# HIST@-L (1

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

A Technical Bulletin for Histotechnology

Vol II No. 1 - Jan. 1972

### Let's Get Our Facts Straight Freida L. Carson, M.S., HT (ASCP)

**Baylor University Medical Center, Dallas, Texas 75246** 

During the past few years we have become aware of the need to make a more exact science of histologic technique and are beginning to see the first steps in that direction with emphasis being placed on recording both the color index (CI) and dye content of stains used in each procedure.

Standardization of terms and proper terminology are also urgently needed. A good example of this is in the use of solutions of ammonia in preparing reagents for use in reticulum stains, Best's carmine stain, and in the removal of formalin pigment.

This solution has been variously referred to as 28% ammonia water,1 2 5 6 7 8 28% ammonium hydroxide,1 2 3 or as ammonia,7 strong ammonia water,2 and ammonium hydroxide4 with no specific strength mentioned. As one can see from the various references, there are inconsistencies both within one book on various procedures and between authors on a given procedure. The 1957 edition of the AFIP Staining Manual<sup>1</sup> tells us to "be sure to dilute 58% concentrated ammonia to 28% ammonia" for Best's carmine stain. By the second edition,3 this note has been omitted and 28% ammonia water is specified for this procedure. This has again been changed in the third edition to 28% ammonium hydroxide. Other authors specify in this same procedure the use of 28% ammonia water<sup>5 6 8</sup> or avoid the problem entirely by just calling for ammonia<sup>7</sup> or ammonium hydroxide<sup>5</sup> in no specific concentration.

Much confusion may be created by these differences and one finds himself in the dilemma of "to dilute or not to dilute."

The facts are:

1. When the gas ammonia is dissolved in water, the following chemical reaction takes place: -NH<sub>4</sub>OH

NH3 + H2O-

Ammonia + Water Ammonium Hydroxide

- 2. Ammonium hydroxide ionizes as follows: NH<sub>4</sub>OH
- 3. The proper term for the solution is ammonium hydroxide, not ammonia water, because a chemical reaction has taken place and a new compound has been formed.
- 4. Concentrated solutions have a concentration of 28% expressed as ammonia (NH3) or 58% expressed as ammonium hydroxide NH4 (OH).
- 5. In staining procedures, the term concentrated ammonium hydroxide or 58% ammonium hydroxide should be used. If a dilution is needed, it should be figured in terms of beginning with a 58% solution of ammonium hydroxide and be expressed accordingly.

All of the procedures referred to above use 58% ammonium hydroxide in reagent preparation, so let's urge this correction in all future editions of textbooks and publications concerning histologic technique.

#### References

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- 4. Humason, G.L.: Animal Tissue Techniques, San Francisco, W.H. Freeman & Co., 1962.
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- 6. Mallory, F.B.: Pathological Technique, New York, Hafner Publishing Co., Reprinted 1961.
- McManus, J.F.A., Mowry, R.W.: Staining Methods: Histo-logical and Histochemical, New York, Paul B. Hoeber, Inc., 1960.
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## Special Staining Control System for Histology

#### Herbert Stevens, HT (ASCP) Oakwood Hospital, Dearborn, Michigan 48124

Special staining techniques are performed at the request of the pathologist. These techniques are invaluable aids in diagnosing or demonstrating a specific organism, mineral deposit, or type of tissue.

However, no special stain should be accomplished without inclusion of a known positive control slide. Laboratories engaged in a variety of special staining techniques should have an equal variety of controls. Technologists must rely on the pathologist for selection and use of stain control material. Considerable time can be saved if a special stain control index system is established within the laboratory. This is accomplished in the following manner, with a 3 x 5 card file.

When the pathologist finds a case positive for melanin, fungi, tubercle bacilli, etc., on the slide label, he notes the case number, product seen and other salient information on a 3 x 5 card. The card is then filed under a specific major head-ing; for example, iron. This procedure should be practiced with every case which may serve as a good control and will result in a convenient system which can be referred to for control material of a specific nature, pigment or pathologic condition.

