HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor — A Technical Bulletin for Histotechnology — Vol II No. 3 - July, 1972

Please Note:
Beginning with this issue, HISTO-LOGIC will be numbered in series. The first 4 issues contained a total of 16 pages which is the reason for this issue beginning with page 17.

Additional Information on Formalin Fixation and H&E Staining

An Editorial

The following remarks were received as supplements to "Artifacts Produced During Tissue Fixation" and "Hematoxylin and Eosin Staining–Problems and Solutions." The editorials appeared in HISTO-LOGIC, Vol. I, No. 2, 1971. The editor is particularly pleased with the contribution from our Canadian friend. A great deal can be gained by an exchange of information between histologists in the U.S.A. and Canada. Contributions from our Canadian colleagues are welcomed.

Explosive Properties of Calcium Carbonate

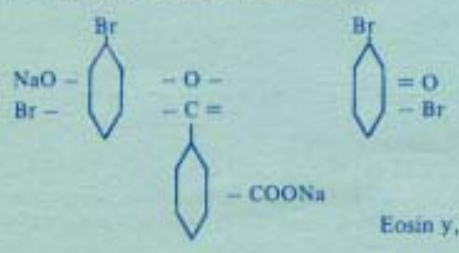
T.A. Morris, F.O.M.L.T., A.I.S.T., F.R.M.S.
The University of Calgary
Calgary, Alberta, Canada

"... I think the dangers of using magnesium carbonate to neutralize formalin, particularly in concentrated form, should be pointed out. If the formalin has broken down to formic acid, CO_2 gas is generated, and, if kept in a closed container will obtain quite a high pressure. A report of a serious explosion due to treating concentrated acid formalin with magnesium carbonate appeared in the Gazette of Inst. Med. Lab. Technology, 1965."
(ED. NOTE: Attempts will be made to include this report in a later edition of HISTO-LOGIC.)

The Stabilization of Eosin Solutions

Eosin, as a staining solution, is known to deteriorate rapidly and requires progressively longer staining time and shorter differentiation. Several methods have been established to overcome this problem; i.e., addition of acetic acid or Lugol's Iodine. However, both methods tend to produce a staining solution which is very difficult to differentiate from the section.

Some years ago while discussing the problem, the late G.T. Gurr offered the following explanation:



Eosin y, C.I. 45380

Solutions of eosin absorb atmospheric carbon dioxide. CO_2 reacts with NaO & COONa radicals on the dye molecule yielding sodium carbonate and thereby blocking the auxochrome. Glacial acetic acid and Lugol's Iodine split the bond. The addition of calcium chloride to an eosin solution provides a compound for binding CO_2 which is more reactive than the dye molecule. An easier remedy is to make the eosin solution in tap water. Hard water renders better results.

The procedure adopted by the author for preparing eosin is as follows:

1. Prepare 1 or 2 liters of 1% eosin in tap water.
2. Filter required amount into staining jar.
   Use for not more than one week, depending on slides stained.
3. Return used stain to stock mixture and refilter before reuse. (It is possibly an old wives' tale, but I do like to have a mold growing in the stock bottle, although I don't think it makes any difference.)

A current lot of eosin has rendered consistent staining/differentiation time with reproducible results for approximately fourteen months. Naturally, sections should be examined microscopically for staining quality.

Fixation Improvement on the Technicon ULTRA[1]

Richard F. Fiester, M.D.
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Milwaukee, Wisconsin 53215

A number of problems with tissue fixation have been encountered after processing surgical specimens on the Technicon ULTRA, with a four hour disc ("clock").

Thirty minutes in a modified formol-alcohol fixative has improved the results considerably. Fresh unfixed tissue is trimmed, as close to 3 mm in thickness as possible, and immersed in formol-alcohol solution on the ULTRA. The cycle is altered to allow for a thirty minute fixing interval.

Modified Formol-Alcohol Fixative

Phosphate-buffered 10% neutral formalin[2]	500.0 ml
Formaldehyde, full strength	250.0 ml
Alcohol, ethyl 95%	250.0 ml

(A pH of 7.1 is obtained.)

After literally hundreds of specimens, there are no difficulties and the sections are superior in quality to previous specimen.

References

1. Fiester, R.F.: Reagent Improvement for Auto-technicon-ultra. Summary Report, *ASCP, 8:* No. 2, 1971.
2. Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., New York, McGraw-Hill Book Co., Blakiston Division, p. 3, 1968.

