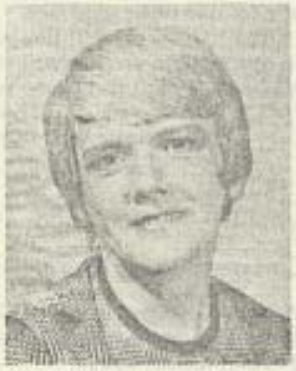
HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor — A Technical Bulletin for Histotechnology — Vol. IV, No. 3 - July, 1974

Announcement



We are pleased to announce that Therese R. Ansman, M.S., Chief Histologic Technician at Hillcrest Medical Center, Tulsa, Oklahoma, will be the recipient of the Golden Forceps Award for an original paper in histopathology. Her paper, "The Vacuum Infiltrator in Routine Fixation, A Preliminary Report," was selected from articles submitted to HISTO-LOGIC. Criteria for selecting the winning paper were originality, clarity, and scientific contribution.

Mrs. Ansman will attend the symposium on Histopathologic Techniques to be held from October 7 to 11, 1974 in Silver Spring, Maryland. Lee G. Luna, D. Lit., H.T. (ASCP) and editor of HISTO-LOGIC, will present her with the Golden Forceps Award.

The Vacuum Infiltrator In Routine Fixation

A Preliminary Report

Therese R. Ansman, M.S.
Chief Histologic Technician
Hillcrest Medical Center
Tulsa, Oklahoma 74104

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 a relatively inexpensive Vacuum Infiltrator.* This unit is compact and can be placed at the cutting bench during the gross description.

Materials

Two 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 and TISSUE-TEK® Base Molds* 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, vaccum 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 fibrofatty 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.

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.

*Lab-Tek Products

Improved Technique in Cell Block Preparation

Bonny A. Solie
Holy Cross Hospital
Salt Lake City, Utah 84102

Preparation of cell blocks has always presented a problem to the histologist. It is difficult to remove the centrifuged sediment from the tube and transfer the intact cell aggregate to a cassette.

I have found the following method a means of solving this problem. If this method is followed, one can easily remove a clotted cell block from a centrifuge tube and have it ready for subsequent processing with very little effort.

Procedure

Centrifuge the specimen at 2,000 RPM for 10 minutes and carefully decant as much of the supernatant as possible.

Add to the sediment:
3 drops of fresh normal plasma. Mix gently with wooden applicator stick.
3 drops of thromboplastin (e.g. Simplastin).
3 drops of .025M $CaCl_2$
Again mix gently with wooden applicator stick.
Let stand for 5 minutes.

Place centrifuge tube upside down on filter paper. (Use the type of paper you normally use to wrap small tissue specimens. I do not recommend using any type of foam pad.) Clot will slide easily from tube.

Wrap clot in filter paper and place in cassette in 10% formalin. Dehydrate, clear and impregnate with paraffin as you would any routine tissue specimen.

The normal plasma, thromboplastin and .025M $CaCl_2$ used in this method are easily obtained from the hematology or coagulation department of the laboratory.

NOTE: Refrigerate all reagents used in this method. The normal plasma and thromboplastin will have expiration dates.

Gaines' Iron Reaction

William K. Gaines, HT (ASCP)
Veterans Administration Hospital
Topeka, Kansas 66622

The unique iron reaction presented below was developed to provide an easy method for demonstrating iron in tissue sections. The method is economical since less than one-fourth of a given solution is used to stain fifty or more slides.

More than 500 suspected cases of iron in tissue have been compared with Gomori's and Perl's reactions over the last 2½ years. The only difference seen microscopically was that Gomori's stained slides appeared to be a slightly deeper shade of blue.

Of the more than 500 cases studied, only four were negative with this procedure and positive by Gomori's reaction.

Materials Required for Preparing Impregnated Strips

1. Filter paper, #110 mm (Cut into strips 24 x 74 mm), 50-70 strips.
2. 400 ml beaker (chemically clean).
3. 50 ml graduate (chemically clean).
4. Paraffin coated forceps.
5. Glass plate measuring 1½" x 1½" (chemically clean).
6. Large mouth jar with top (chemically clean).

Method for Preparing Strips

16% Potassium Ferrocyanide

Potassium ferrocyanide	8 gm
Distilled water	50 ml

1. Dip filter paper strips in potassium ferrocyanide solution until completely wet; 1 to 2 minutes.
2. Place strips on glass plate singly with the aid of paraffin-coated forceps. A small amount of remaining solution should be poured over strips.
3. Place plate containing strips in 58°-65°C oven until strips are completely dry.
4. Store impregnated strips in large mouthed jar and keep jar closed tightly.

Fixation 10% buffered neutral formalin.
Microtomy Cut paraffin sections at 6 micra.
Solution

20% Hydrochloric Acid

Hydrochloric acid	20 ml
Distilled water	80 ml

Unused solution may be stored.