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When a control is needed, the technician consults the card file under the major heading and uses the information to obtain corresponding microscopic slides. The slides are checked microscopically to determine the most suitable control. The paraffin block is withdrawn from file and sectioned to produce control slides.

This system provides a quality control method for your special staining procedures and saves considerable time for both pathologist and technologist.

One may write the Armed Forces Institute of Pathology, Washington, D.C. 20305, for control material difficult to obtain. Take into account, however, this service is a time-consuming effort on the part of the laboratory staff and only requests of a "last resort" type should be made. Since the AFIP has exhausted much of the scarce control

Since the AFIP has exhausted much of the scarce control material, laboratories throughout the country should make every effort to assist by sending control material to AFIP for dissemination. Material should be in the form of paraffin blocks or wet tissue mailed with an explanatory letter addressed to: Director, Armed Forces Institute of Pathology, Washington, D.C., 20305, ATTN: Mr. Lee G. Luna.

## Serial Section Separator

Luise G. Bohacek, HT (ASCP) State University of New York at Buffalo Buffalo, New York 14223

The serial section separator shown below has been used by the authoress for many years with great success. The suggestion is presented here in the hope that it will be beneficial to techicians doing serial sectioning.

Typewriter paper is cut into four strips, each measuring 8-1/2 x 3/8 inches. The strips are then stapled together as shown in the diagram below. Each projection of the separator is then numbered 1 through 8. Separator is floated on the water of a round flotation bath. The first section cut is placed in the space numbered 1, second section in space 2, third section in space 3, etc.

Sections are allowed to expand completely, after which they are transferred to microscopic slides in the usual manner. The authoress finds that by separating the sections into numbered spaces mix-ups in sequence are prevented, thereby producing *true* serial sections.



## Helpful Hint

#### HOW TO HOLD ONTO TISSUE BIOPSY SECTIONS

Processing smaller tissue specimens such as liver biopsies, skin curretings, etc., in plastic and metal cassettes (capsules) can lead to problems.

Placing just the tissue into the cassette can result in its being lost through one of the many cassette holes provided for the proper exchange of fluids. To overcome this problem, some have wrapped the specimen in lens cleaning tissue (such as is used to clean eyeglasses and microscope lenses) before placing the tissue into the cassette. However, this method often results in poor processing since the lens cleaning tissue interferes with proper fluid exchange.

To avoid lost tissue specimens — while assuring adequate fluid exchange — sandwich the section between two pieces of polyfoam. (An ideal source for the polyfoam is your coverslip orders, polyfoam is used as packing material by most manufacturers, and this material is of a satisfactory thickness.) This material is quite porous and inert to processing solutions. Just cut two pieces of polyfoam to fit the cassette, place one piece in the bottom of the cassette, position the section, then place the second piece on top, close the cover and process as usual. Tissue sections will be adequately processed and easily retrievable for embedding.



## A New Seminar-Workshop for Cytology and Histology Instructors

A Seminar-Workshop designed expressly for cytology and histology instructors will be held in early February, 1972, at the Charles S. Beardsley Research Center, Elkhart, Indiana, under the joint sponsorship of the Ames Company Technical Services Department and Lab-Tek Products. The two-day session will offer the latest innovations in teaching aids and technics designed to assist the instructor in teaching cell and tissue handling practices. Instead of being bogged-down with the examination and interpretation of stained cells or tissues, the Seminar-Workshop will focus on supplying the instructor with new ideas for teaching students the basic concepts of cell and tissue technology. Enrollment is limited, so reservations should be made early.

