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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) | Technical Bulletin for Histotechnology Published: January, April, July, October | Vol. VIII, No. 4 - October, 1978

Recommended Procedure for Demonstrating Hepatic B Antigen (HBAg) in Paraffin Sections

An Editorial

The information provided below concerning the demonstration of HBAg in paraffin sections is presented in response to the following letter.

"I much appreciate receiving HISTO-LOGIC® and find it most helpful for reviewing problems in histology. I would be most appreciative if in one of your issues you would discuss different histological techniques for demonstrating viral inclusion bodies in liver tissue from cases of viral hepatitis." (Paul Ortega, M.D.)

The Editor and Lab-Tek, publisher of HISTO-LOGIC, appreciate Dr. Ortega's interest and encourage other readers to suggest topics they wish discussed in future issues of HISTO-LOGIC.

Remarks contained in this article will be limited to our experience with the staining procedures. For more detailed information on various aspects of this subject, you are encouraged to read the references cited,[1, 2, 3, 4] particularly the one by Ishak.[1]

"The discovery[2] of 'ground-glass' liver cells in the HBAg carrier and the specific identification of the surface antigen by special stains in paraffin sections, have been extremely useful to the pathologist. Ground-glass hepatocytes are characteristic of the HBAg carrier. They may be present in chronic persistent and chronic active hepatitis and in cirrhotic livers with or without hepatocellular carcinoma. Ground-glass cells, which contain the surface component of the HBAg, can be stained specifically by a number of special stains."[1]

The aldehyde fuchsin and orcein procedure have been evaluated extensively and in the editor's view, the aldehyde fuchsin procedure is preferred for the demonstration of HBAg in paraffin embedded tissue sections. This procedure is simple to perform, the results are more conclusive and the tinctorial qualities are more definite.

The following problems were encountered with the orcein procedure: A. The orcein solution has a tendency to break down sooner than the aldehyde fuchsin. B. The acquisition of the right brand of orcein dye is a problem. C. The intensity of the reaction is not always good. D. The differentiation between the HBAg and background is not always well delineated. While we prefer the aldehyde fuchsin procedure, only personal experience can dictate an individual's preference. Therefore, both procedures are included.

A Modified Aldehyde Fuchsin for HBAg[3]

Fixation: Any well fixed tissue (10% buffered neutral formalin preferred).

Microtomy: Cut sections at 6 microns.

Solutions:

Aldehyde Fuchsin

Basic fuchsin	1.0 g
Alcohol, 70%	200.0 ml
Hydrochloric acid (concentrated)	2.0 ml
Paraldehyde	2.0 ml

Let solution stand at room temperature for 3 days or until stain is deep purple. Filter solution and refrigerate for 1 day before use.* Store solution in refrigerator.

0.15% Potassium Permanganate

Potassium permanganate	0.15 g
Distilled water	100.0 ml
Sulfuric acid (concentrated)	0.15 ml

1.5% Oxalic Acid

Oxalic acid	1.5 g
Distilled water	100.0 ml

Staining Procedure:

1. Decerate and hydrate slides to distilled water.
2. Place slides in 0.15% potassium permanganate solution for 5 minutes.
3. Rinse slides in 2 changes of distilled water.
4. Place slides in 1.5% oxalic acid until sections are colorless, usually 10 to 15 seconds.
5. Wash slides gently in tap water for 1 minute.
6. Rinse slides in distilled water (2 changes) by pouring on and off.
7. Rinse slides in 3 changes of 95% alcohol.
8. Stain slides in aldehyde fuchsin for 1 hour.
9. Remove excessive aldehyde fuchsin by rinsing slides twice in 95% alcohol.
10. Rinse slides twice in absolute alcohol (2 changes) by pouring on and off.
11. Clear slides in xylene, 3 changes.
12. Mount coverslip in resinous media.

Results:

Purple staining of all or part of the cytoplasm of liver cells which have "ground-glass" cytoplasm with the hematoxylin and eosin stain. Positively-stained cytoplasm corresponds to localization of hepatitis B surface antigen; as determined by other studies (immunofluorescence, immune electron microscopy). Ground-glass cells can also be stained by the orcein stain for elastic tissue.