The Vacuum Infiltrator In Routine Fixation

A Preliminary Report

Therese R. Ansman, M.S.
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Abstract

A report is made on the use of heat and vacuum in the fixation of routine histopathology specimens. A simple and relatively inexpensive method for quality control of the fixation process is described.

Introduction

One of the problems which plagues both pathologist and histologic technician is the specimen which arrives from surgery after the tissue processor has begun its cycle. Since most tissue processors are set for a four-hour fixation period, a similar problem occurs if there is an unusually large number of surgical specimens, or if the pathologist is late starting the gross description. Since these specimens are subjected to a shortened, inadequate fixation period, the result is poor processing, poorer sections, and frustration.

It is well known that the rate of fixation varies directly with the temperature of the fixative,[1,2] so heat was first considered as a solution to the problem. It was also hoped that the staining qualities would be enhanced since at room temperatures "relatively little use is made of the capacity of formalin to form addition compounds and bridges."[3] Although Combs[4] concluded that fixation was most often good at 30° C or 75° C, it was decided not to exceed 60° C since the higher temperatures "hasten autolysis, involve heat coagulation, and speed the loss of formaldehyde, through volatilization."[2] At the lower temperatures, time was still a factor to be considered. As early as the 1800's Francotte[5] and Hoffmann[6] showed that the addition of vacuum speeded fluid exchange and shortened processing time; so it was decided to combine heat and vacuum.

The ideal solution seemed to be a rapid tissue processor which included these features. However, the initial cost of this type of processor is prohibitive to many laboratories; and even with the heat and vacuum processor available, the problem of the late-arriving specimen is not completely overcome. Too many pathologists consider the usual four-hour fixation period in overnight processing not as a necessary step, but more as a "grace period" to give them time to complete the gross description. Thus, it was decided to use the relatively inexpensive Vacuum Infiltrator (manufactured by Lab-Tek Products, Westmont, Illinois). This unit is compact and can be placed at the cutting bench during the gross description.

Materials

Two LAB-TEK® TISSUE-TEK II Vacuum Infiltrators
Vacuum pump or vacuum source
Routine pathology specimens

Methods

Method #1 – At the beginning of the study, two adjacent tissue sections were cut at the time of gross description. One group was placed in formalin at room temperature in the routine manner. The others were placed in the basket in the Vacuum Infiltrator containing formalin heated to 43° C. At the end of the gross description, the tissues in the Infiltrator were subjected to vacuum for 15 minutes and then placed on the tissue processor at the same time as the routine specimens. The second Vacuum Infiltrator was used for paraffin infiltration at the end of processing on half of the routinely handled specimens. The tissues were then blocked in Paraplast® (a product of Sherwood Medical Industries, Inc.) using the TISSUE-TEK® Rings (Lab-Tek Products) and TISSUE-TEK® Base Molds (Lab-Tek Products) in the manner described in a previous paper.[7]

Method #2 – All surgical specimens were placed in the Vacuum Infiltrator containing heated formalin as soon as they were sectioned and left in the heated formalin until the basket was filled. They were then transferred to formalin at room temperature until all specimens had been grossed earlier in the day. The tissues were then processed, vacuum infiltrated with paraffin, and blocked as in Method #1. The temperature of the formalin was varied to determine the optimum temperature for fixation.

Results

Method #1 – There were no grossly apparent differences noted in the four groups of slides. They appeared very much the same as those produced from any well-fixed, well-processed tissue. Differences *were* noted in the *ease of sectioning*. The tissues treated with heat and vacuum for *both* fixation and paraffin infiltration produced slightly better sections and were much less difficult to section than those produced by the standard method. This was particularly true with tough tissues such as uterus and cervix.

Method #2 – The greatest differences were noted in fibro-fatty tissues such as breast, and again in the tougher tissues. Even when the fixation period on the tissue processor was shortened to as little as one hour or less, the tissue was adequately fixed and the quality of the slides unimpaired. This was especially notable when these specimens were compared to previous specimens that had a shortened period of fixation. The best results in sectioning were obtained when the formalin was heated to 54° C.

Discussion

A vacuum of 15 inches for a period of 15 minutes is recommended for the Vacuum Infiltrator. At our laboratory, the vacuum source averages 14 inches. Since the primary purpose of this work was to find an inexpensive and simple way to provide quality control in fixation, it was decided to not request a vacuum regulator since this would add to the initial cost.