#### TEACHING CELL AND TISSUE TECHNOLOGY HANDLING PRACTICES SEMINAR AND WORKSHOP

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FOR INFORMATION OR RESERVATIONS, CONTACT: Alfred H. Free, Ph.D. Vice-President and Director, Technical Services Ames Company, Division Miles Laboratories, Inc. Elkhart, Indiana 46514 (219) 264-8696

## Remarks on the Birth of Histo-Logic

An Editorial

The birth of anything produces many side effects, one of which is a desire to express delight or dislike of that event. The birth of HISTO-LOGIC has produced expressions from

The birth of HISTO-LOGIC has produced expressions from many histologists throughout the country. Fortunately, these expressions have been of the delight type, and being only human we though it would be well to share them with you.

"I want to first say how happy I was to receive my first copy of HISTO-LOGIC. It's good to know that finally there's a technical publication devoted entirely to our profession. I want to say that your undertaking this publication should be congratulated. Again, I want to congratulate you on this fine endeavor!"

Rosalind F. Weiss Philadelphia, Pennsylvania 19125

"I was delighted upon receiving my first publication of HISTO-LOGIC. This is what the field of Histotechnology has needed for many years, and I shall do everything I can to contribute and support the bulletin via control slides and in any other way possible."

Josephine G. Frier West Palm Beach, Florida 33406

"May I also take this opportunity to tell you how much I enjoyed reading the first edition of HISTO-LOGIC. My best wishes for all future editions." Willa Mikowski

Traverse City, Michigan 49684

"Please let me first of all congratulate you on HISTO-LOGIC. I am very happy about its appearance and I am also very sure it will be a success and big help to many people. Thank you so much for your trouble and GOOD LUCK with HISTO-LOGIC."

Mrs. Ernestine Henkel Camp Hill, Pennsylvania 17011

"I would also like to congratulate you on the first issue of HISTO-LOGIC. I received my copy Tuesday and I was greatly impressed. The hints and procedures will be of great help to me. I am looking forward to the next issue. Thank you for your time and trouble." Wayne R. Kampa

Rochester, Minnesota 55901

"We read the announcement of the Symposium in the new publication HISTO-LOGIC which we found most interesting and one which should fulfill a very real need for technicians in the field of histology and pathology." Martha Q. Smythe Darby, Pennsylvania 19023

"This is to let you know that I have received my first copy of HISTO-LOGIC and I am happy to know that you have started the ball rolling for the unification and organizing of the Histopathology Technologists." Lena M. Cohen Denver, Colorado 80220

"The bulletin HISTO-LOGIC you are editing looks good. Thank you for your work in our field." Imogene R. Pettit Houston, Texas 77002 "Thanks for the histotechnology paper (newsletter). I think this paper is needed and appreciated by all histologists." Elaine Jensen Salt Lake City, Utah 84112

"Received the first copy of HISTO-LOGIC and it is just great! Thank you so very much for all your special efforts." Doris S. Castle Ashland, Kentucky 41101

"I would like to take this opportunity to commend and thank you for your part in the publishing of HISTO-LOGIC. Communication as you presented in July and your plans for the future issues is long overdue." Donald Hammer Minneapolis, Minnesota 55404

"I received my issue of HISTO-LOGIC, it is very well done, it will be of great value to our profession as a binding link." Herbert Stevens Madison Heights, Michigan 48071

"Allow me to congratulate you on the wonderful job you did on HISTO-LOGIC. You really got off to a flying start." Dominic L. Europa New York, N.Y. 10016

"I received a copy of HISTO-LOGIC. It is good to see a newsletter designed for Histo-Technologists." Edgar L. Kitchen Whittier, California 90602

"I was very pleased to receive the technical bulletin for histotechnology, HISTO-LOGIC. To find you as the editor is marvelous and I am sure that we all will gain from your editing the bulletin." Luise G. Bohacek Buffalo, New York 14223

"We have all enjoyed the bulletin here at our laboratory. I hope you are able to keep it going as we will be looking forward to it." Loretta Cole Flint, Michigan 48502