(*Refrigeration before use will eliminate some section background staining.)

A Modified Orcein for HBAg[3]

Fixation: Any well fixed tissue (10% buffered neutral formalin preferred).

Microtomy: Cut sections at 6 microns.

Solutions:

Orcein*

Orcein	1.0 g
Alcohol, 70%	100.0 ml
Hydrochloric acid (concentrated)	1.0 ml

Let solution stand at room temperature for 3 weeks. Store solution in refrigerator.

0.15% Potassium Permanganate

Potassium permanganate	0.15 g
Distilled water	100.0 ml
Sulfuric acid (concentrated)	0.15 ml

1.5% Oxalic Acid

Oxalic acid	1.5 g
Distilled water	100.0 ml

Staining Procedure

1. Decerate and hydrate slides to distilled water.

2. Place slides in 0.15% potassium permanganate solution for 5 minutes.
3. Rinse slides in 2 changes of distilled water.
4. Place slides in 1.5% oxalic acid until sections are colorless, usually 10 to 15 seconds.
5. Wash slides in gently running tap water for 1 minute.
6. Rinse slides in distilled water (2 changes) by pouring on and off.
7. Rinse slides in 2 changes of 95% alcohol.
8. Stain slides in Orcein solution for 16 hours.
9. Rinse slides in absolute alcohol (6 changes) by pouring on and off.
10. Clear slides in xylene, 3 changes.
11. Mount coverslip in resinous media.

Results:
Dark brown staining of all or part of cytoplasm of liver cells which have "ground-glass" cytoplasm with the hematoxylin and eosin stain. Positively-stained cytoplasm corresponds to localization of hepatitis B surface antigen; as determined by other studies (immunofluorescence, immune electron microscopy).

*Purchase from: British Drug House (BDH), Chemicals Ltd., Poole, England (Product No. 34063)

References:

1. Ishak, K.G.: Light Microscopic Morphology of Viral Hepatitis. Am. J. Clin. Path., 65: No. 5, 787-827, May 1976.
2. Hadziyannis, S., et al: Cytoplasmic Hepatic B Antigen in Ground-Glass Hepatocytes of Carriers. Arch. Path., 96: 327-330, 1973.
3. Shikata, T., et al: Staining Methods of Australia Antigen in Paraffin Sections. Jap. J. Exp. Med., 44: 25-36, 1974.
4. Deodhar, K.P., et al: Orcein Staining of Hepatitis B Antigen in Paraffin Sections of Liver Biopsies. J. Clin. Path., 28: 66-70, 1975.

H.T. Students Speak Out

Editorial Comment:
The following three articles by Deborah Matyas, Suzanne Dunbar and Glen Kauwell, were submitted to me while these individuals were students at Geisinger Medical Center, Danville, Pennsylvania. The procedures are being published since I feel it is important that we allow our students to contribute to the field of Histotechnology. In addition, it behooves all of us to encourage this type of participation. Therefore, any reference to the training facility should not be misinterpreted to indicate the editor or publisher has any preference for this training program or training activity. The articles are published with the approval of the Medical Director and Laboratory Supervisor.

Simple Solutions to Problems in Cutting

Deborah Matyas
Often, cutting good tissue sections can be a problem. I would like to review some of these problems and make some useful suggestions on how they may be remedied.

Difficulty in obtaining a ribbon is often due to a dull knife, loose knife clamps or a loose block holder. Lines through tissue sections are often caused by a nick in the knife bevel. Nicks in the knife are often produced when cutting hard tissue or tissue containing sutures. These two problems can be solved by insuring that every screw on the microtome is tightened and that knives are optimally sharp.

Certain types of tissue may be difficult to cut, even though good equipment is used. However, with the aid of a few simple steps these same specimens can be made much easier to cut. For example, tissues which are brittle and fragments, such as thyroid colloid and blood-containing specimens, may pose some problems when sectioning. Dabbing warm water on the surface of the specimen may remedy the shredding. If not, soaking them in warm, soapy water may solve the difficulty.

