml |
| Glacial acetic acid..... | 0.5 ml |
| Picric Acid - Acetone | |
| Picric acid..... | 0.5 gm |
| Acetone..... | 1000.0 ml |
| Acetone - Xylene | |
| Acetone..... | 50.0 ml |
| Xylene..... | 50.0 ml |

Staining Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Stain slides in 1% crystal violet for 2 minutes.
3. Wash slides in tap water to remove excess crystal violet.
4. Place slides in Gram's iodine for 5 minutes.
5. Wash slides in tap water to remove excess iodine.
6. Blot, but not to dryness.
7. Differentiate slides in acetone until blue color ceases to run from the slide. This is best accomplished by vertically dipping the slide approximately two times per second in a Coplin jar filled with acetone. Only a few seconds is necessary to accomplish this.
8. Rinse slides quickly in tap water and wash thoroughly to remove acetone.
9. Place slides in working basic fuchsin solution for 5 minutes.
10. Wash slides briefly in tap water.
11. Place slides in Gallego's solution for 5 minutes (blowing on solution occasionally to agitate helps this stage of differentiation).
12. Wash slides thoroughly in tap water and blot, but not to dryness.
13. Acetone, 3 quick dips.
14. Picric acid-acetone, 3 quick dips.
15. Acetone, 3 quick dips.
16. Acetone-xylene, 5 quick dips.
17. Xylene, 10 quick dips.
18. Xylene, 1 minute.
19. Mount in resinous mounting media.

Results:

Gram-positive bacteria — stain blue
 Gram-negative bacteria — stain red
 Background tissue — generally yellow
 Nuclei and epithelium — stain light red

Notes:

In steps 2, 4, 9 and 11 solutions are applied to slides which are lying flat.

In steps 1, 7 and 13 to 18, the slide is dipped in a Coplin jar containing solution.

Short duration (3 quick dips) in 0.05% picric acid-acetone gives reliable differentiation of Gram-negative bacteria and a desirable yellow background. Higher picric acid concentrations (even 0.1% picric acid-acetone) result in poorly controlled differentiation with poor staining of Gram-negative bacteria. Rapid dehydration in acetone (as indicated) is essential for good results.

The more concentrated basic fuchsin Working Solution B is essential for good staining of Gram-negative bacteria when Formal-Zenker's fixative is used. It is emphasized that *Gallego's differentiator is a critical reagent in this stain procedure.*

Editor's Note:

This stain has been used in the AFIP Laboratories for some time with excellent results.

Reference:

Brown, R.C. & Hopps, H.C.: Staining of Bacteria in Tissue Sections: A Reliable Gram Stain Method. *Am. J. of Clin. Path.*, Vol. 60, No. 2, August, 1973.
 Printed with permission from Editor-in-Chief, American Journal of Clinical Pathology.

Histotechnology Seminar Offered

The First Annual Histotechnology Continuing Education Seminar will be held February 23, 24 & 25, 1978 at the University of Texas Health Science Center at San Antonio, Medical School Component. Fourteen (14) contact hours will be accumulated. Course number 9020 will be directed by Sue Judge, HT (ASCP) and Lyn Richardson, HT (ASCP).

The course is sponsored by the University of Texas Health Science Center at San Antonio in cooperation with the University of Texas Medical Branch at Galveston, University of Texas M.D. Anderson Hospital and Tumor Institute, and University of Texas Health Science Center at Dallas.

The concept of the course is to present up-to-date methods and scientific advances in the field of histotechnology, covering basic as well as advanced information to improve standards in the histopathology laboratory of large and small hospitals as well as research laboratories. Outstanding technologists and faculty members will present both lectures and workshops. The latest equipment and products for the laboratory will be available for examination.

Fee for the course is \$45. Students from any of the four participating institutions may pay a special fee of \$20.

Fungal Growths Due to Excessive Washing

An Editorial

The three photographs (Figs. 1, 2 and 3) demonstrate a growth which, though unlikely, can occur and may result in diagnostic problems. The fungi-like organisms seen in the photographs were produced by exposure of the tissue specimen to a running tap water wash which exceeded four days. It is unlikely that tissue of any kind used for the production of microscopic slides will ever require four days of washing in running water, but there are several instances in which a mistake can be made which may result in excessive tissue washing in tap water. Because of this possibility, the problem is being brought to your attention.

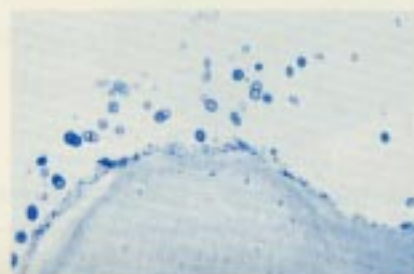


Figure 1:
This photograph shows the fungi growing outside the tissue specimen. Skin, H&E X300

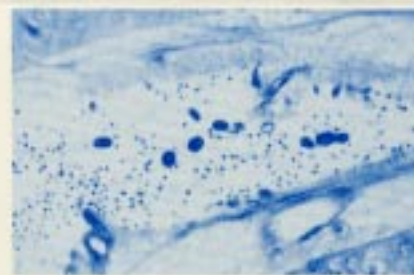


Figure 2:
Bacteria-like structures can be seen with the fungi in a hair follicle. Skin, H&E X440

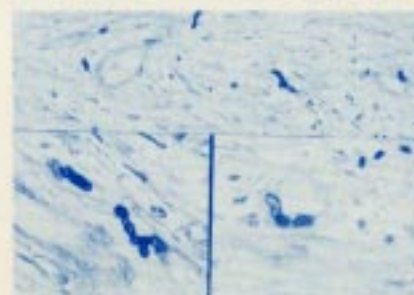


Figure 3:
The fungi can be seen growing in the dermis. Note that the fungi appears to be embedded in the tissue structure. Skin, H&E X300 and 575

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Technically Speaking

Robert A. Clark, Technical Services
 Lab-Tek Products, Naperville, IL 60540

The use of vacuum infiltration with the aid of heat has proven to be most beneficial in the preparation of tissue specimens, especially those that are porous and of low density.

Tissue-Tek® II Vacuum Infiltrator serves the histology laboratory in several capacities.

First, paraffin impregnation is greatly enhanced by using the unit with melted paraffin at 60°C and 15 inches of vacuum. The amount of air trapped in the tissue specimen is often surprising.

Second, the vacuum infiltrator can be used to enhance formalin fixation before actual processing begins. Late surgical specimens can often delay the entire processing schedule. With the addition of 10% formalin into the chamber, heat and vacuum, those late specimens can be fixed in 20 minutes or so.

And, the unit can be used as a work pot. Placed beside the Tissue Embedding Center, Tissue-Tek II Vacuum Infiltrator becomes a welcome accessory item. It eliminates the need to remove the paraffin pots from the Tissue Processor.

Like any piece of equipment, various parts of the Vacuum Infiltrator can wear out. Listed below are the part numbers for the most commonly replaced parts:

| | |
|----------|--------------------------------|
| 95000250 | Cover (with valve assembly) |
| 50062036 | Cover (without valve assembly) |
| 40570023 | Thermostat |
| 95000253 | Line trap |
| 40211021 | Heat Element |
| 50361008 | Rubber magnet |

These parts may be obtained through any authorized Lab-Tek Service Certified Distributor.

To receive your own personal copy of HISTO-LOGIC, or to have an associate added to the mailing list, submit home address to: Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 North Aurora Rd., Naperville, Illinois 60540.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.