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"I enjoyed the first issue of HISTO-LOGIC, Best of luck." John Ashton Plainfield, New Jersey 07061

"I enjoyed the issue of HISTO-LOGIC very much. Thank you." E. Frances Freund Richmond, Virginia 23229

"It is with pleasure that I commend you upon the publication HISTO-LOGIC. This will be an invaluable aid in updating and establishing control standards." Jeroline C. Rice Greenville, South Carolina

"I congratulate you on your achievement as Editor of HISTO-LOGIC, a very helpful bulletin specifically for Histo-pathology laboratory." Harold Bolt Saginaw, Michigan 48603

"Congratulations on HISTO-LOGIC. This is a big step forward in the education of the Histotechnicians." Vivian McClure San Francisco, California 94110 "Thanks so much for your latest contribution to the world of Histology. Your new HISTO-LOGIC is simply terrific and I shall look forward to receiving each forthcoming copy. It's great!"

Grace K. Thompson Bay Pines, Florida

"Congratulations on your first issue of HISTO-LOGIC. I find it not only informative, but very useful in sales. Keep up the good work, and I wait with anticipation for the next issue." Sam Pavlak

Rochester, New York 14603

"Today I received my first copy of HISTO-LOGIC and I must admit that I am thrilled. A publication of this sort is certainly very much needed and your choice to place Mr. Luna in charge was in my humble opinion excellent. I am sure it will be widely appreciated. Good Luck!" Ernestine Henkel

Camp Hill, Pennsylvania

"I was very happy to receive your newsletter HISTO-LOGIC and am so glad you have printed this useful letter for us." Virginia Puffer Ogdensburg, New York 13669

"I have received my first copy of HISTO-LOGIC and wish to thank you for sending it. This is to let you know that I think this is an excellent idea." Viola Flanagan Kanaas City, Missouri 64108

"Thave enjoyed your first copy and found it very worthwhile." Katheryn Copeland Wichita, Kansas 67218

"This week my supervisor showed me a copy of your new technical bulletin, HISTO-LOGIC. I was very impressed with it, and pleased to see that a paper dealing specifically with our field is now available. Thank you and I look forward to receiving HISTO-LOGIC. Susan Weston

Gorham, Maine 04038

"I just read Vol. 1, No. 1, July, 1971 HISTO-LOGIC and think it is an excellent idea." Kathleen Johnston Denver, Colorado 80206

"The first volume and issue of the Technical Bulletin for Histotechnology was forwarded to me by John Gagliardi of Lab-Tek. Congratulations to you for accepting the position as editor for this publication. I am sure your stature in the field of histology will make this intra-professional communication a successful endeavor." Jack D. Closson

Manager Biomedical Products Curtin Scientific Company

"The first issue was quite impressive." Kenneth E. Lyle South Gate, California 90280

"I received your first copy of HISTO-LOGIC and 1 find it most informing and useful." L.J. Mason Bayside, New York 11361

"Have just seen your first issue of HISTO-LOGIC. Excellent!" L.O. Binder Bethesda, Maryland 20014 "Just want to let you know how much I liked my copy of HISTO-LOGIC and say that I would appreciate getting a copy each time it is printed." Kay Briggemdon Sacramento, California 95824

"Congratulations on your new publication HISTO-LOGIC! A better thing could not have happened for all histology technicians." Ralph Snyder New York, N.Y. 10021

"The sample copy of HISTO-LOGIC was very much appreciated and enjoyed. It has long been needed and has been long overdue." Mary Knight Atlanta, Georgia

"Volume I, No. 1 of HISTO-LOGIC, edited by Mr. Lee G. Luna, is quite an excellent publication." S.W. Kowierschke Bryan, Texas 77801

"I want to take this opportunity to express my appreciation to you for your sponsorship of the new publication HISTO-LOGIC. I received my first copy yesterday and enjoyed it very much. I know it will be a worthwhile endeavor and will make a great contribution to the profession of Histotechnology." Billie Swisher