Staining Procedure

1. Decerate and hydrate slides and iron control to distilled water.
2. Place slide on staining rack (tissue up) covered with parafilm. Place one impregnated strip on each slide.
3. Pipette 0.5 ml distilled water onto each slide.
4. Pipette 1 ml of 20% HCl on each slide and leave for 30 minutes.
5. Remove strips, with forceps, rinse slide with distilled water.
6. Counterstain in alcoholic eosin 2 to 3 dips.
7. Dehydrate in 95% absolute alcohol, 2 changes each.
8. Clear in xylene, 3 changes.
9. Mount coverslip with resinous media.

Results Iron –blue
Cytoplasm –pink

Remarks The impregnated strips are stable for 3 to 4 months if kept in a tightly stoppered container.

Improving the Method for the Demonstration of Adenosine Triphosphatase

John P. Koski, HT (ASCP)
University of Michigan Medical Center
Ann Arbor, Michigan 48104

Khan,[1] et al., have recently described a method for the demonstration of myosin ATPase which employs a paraformaldehyde-pyrophosphate fixative. Although the method works well, especially when using the fixative recommended, we have found that a slight modification of the incubating medium produces equivalent results, is easier to prepare, and easier to develop during the exposure to ammonium sulfide.

The addition of gelatin to the medium prevents diffusion of hydrolyzed phosphate in the tissue sections. Gelatin, however, cannot be well used in media at room temperature since it tends to solidify the medium. Sodium hydroxide is added to the solution to adjust the pH; however the viscosity of a gelatin solution prevents rapid mixing of the sodium hydroxide. The adenosine triphosphate is precipitated in the solution, thus decreasing the concentration of available substrate.

Adding polyvinylpyrrolidone (PVP) to the solution in a concentration of 60 mg/ml of medium prevents these problems. Polyvinylpyrrolidone (MW 10,000) is extremely soluble in water. When the section is rinsed after incubation, PVP aids in rapidly clearing unhydrolyzed substrate from the section and at the same time does not remove the calcium hydroxyapatite complex.

The modified medium will keep well in the refrigerator for two weeks with little change in reaction quality. After two weeks, the solution is filtered, 100 mg of adenosine triphosphate is added, and the pH checked.

A serial section is used to demonstrate nonspecific alkaline phosphatase for comparison since it is well known that nonspecific alkaline phosphatase occurs perinuclear in most organs, and in small blood vessels.

Sections which were not fixed with the recommended fixative showed ATPase activity. However, they required longer times of incubation. In liver, we have found that 2% glutaraldehyde fixation (pH 7.4) diminished hepatocyte ATPase. Activity was seen well in bile canaliculi.

Adenosine triphosphatase is also a useful tool for identifying fiber types in muscle biopsies. Most fiber atrophies affect type II fibers (those which use the glucose-glycogen system for energy). However, several cases of type II selective atrophy have been found. (Type I fibers use the Krebs cycle for energy.) The ATPase reaction is useful for distinguishing them.

Reference

1. Khan, M. A.; Papidimitriou, J. M.; Holt, P. C. S., and Kakulas, B. A.: A calcium citro-phosphate technique for the histochemical localization of myosin ATPase. Stain Technol., *47*:277-281, 1972.

Eliminating Cytoplasmic Staining in Sections Stained with Weigert's Iron Hematoxylin

Charles J. Churukian
University of Rochester, School of Medicine
Rochester, New York 14642

Because of its resistance to the action of various staining solutions, Weigert's iron hematoxylin[1,3] is used extensively as a nuclear stain in many special stain methods. Among these are Mayer's mucicarmine, Van Gieson's[1,3] connective tissue, and the trichrome methods of Masson[2] and Gomori.[4]

Weigert's iron hematoxylin can be used as either a progressive or a regressive stain, depending on the special stain or modification of the special stain being performed. When used as a progressive stain there is a tendency for a bluish or blackish gray precipitate, or sheen, to form on both the microslide and tissue section. This staining artifact is more likely to occur if an excess of gelatin is used as the adhesive in the flotation bath. The precipitate is not likely to occur in regressive staining if the section is differentiated in some acid solution, or if acids are contained in staining solution, as in Gomori's trichrome.

Cytoplasmic staining due to Weigert's iron hematoxylin can be removed by the following method:

1. Stain with Weigert's iron hematoxylin for the usual time.
2. Wash well with distilled water.
3. Differentiate cytoplasm in 0.5% HCl in 70% ethyl alcohol for 10 seconds.
4. Wash thoroughly with tap water and rinse in distilled water.
5. Proceed with staining procedure.

Sections treated in this manner have a clearer, cleaner appearance, and there is no noticeable effect upon the staining quality of subsequent special stain performed.