Another problem is tissue which will not cut properly because of the presence of keratin. Keratin can be softened by smearing a small amount of depilatory creme on the hard spots and allowing the specimen to set at room temperature for twenty to thirty minutes. At this point the depilatory creme is wiped off and the tissue placed in the freezer until it is hard enough to cut.

Very often, tissue will not section on the first attempt. If this is the case, it may be helpful to return the block to the refrigerator. Freezing the block allows for easier cutting and better sections. There are times when tissue will not cut the first day no matter what techniques are used. During these times it is best to allow the moisture to evaporate out of the tissue. This can be done by allowing the tissue block to sit out at room temperature overnight.

These few simple suggestions frequently bring about improvement in the sectioning of a variety of difficult-to-cut specimens and result in improvement of the microscopic tissue section.

Suggestions for Conserving Chemicals

Suzanne Dunbar
Due to the ever increasing cost of patient care, the need for conservation in every department of the hospital is essential. Since hospital histology laboratories require a great deal of equipment, the laboratory expense in many instances is astronomical. Some of these expenses can be mininized by the laboratory personnel if certain simple practical techniques are practiced. For example:

Complete disposal of all solutions used in the tissue processor is not necessary on a daily basis. Generally, only the first container of each solution needs to be changed daily. The other containers are then moved one space backward, allowing the last containers of each chemical to contain fresh solution. The clearing agent (xylene), which is used on the tissue processor, is then used for removing paraffin from the embedding equipment. Not all clearing agents can be used this way, so it is better to check the advantages and disadvantages of the clearing agent that is used in your laboratory.

Once a week, the containers are emptied of solutions and washed. Washing is recommended in order to remove any deposits that may build up during the week. This technique of conserving chemicals can also be applied to some of the staining procedures.

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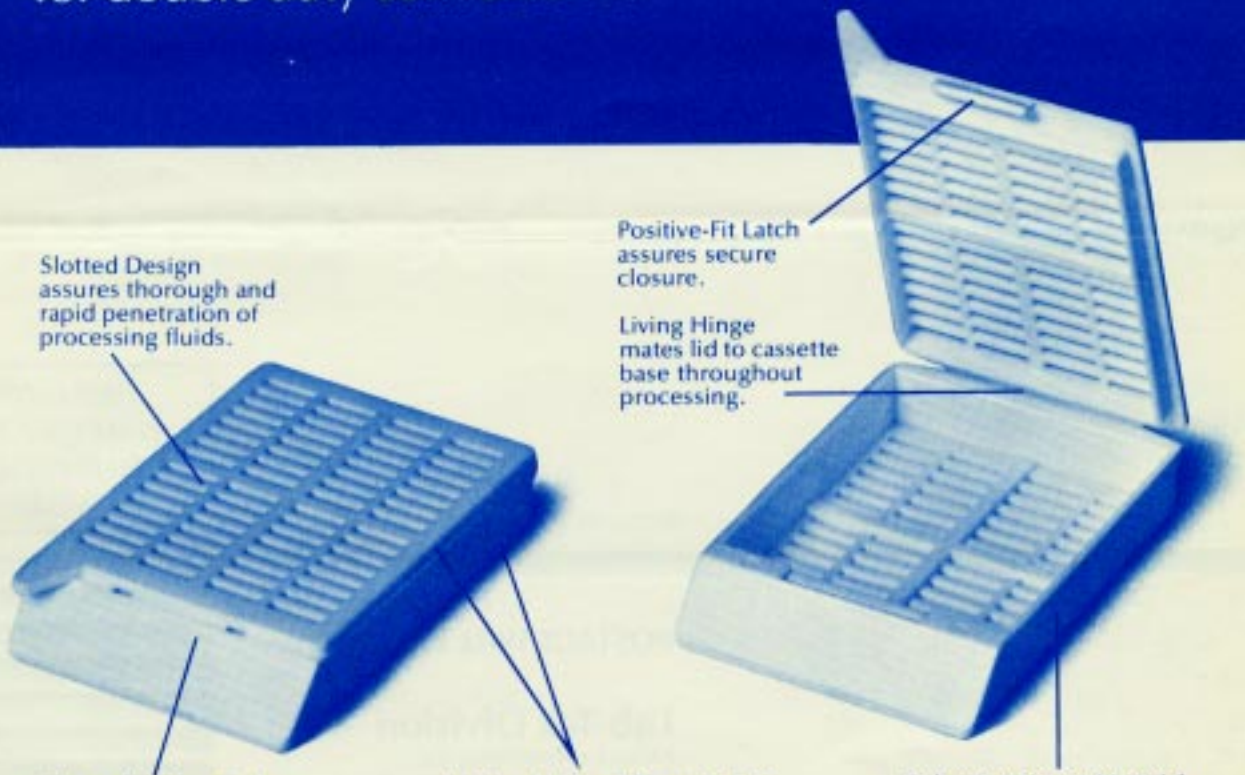