Atlanta, Georgia 30312

"This is to acknowledge receipt in yesterday's mail of Vol. 1, No. 1, July 1971 of the new HISTO-LOGIC technical bulletin, and I must say that this is something that all of us who are working in histology laboratories need very much. I am very enthused over this bulletin as it will be a great help to me in my histology work." Marjorie Farlow

Napa, California 94558

"I was most pleased to receive the initial copy and felt that it was very well done. This is an instrument desperately needed in the field and I look forward to future editions. Thank you so much." Eleanor Oldham

Cleveland, Ohio

"I am the head of the histology department at St. Joseph's Hospital and just saw a copy of your HISTO-LOGIC bulletin. It is very well put together and long needed." Betty Rockower Tarrytown, New York

"Our laboratory has just seen a copy of HISTO-LOGIC. We find it to be an up-to-date and important bulletin for added information in the histology laboratory. Thank you and we hope you continue to edit an excellent paper." Sharon Taylor

Columbia, Missouri 65201

"Thank you and congratulations on your publication of HISTO-LOGIC. 1, for one, am most grateful for your foresight in giving the histology technician a newsletter that meets a nation-wide need." Vivian McChure San Francisco, California 94110

"I recently read your HISTO-LOGIC Technical Bulletin and really enjoyed it." Mary Madrid Houston, Texas 77002

## Better Paraffin Sections with the Aid of Vacuum

L. G. LUNA and E. F. BALLOU, M.T. (ASCP) Histopathology Laboratories, Armed Forces Institute of Pathology, Washington, D.C. 20305

The value of the use of vacuum in processing tissues has been recognized for about a decade. When used in fixation, processing time is reduced and more complete penetration is accomplished.<sup>1</sup> Again, use of the vacuum to de-gas the tissue during infiltration gives better impregnation with paraffin. Control of the pressure with a standard vacuum oven has proved to be satisfactory when using this procedure on whole organs or large pieces of bone.

Adapting this principle with the use of a simple, inexpensive unit similar to that described by Weiner,<sup>2</sup> the authors have found it possible to produce better sections of a variety of tissues, and they can be cut with less difficulty than those that are handled by conventional methods. The use of vacuum has, therefore, been adopted by this installation as a routine step in the processing of all types of tissues, including human and animal eyes, bone, lung, nerve, and other soft tissue.

The vacuum head consists of a one-half inch plastic disc about 6 inches in diameter with a central opening. On the under side of this disc is a rubber ring three-sixteenths of an inch thick and seven-eighths of an inch wide, placed flush with the outer edge of the disc, which acts as a gasket. Through the central opening is a brass cross joint, at each end of which is a gas stopcock. To one is attached the hose from the vacuum or motor pump, while the other serves as an air valve. This assembly can be used on any metal container of a size similar to a paraffin pot, which is standard equipment on most of the various tissue processoes.\*

Following the two paraffin baths usually employed by most technics, the basket of tissue is placed in a third paraffin bath, to which is attached the vacuum head described. Application of a moderate, even pressure is made and kept constant for about 1 hour. Tissue is then embedded in the usual manner.

After use of the vacuum, the tissue is found to be free of tiny air holes or bubbles frequently encountered in other embedded tissue. This reduces the tendency of the tissue to adhere to the cutting edge of the knife, to pull or tear as it contacts the knife edge through such holes, and to be only partially infiltrated with paraffin.

When "rush" reports are required the same day on which the material is received, it is possible to use the vacuum head to advantage while processing the tissue by hand. It speeds up both dehydration and impregnation. (Because of the deleterious effect of most clearing agents on both the rubber gasket and the plastic disc, the use of the vacuum head during the step of clearing is omitted.) The seeming loss of time at this point is compensated by the value of its use during the dehydration and impregnation steps respectively.