Method #1 was postponed until a later date, since it was felt that more controlled conditions and more intensive investigation are needed to determine the effects on the staining qualities of the sections, the optimum temperature, and optimum vacuum. Method #2 was continued, and forms the basis of this preliminary report. The Vacuum Infiltrators are the only equipment required, and no special training is needed for their operation. Method #2 has proven to be so effective that it is now routine procedure in our laboratory and assures us of quality control in tissue fixation.

Acknowledgment

The author wishes to thank Mr. John Gagliardi and Lab-Tek Products for the equipment and encouragement furnished by them.

References

1. Dempster, W.T.: Rates of Penetration of Fixing Fluids. *Amer. J. Anat., 107*:59-72, 1960.
2. Lillie, R.D.: Histopathologic Technic and Practical Histochemistry. McGraw-Hill, Inc., New York, 1965.
3. Pearse, A.G.E.: Histochemistry Theoretical and Applied. Little, Brown and Company, Boston, 1960.
4. Combs, R.M.: Hyperthemia as a Factor in Cytological Fixation of the Oyster Mantle. *Tr. Amer. Micr. Soc., 80*:400-14, 1961.
5. Francotte: *Bull. Soc. Belg. Mic.*, p. 45, (1884). (Quoted in Bolles-Lee: *The Microtomist's Vade-Mecum*. The Blakiston Co., Philadelphia, 1946).
6. Hoffmann: *Zool. Anz.*, p. 230. (Quoted in Bolles-Lee, ibid.)
7. Ansman, T.R.: Blocking Procedures in Histopathological Technique. *Amer. J. Med. Tech.*, March-April, 1967.

AFIP Symposium

The 8th Annual Symposium on Histopathologic Techniques will be conducted by the Armed Forces Institute of Pathology from October 2-6, 1972. Listed below are the titles and faculty members for the scientific sessions and workshops. Your attention is directed to the Thursday evening activity which will consist of a banquet and complimentary cocktails by Lab-Tek Products, Naperville, Illinois.

Preliminary programs were mailed in June. *If you have not received a preliminary program and would like one, please write:* The Director, Armed Forces Institute of Pathology, Washington, D. C., 20305, ATTN: Lee G. Luna, Room 2074.

Workshops

WORKSHOP NO. 1: MONDAY, OCTOBER 2, 1972
STAIN TECHNOLOGY: SELECTED SPECIAL STAINING TECHNIQUES
Mr. B. Wenger

WORKSHOP NO. 2: MONDAY, OCTOBER 2, 1972
RESULTS ORIENTED MANAGEMENT FOR HISTOLOGY SUPERVISORS
Mr. R. Rike

WORKSHOP NO. 3: TUESDAY, OCTOBER 3, 1972
TISSUE IDENTIFICATION
Mrs. E. Prophet

WORKSHOP NO. 4: WEDNESDAY, OCTOBER 4, 1972
TO BE ANNOUNCED
Colonel C. Goings

WORKSHOP NO. 5: WEDNESDAY, OCTOBER 4, 1972
CRYOTOMY (CRYOSTAT TECHNOLOGY)
Mr. E. Cunningham

WORKSHOP NO. 6: WEDNESDAY, OCTOBER 4, 1972
MICROTOMY (TISSUE SECTIONING)
Mr. K. Russell

Thursday evening, October 5, there will be a Histopathology Symposium Banquet at 7:30 P.M. The guest speaker for the banquet will be Vice Admiral G. M. Davis, Medical Corps, United States Navy Surgeon General, United States Navy.

Preceding the banquet Lab-Tek Products, Naperville, Illinois, will sponsor complimentary cocktails from 6:00 - 7:30 P.M.

Scientific Sessions

WEDNESDAY, OCTOBER 4, 1972:

HISTOCHEMISTRY TODAY
Mr. Pickett

METHODOLOGY IN THE USE OF GLUTARALDEHYDE FIXATION IN ROUTINE HISTOPATHOLOGY
Mr. Slocum

CELL BLOCK PREPARATIONS
Mrs. Hannon

UNDERSTANDING SPECIAL STAINS
Mr. Raney

REAPPROPRIATION OF SPACE FOR LABORATORY USE: PLANNING AND EQUIPPING
Mr. Brown

IMPROVED STAINS FOR SEX CHROMATIN
Mr. Romieka

LESIONS OF ELASTIC TISSUE
Dr. Budinger

KIDNEY BIOPSY METHODOLOGY AND BASEMENT MEMBRANE STAINING
Mr. Avallone

METHOD OF TEACHING PRINCIPLES AND MECHANICS OF SPECIAL STAINS
Mr. Murdock

HISTOCHEMISTRY
Dr. Johnson

THURSDAY, OCTOBER 5, 1972:

IMPROVED TEACHING TECHNIQUES FOR ADVANCED HISTOTECHNOLOGY
Mr. Haas

HISTOCHEMISTRY OF THE HEMATOXYLIN AND EOSIN STAIN
Mr. Elias

ADVANCES IN SCANNING AND TRANSMISSION OF ELECTRON MICROSCOPY
Dr. Fawcett

FAT STAINING METHOD FOR OIL SOLUBLE AZO DYES
Miss Winkler

METALLIC IMPREGNATION STAINS FOR C.N.S. TISSUE
Mr. Europa

LABORATORY SAFETY
Mr. Russell

FRIDAY, OCTOBER 6, 1972:

A SIMPLE FIXATION-DECALCIFICATION AND IRON STAINING TECHNIQUE FOR BONE MARROW SPECIMENS
Mr. Kovalchek

THE MANY USES OF ATTWOOD'S METHOD FOR AMNIOTIC FLUID EMBOLISM
Mr. Kiel

INFECTION HAZARDS IN THE HISTOPATHOLOGY LABORATORY
Dr. Zimmerman

SLIDES FOR TEACHING
Dr. Besant-Matthew

ENZYME HISTOCHEMICAL AND HISTOLOGIC PROCEDURES IN THE EVALUATION OF MATERIALS FOR SURGICAL IMPLANTATION
Mr. Salthouse

REGRESSIVE VS. PROGRESSIVE HEMATOXYLIN STAINING
Mr. Womack

A NEW STAINING PROCEDURE FOR THE DETECTION OF EARLY MYOCARDIAL ISCHEMIA
Mr. Kampa

FRIDAY, OCTOBER 6, 1972:

HISTOQUICKIES

AUTOMATION IN ROUTINE TISSUE STAINING
Dr. Ceremsak

AMYLOID
Mr. Haas

OBTAINING AND PROCESSING UNIMPLANTED EMBRYOS
Mrs. Russell

THE IMPORTANCE OF SELECTIVE STAINING OF CURIOUS BODIES ASSOCIATED WITH SARCOID LESIONS
Mr. Carter

SUCCESS OR FAILURE – HISTOLOGICALLY SPEAKING
Mr. Luna

Teaching – Seminar – Workshop

A seminar workshop on teaching various disciplines of the clinical laboratory is being sponsored in the Fall of 1972 by Ames Company and Lab-Tek, Divisions of Miles Labs., Inc. This Seminar Workshop is:

Cytological and Histological Teaching Technique
December 1–2, 1972

The Seminar Workshop will be held in Elkhart, Indiana and will begin on Friday morning at 9:00 a.m. and end at noon on Saturday. The registration fee for each person is $25 and this includes all teaching materials, the Friday luncheon, a reception, and a banquet and a continental breakfast on Saturday. This program is specifically geared for those involved in teaching.

For further information contact:

Dr. Alfred H. Free, Vice-President
Technical Services & Scientific Relations
Ames Company, Div. Miles Labs., Inc.
1127 Myrtle Street
Elkhart, Indiana 46514
Area Code (219) 262-7339

Gomori's Trichrome for Staining E. Histolytica

Edgar L. Kitchen
Senior Histotechnologist
Presbyterian Intercommunity Hospital
Whittier, California 90602

Gomori's[1] one step trichrome has been used in our laboratories to stain Endoamoeba histolytica in paraffin embedded sections. It has proven more valuable than iron hematoxylin because E. histolytica is more distinct. The stain produces a variety of colors; staining amoeba internal structures gray to blue, and ingested erythrocytes red. Conversely, the iron hematoxylin stains everything blue-black.

Fixation
Phosphate-buffered 10% neutral formalin or Zenker's.

Microtomy
Paraffin sections at 6 microns.