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The Histology Training Program at Geisinger

Glen L. Kauwell

This article outlines a portion of the training program presented at the School of Histotechnology, Geisinger Medical Center.

The school provides a year of intensive training both in the theoretical and practical aspects of Histology. Lectures are given by a number of Staff and Resident Pathologists on the various procedures and techniques used in the laboratory.

The laboratory instructors consist of four Histology Technicians and the Histology Supervisor; all are certified H.T.'s by the Board of Registry of ASCP. Each student is assigned to a different technician for a period of three months, so he/she can learn different ways in which a given procedure may be done due to possibly different approaches used by the four technicians. Since all the technicians are involved in special stains, microtomy, embedding, knife sharpening, care of the equipment, and all the other tasks in the laboratory, the students are involved at all times, in the work they will do as Histology Technicians. This produces a situation where the students know all the technicians and feel free to readily ask questions when they arise.

The students also spend time in the Surgical Pathology Laboratory, where they are taught the procedures of staining frozen sections and the use of the cryostat. Since this is the starting point for the surgical specimens, the student can actually follow the specimen from the initial sectioning through the final coverslipping of the stained tissue. This provides, in my opinion, a thorough understanding of what we as students are doing and the sense of knowing why all the different steps in processing are necessary.

Students are also given the opportunity to observe autopsies through the year. This is an interesting, as well as important part of the training, as it helps in being able to identify gross specimens as well as giving the students a chance to question the Pathologist on the function of different organs. Students may also learn why certain special stains are helpful on certain types of tissue.

Another aid which I have found to be particularly interesting and helpful, is that on occasion we are shown slides of tissue sections with various stains and asked to identify the stain and the tissue. This provides us with a means of studying the end result of our work and learning to identify what a proper stain should look like. This enables us to check our stains and determine whether or not they are of acceptable quality, and if in fact the stain was done properly at all.

At the end of what has proven to be a very interesting year, I have found that my interest in Histology has grown. Also, I believe we are prepared to do any work that would be expected of us in a routine Histology Laboratory.

Erratum

In the article "A Modified Thiosulfation-Aldehyde Fuchsin Method for Pancreatic Beta Cell Granules", page 116 of the July, 1978 issue of HISTO-LOGIC, the formula for Wenger's Aldehyde Fuchsin should read 5.0 gm. of Potassium dichromate, not 5.0 ml.

"Histology Sigma" Association

A proposed "Histology Sigma" invites potential members now to form this Association. Everyone is invited to this Association provided he/she can present a paper of one's chosen field of research or specialty. All papers will be published as a book entitled "Recent Proceedings in Histology".

Meeting will be held during the summer months at a designated place every two years, depending upon the availability of papers generated by members. No fee is required to join this Association, but you will have to pay your own expenses (unless paid by employer) to go to this meeting. If you are interested in joining this Association, contact:

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An Editorial View on H&E Consultation

Of the various problems facing the pathologist regarding the quality of microscopic slides produced in the histology laboratory, poor Hematoxylin and Eosin (H&E) preparation has to be the most disconcerting. Poor H&E staining is not an uncommon problem, and therefore some steps should be taken to eliminate it.