#### Summary

This installation has adapted a unit that it uses routinely to de-gas various kinds of tissue by means of vacuum during infiltration. The use of this unit as a regular step in processing accomplishes the following:

- 1. Reduces the total time of processing.
- 2. Gives more complete impregnation.
- Eliminates distortion of tissues that results from precutting the gross specimen for processing in order to get complete infiltration.
- Produces smoother sections without tears or holes.
- 5. Reduces time for sectioning blocked material.

The choice of plastic over glass for the disc has been made on the basis of its strength and durability, its lower cost, and the case with which it can be fabricated.

#### Bibliography

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- Weiner, S.: A Simplified Vacuum Embedder. Stain Technol., 32: 195-196, July, 1957.

\*Acknowledgment is given to Mr. R. F. Davis, Chief, Construction Branch, Scientific Illustration Division, Armed Forces Institute of Pathology.

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### Replies to: Can you help?

The editor's corner of HISTO-LOGIC, Vol. 1, No. 2, October, 1971, contained a query for which the following answers have been submitted.

"Whenever we receive a specimen of which the resection lines need to be identified in the sections, such as cone biopsy of cervix or a portion of skin, we rub the surgeon's resection line with a silver nitrate applicator. This is the same type as used clinically for the cautery of small haemorrhages such as nose bleeds. I am sure your Pharmacy Department will keep a supply of these applicators. (Silver nitrate solutions don't workas well. They would need to be about 70-80% and they dilute, contaminate or deteriorate rather quickly.) The silver nitrate can be applied to an unfixed, partially fixed or totally fixed specimen. After the application the specimen is returned to formalin which will result in reduction of the silver nitrate and deposition of very fine metallic silver at the resection line. Tissues can be processed and sectioned as usual. In the final section there will be no problem identifying the resection line as the silver deposit shows up clearly. This silver deposit is of a very fine nature and allows observation of histological details through the deposits."

J. Nyssen, B.A., R.T. University Hospital Saskatoon, Saskatchewan

Canada

"The use of India Ink has proven very unsatisfactory for the use of marking margins in the situations you mentioned in HISTO-LOGIC, Vol. 1, No. 2, as it tends to fade away and becomes fairly indistinguishable on the finished slide. In our laboratory, we have used "Labink" and have found it very distinguishable in the finished slides microscopically. It is also very useful for marking slides permanently. "Labink" is produced by the Kayes Scientific Corporation and is probably available from your local supply house."

Robert E. Williams

U.S. Public Health Service Hospital, H.E.W. P.O. Box 3145

Seattle, Washington 98114

"In my laboratory I have had your problem of not being able to identify surfaces that we wish to examine microscopically. I use mercurochrome, using a glass rod, and touching only the area that is to be identified. Mercurochrome will stain the area touched and will not wash out in the fixation or the dehydration process."

E. Louise Russell The University of Tennessee Agricultural Research Laboratory Oak Ridge, Tennessee 37832 JIDOJ-@LSIH

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# **Editor's Corner**

Second Annual Midwest Histology Conference Histopathologic Technique, March 25, 1972, University of Iowa, Iowa City, Iowa, For information contact Mr. James A. Raney, University of Iowa, 133 Medical Laboratory Bldg., Iowa City, Iowa 52240.

Virginia Society of Histology Technicians will meet April 21-22, 1972, Portsmouth, Virginia. For information contact Patricia K. Harlow, 113 Shore Drive, Portsmouth, Virginia 23710.

Southwestern Pennsylvania Histology Society will meet May 24, 25 and 26, 1972, in Pittsburgh, Pa. For information contact Mrs. Elaine Kurtz, P.O. Box 26, Munhall, Pennsylvania 15120.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, Box 552, Hyattsville, Maryland 20782. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.

To receive your own personal copy of HISTO-LOGIC, or to have an associate added to the mailing list, write: Lab-Tek Products, Division Miles Laboratories, Inc., 39 E. Burlington, Westmont, Illinois 60559.