References

1. Brown G. B.: Primer of Histopathologic Technique. Appleton-Century-Crofts, Educational Division, Meredith Corp., New York, 1969.
2. Masson, P. J.: Some histological methods. Trichrome stainings and their preliminary techniques. J. Tech. Meth., *12*:75, 1929.
3. 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.
4. Gomori, G.: A rapid one-step trichrome stain. Am. J. Clin. Path., *20*:661, 1950.

A Technical Bulletin for Histotechnology Vol. IV, No. 3 - July, 1974 LTM 21-771 Printed in the U.S.A.

HISTO-LOGIC™

Editor's Corner

California Histologic Symposium

The California Histology Society will sponsor a Histologic Symposium on November 15, 16, and 17. For information contact: Mr. Irwin Haas, UCLA Health/Science Center, Surgical Pathology B-3249, Los Angeles, California 90024.

Massachusetts Histotechnology Seminar

The Massachusetts Society for Histotechnology is presenting its first Statewide Meeting and Scientific Seminar on October 26-28, 1974, at the Sheraton Regal Inn, Cape Cod, Hyannis, Massachusetts. For information contact Scientific Chairman: Ms. Norma King, Beth Israel Hospital, Pathology Department, 330 Brookline Avenue, Boston, Massachusetts 02215.

Minnesota Histotechnologist Conference

The Minnesota Society of Histotechnologists will hold its Third Annual Conference on Histopathological Techniques November 13-16, 1974, at the Sheraton-Ritz Hotel, Minneapolis, Minnesota. Principal speakers are Mr. Lee Luna and Mr. Dominic Europa. For information contact: Mr. Donald Hammer, 12805 13th Avenue North, Minneapolis, Minnesota 55441.

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.

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.

HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor A Technical Bulletin for Histotechnology Vol. IV, No. 3 - July, 1974

Addendum

Attention - State Histology Societies

This year was a very important first for the histotechnologist since we now have an incorporated, viable society. Another important first is the combined National Convention/Symposium which will be held in Silver Spring, Maryland. AT THIS MEETING VERY IMPORTANT DECISIONS CONCERNING THE FUTURE OF THE NATIONAL CONVENTION will be made. However, we would like all members and potential members to become involved in policy-making decisions. This, of course, requires a great deal of exchange of information. We are most anxious to keep everyone informed. We are therefore requesting all state societies or groups of histotechnologists interested in receiving a copy of the business meeting agenda, to send the name and mailing address of one representative they wish to receive this information. The representative can then disseminate the information to their own state or group membership. Please send name and address to:

Dominic L. Europa
Department of Pathology
Bellevue Hospital Center
400 E. 29th Street
New York, N. Y. 10016

National Convention and Symposium on Histopathologic Techniques

This is a reminder that The First Convention of the National Society for Histotechnology and the Tenth Annual Symposium on Histopathologic Techniques will be conducted at the Sheraton and Holiday Inn, Silver Spring, Maryland. The dates for this year's program are October 7-11, 1974.

Registration Application: The registration information appears in HISTO-LOGIC, Vol. IV, No. 3-July, 1974. Registration for scientific sessions is limited to 600. We request that you file your registrations by September 15th. Late applications, however, will be accepted provided the quota has not been filled.

Registration forms may be photocopied if more than one individual from the same activity wishes to attend. Mail registration and check to Registrar, P.O. Box 36, Lanham, Maryland 20801.

DO NOT USE THIS SPACE

NAME _____
Last First Initial (Mr,Mrs,Miss,Dr, Mil Rank)

HOME ADDRESS _____
Street City State Zip

EMPLOYED BY _____

PLEASE CHECK FUNCTIONS YOU DESIRE TO ATTEND:

SCIENTIFIC SESSIONS_____ $30.00 (Wed,Thur,Fri)

BANQUET_____ $12.00 (Thursday Evening)

WORKSHOPS

No. 1	–	$20.00	(Monday)	No. 5	–	$20.00	(Tuesday)
No. 2	–	$20.00	(Monday)	No. 6	–	$20.00	(Tuesday)
No. 3	–	$20.00	(Monday)	No. 7	–	$20.00	(Tuesday)
No. 4	–	$20.00	(Tuesday)	No. 8	–	$20.00	(Tuesday)

CHECK OR MONEY ORDER MUST ACCOMPANY REGISTRATION APPLICATION. MAKE PAYABLE TO: V.S.H.T.

MAIL REGISTRATION TO: REGISTRAR, P.O. BOX 36, LANHAM, MARYLAND 20801.

A Technical Bulletin for Histotechnology Vol. IV, No. 3 - July, 1974 LTM 21-771 Printed in the U.S.A.

HISTO-LOGIC T.M.

Addendum

Address Correction Requested

Lab-Tek Products
Division Miles Laboratories, Inc.
30W475 N. Aurora Rd., Naperville, Illinois 60540