Solutions

Gomori Trichrome

Chromotrope 2R	0.6 gm
Light green	0.3 gm
Glacial acetic acid	1.0 ml
Phosphotungstic acid	0.8 gm
Distilled water	100.0 ml

0.5% Acetic Acid

Glacial acetic acid	0.5 ml
Distilled water	100.0 ml

Staining Procedure

1. Decerate to distilled water.
2. Mordant in Bouin's[2] solution at 56° C for 1 hour.
3. Wash in running tap water until yellow disappears.
4. Stain in Weigert's hematoxylin[2] for 10 minutes.
5. Wash in running tap water for 15 minutes.
6. Stain in Gomori's trichrome for 15 minutes.
7. Place in distilled water for 60 seconds.
8. Differentiate in 0.5% acetic acid water for 5 minutes.
9. Place in distilled water for 2 minutes.
10. 95% ethyl alcohol, 2 changes 1 minute each.
11. 100% ethyl alcohol, 2 changes 1 minute each.
12. Xylene, 3 changes 2 minutes each.
13. Coverslip.

Results

Muscle	Red
Collagen	Green
Nuclei	Blue to black
Amoeba capsule	Red
Amoeba cytoplasm	Grayish green
Erythrocytes	Red

References

1. Gomori, G.: A Rapid One-step Trichrome Stain. *Amer. J. Clin. Path., 20:* 661-664, 1950.
2. Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd. ed., New York, McGraw-Hill Book Co., Blakiston Division, 1968.

Did You Know?

An Editorial

. . . that the demonstration of hyaluronic acid with alcian blue or colloidal iron may be difficult or impossible after Bouin's or Zenker's fixation.

. . . that more instrumentation for the histology laboratory has been developed during the last two decades than during the previous three hundred years.

. . . that formaldehyde was discovered by Mikhailovich Butlerov in 1859.

. . . that the first recorded use of formaldehyde as a tissue fixing agent was in 1893 by F.Z. Blum.

. . . that the first recorded use of a tissue embedding matrix was in 1873 by Flemming, who embedded in soap. He later embedded in paraffin-turpentine (1876).

. . . that many histologists consider the terms decolorize and differentiate synonymous. They do not mean the same in histology: *to decolorize* is to remove excess dye from tissue, while *to differentiate* means to distinguish various tissue components.

. . . that the first recorded use of celloidin dates back to 1879 when Duval used it as an embedding matrix.

. . . that animal tissue is more difficult to section than human tissue. (Additional comments will be made on this subject in a future edition of HISTO-LOGIC.)

. . . that some biological dyes can be purchased with a wide range of dye contents. This variation can affect tissue staining in some procedures.

. . . that Roberta Mosedale is associate editor of HISTO-LOGIC and is responsible for all secretarial work connected with the newsletter.

. . . that BIOLOID (the recommended embedding media for eye globes) is available, and may be purchased from VWR Scientific.

A Technical Bulletin for Histotechnology Vol II No. 3

HISTO-LOGIC T.M.

LTM 21-771 Printed in the U.S.A.

It's easy to see...

why our newly improved TISSUE-TEK® II Cassette makes tissue processing and embedding more efficient:

- in keeping with the most advanced histologic techniques, the new style Cassette can be used with any processor
- the new transparency allows you to see the tissue through the Cassette for better positioning
- the see-through feature lets you detect air bubbles or debris which could cause confusing artifacts in the section

In ordering the new-style Cassette, use the same #4191.

The new Cassette utilizes the same metal cover #4194.

In order to write on the new Cassette, a soft-leaded art pencil is recommended.

NOTE: Tissue-Tek II Processing/Embedding Cassette is a plastic "disposable" designed for one-time use. Exposure to chloroform and/or molten paraffin contaminated with an excess of any clearing agent may cause Cassette distortion.

Editor's Corner

"Good Histology Workshop"

The American Society of Clinical Pathologists will present a one day "Good Histology Workshop" in San Francisco, California, during the week of October 16, 1972. For information contact: The American Society of Clinical Pathologists, 1300 West Harrison Street, Chicago, Illinois 60612.

Chicago Histology Conference

The Chicago Histology Society will present its Fifth Annual Conference on Histologic Technique on September 8,9, 1972 at St. Joseph Mercy Medical Center in Aurora, Illinois. For information contact: Mr. Kenneth H. Urban, 2141 N. Magnolia Avenue, Chicago, Illinois 60614

Northern California Histology Seminar

The Northern California Society of Histopathology Technologists will conduct an all-day Histology Seminar on Saturday, October 21, 1972, during the *33rd Annual Convention of the California Association of Medical Laboratory Technicians* meeting at the St. Francis Hotel in San Francisco on October 18-21.

For information contact: Mrs. Vivian McClure, Department of Pathology, San Francisco General Hospital, 1001 Potrero Avenue, San Francisco, California 94110.

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., 30W475 N. Aurora Rd., Naperville, Illinois 60540.