It is ironic that the problem exists at all, particularly since this is the most common and most often performed procedure in the histology laboratory. It is unimaginable to think that a day goes by in any laboratory where an H&E is not performed. This being the case, one can guess that there must be several million slides produced annually by 4-6 thousand laboratories. If we as Histotechnologists possess this much experience in performing this procedure, what then accounts for the problem of poor quality H&E's? We certainly cannot attach any blame on the lack of good staining procedure, since the histology literature provides many excellent methods. We should also stop blaming poor H&E's on the quality of the hematoxylin dry powder or chemicals used to formulate hematoxylin staining solutions. I find little to substantiate that the dyes and chemicals available to us today are involved in determining the final quality of the H&E. Most chemicals are of high quality and quite acceptable for compounding hematoxylin solutions. The problem lies elsewhere and I offer the following as possible answers to this intriguing question.

A. Many technicians do not recognize the difference between a good and a poor H&E.

B. Many laboratory supervisors or service chiefs do not allow a change to better H&E procedure, if to produce a better quality slide involves an additional 15 to 30 minutes of staining time.

These might seem basic answers, but let me explain further. In my position I receive many requests for consultation on all facets of histological techniques. Roughly 40 percent of the consultation requests involve the quality of H&E stained slides. In almost every instance, my answer is simply "use the Mayer's progressive hematoxylin with an eosin-phloxine counterstain." This procedure appeared in Histo-Logic, Vol. VI, No. 2, p. 81, April 1976.

Although I am convinced this is the best H&E staining procedure available, it is difficult to get people to use it routinely. This is primarily due to its one drawback, namely, that it takes one hour to perform, while regressive H&E staining procedures can be altered and performed in considerably less time. The universal reply I receive when suggesting this technique is: "I like the stain but can't use it since it takes so long to perform." I realize surgical services are pushed to produce H&E stained slides in the shortest time possible, but I maintain that many laboratories are substituting high quality, simplicity and reproducible results for an additional 20 to 30 minutes of staining time. My question is: Are we providing the best patient care possible by taking this approach?

A Technical Bulletin for Histotechnology

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Prevention of Mold and Bacterial Growth in the Histology Laboratory

An Editorial

The growth of mold and bacteria in staining solutions is a common problem in many histology laboratories. It can be troublesome to the pathologist when he studies microscopic slides, particularly if the slides under study have been stained with procedures for the demonstration of fungi and bacteria. And it can be prevented to a large extent, by the use of a few crystals of thymol in the staining solutions (generally, approximately 0.25 gm of thymol for every 500 ml of solution will suffice).

To my knowledge, there are no solutions in which thymol cannot be used. However, controlled studies have not been performed and therefore the use of thymol should be discontinued if staining procedure or solutions are altered in any way. In addition, thymol should not be used at will, but its use should be limited to known problem solutions; i.e., solutions in which one has experienced the growth of bacteria or mold.

One other source of bacterial growth is the tissue floatation bath in which gelatin is used as the section adhesive. Gelatin and the warm water can serve as a good media for bacterial growth. This bacteria poses a serious problem microscopically and every effort should be made to prevent its development. Here again, the addition of a few grains of thymol to the floatation bath will limit or prevent bacterial growth. The amount used is the same as provided above.

There is one objectionable problem. Namely, the fact that the thymol produces a slight odor for a short period when first added to the warm water. To alleviate any potential questions on the use of thymol, the following is reprinted from the Merck Index. "USE: For destroying mold and herbarium parasites, *preserving anatomical specimens*, urine; for embalming. Has been recommended for the prevention of mildew growth and as an antimold for paper."

Examples of bacterial and mold growths, plus many other artifacts are photographically illustrated in the following atlas.

Thompson, S.W. & Luna, L.G.: *An Atlas of Artifacts Encountered in the Preparation of Microscopic Tissue Sections*. Charles C Thomas, Springfield, IL 62717, 1978.

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 Division, Miles Laboratories, Inc., 30W475 North Aurora Rd., Naperville, Illinois 60540.
Printed in U.S.A.

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.