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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. X, No. 3 - July, 1980

GOLDEN FORCEPS AWARD WINNER

We are pleased to announce that Charles Churukian has been selected as the recipient of the Golden Forceps Award for 1980. Mr. Churukian, who is Supervisor of the Special Stains Laboratories at the University of Rochester Medical Center, Rochester, New York, has submitted numerous articles for publication in **Histo-Logic®**. Criteria for this award were clarity, originality, scientific content and continued contribution.

The Golden Forceps Award will be presented at the Symposium/Convention of the National Society for Histotechnology to be held in Atlanta, Georgia, October 27-31, 1980. Mr. Churukian's current article, "Prolonging the Shelf-Life of Solutions Containing Silver Nitrate," appears in this issue. Reprints of all his articles are available from Lab-Tek Division, Miles Laboratories, Inc., 30 W 475 North Aurora Road, Naperville, IL 60540.

Prolonging the Shelf-Life of Solutions Containing Silver Nitrate

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It is generally accepted that refrigerator storage of solutions containing silver nitrate will considerably extend their shelf-life. The refrigerator-stored shelf-life of Fontana-Masson[1] ammonical silver and Gomori-Grocott[2] methenamine silver (GMS) has been reported to be about one month.[3,4] Most ammonical silver solutions used in various reticulum methods are unstable, especially those containing sodium or potassium hydroxide. Because of this, most workers have overlooked the possibility of refrigerator storage of ammonical silver. Usually, ammonical silver is used shortly after it has been prepared and discarded after use. This necessitates the preparation of ammonical silver each time a reticulum stain is requested which, in laboratories where a large volume of special stains are done, could be several times a week, involving considerable time and expense.

Gomori[5] reported that ammonical silver containing sodium or potassium hydroxide may be used for two or three days when stored at room temperature. In the same report, he indicted that ammonical silver which contained a soluble carbonate would be usable for at least five or six days. According to Lillie[6] the ammonical silver of Laidlaw,[7] which contains lithium carbonate, keeps well for weeks and may be reused a dozen or more times. We have found that the ammonical silver used in a modification of Gomori's[8] reticulum method and that used in Wilder's[9] method can be reused for ten to fourteen days if kept refrigerated. Just prior to use the solution is brought to room temperature and immediately after use it is returned to the refrigerator. On a number of occasions, we have observed that reticulum fibers stain better after the ammonical silver has aged in the refrigerator for two or three days. After about five days, the solution begins to take on a pale yellow-brown color which may or may not become progressively darker. This coloration does not seem to adversely affect the impregnation properties of the ammonical silver.

It is recommended that ammonical silver, especially that which contains sodium or potassium hydroxide, be discarded when its shelf-life (not more than two days at room temperature in the dark and ten to fourteen days in the refrigerator) has expired. According to Smith[10] and Wallington[11] this type of ammonical silver has a tendency to form explosive silver compounds if the solution is exposed to light and is kept at room temperature for several days. According to their reports, explosions have occurred in some laboratories where precaution has not been exercised. Refrigerator storage of ammonical silver retards the formation of the explosive silver compounds but, according to Wallington's report, does not always prevent their formation. The formation of a dark precipitate in ammonical silver is an indication that explosive silver compounds have formed. Should this occur, extreme care should be exercised in discarding the solution because the potential for an explosion is great.

We have observed that silver nitrate crystals stored at room temperature will gradually take on a greyish-violet color. This does not occur when silver nitrate is kept refrigerated, even after several years. Solutions made with silver nitrate which has become discolored are usable, but from our observation, seem to be less stable than those prepared with nondiscolored silver nitrate.

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American Registry of Pathology Histopathology Staining Control Slide Program

In 1976 the American Registry of Pathology (ARP) was chartered by Congress to be established at the Armed Forces Institute of Pathology as a "non-federal corporation to serve as a focus for interchange between military and civilian pathology and encourage the participation of medical, dental, and veterinary sciences in pathology for the mutual benefit of military and civilian medicine."

In response to requests from a number of pathologists and a state society of pathology the American Registry of Pathology decided to initiate a program to produce slides for the control of stains used in histopathology. The first slides prepared are for *M. tuberculosis*, gram negative bacteria, gram positive bacteria, fungus, and amyloid. Next, slides for other special stains, including Warthin-Starry for spirochetes, will be prepared.

In diseased human tissues, microorganisms of varying ages and states of degeneration may be more difficult to stain than in tissues of animals experimentally inoculated. Therefore, insofar as possible, slides prepared from human tissues will be used. An exception, of course, will be in providing control slides for spirochetes.

The slides for each special stain will be packaged in boxes containing 25 slides. Each box will contain one slide stained by the method used routinely at the AFIP and a copy of the method currently used in the histopathology laboratory at the AFIP. For some organisms, e. g. fungi, two stains (PAS and GMS) will be included. Each box of 25 slides will therefore contain 24 or 23 unstained paraffin sections.

The price to laboratories (federal and non-federal) for a box of 25 slides, when payment accompanies the order, is $45.00. If purchase order is sent, to cover administrative costs, the price is $50.00. Payment (U.S. dollars) should be made to the American Registry of Pathology and addressed to Histopathology Staining Control Slide Program; American Registry of Pathology; Armed Forces Institute of Pathology; Washington, DC 20306.

Preparation of Fluid Material for Cytologic Evaluation

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In many small hospitals the histotechnologist is responsible for the preparation of non-gynecological materials for cytologic examination. This is a very responsible task since without proper cyto-preparatory techniques, cytologic diagnosis is impossible. A few important technical considerations of which the histotechnologist should be aware are provided below.

Appearance of Gross Specimen

When a body fluid is received in the laboratory, the volume and the gross appearance of the specimen should be noted and recorded. Sputum should be carefully examined for particulate material and blood-streaked areas. These areas should be chosen for cytologic examination. It should be noted if a pleural effusion is spun down and a large white button is obtained. This should also be done if a urine specimen is clear and no button is obtained on centrifugation.

Specimen Handling

Fluids that are received unfixed should not be allowed to sit in the laboratory, since exfoliated cells decompose rather rapidly. If there is an unavoidable delay, the specimen should be stored in the refrigerator. However, smears should be made as soon as possible after the fluid reaches the laboratory.

Smear Preparation

Viscid specimens, such as sputum, can be spread on a plain glass slide. When it becomes necessary to centrifuge a specimen to obtain sufficient cellular material for examination, the sediment can be spread on a plain glass slide coated with a thin film of egg albumin, or spread on a fully frosted slide. This will keep the specimen from washing off during the fixation and staining processes. It is important to spread the material thinly and evenly, as a thick smear will not stain properly and is difficult or impossible to read.

Fixation

Fixation must be undertaken **immediately** after making the smear. Slide/specimen drying produces artifacts which impair staining and interpretation. The slides may be either immursed in 95% ethyl alcohol or fixed with a commercial fixative.

Gross Specimen Storage

It is recommended that a few extra slides be made while the specimen is still fresh. These slides are set aside in case special stains or additional slides are required for diagnosis. The unfixed gross specimen should be refrigerated and saved until a diagnosis has been rendered and reported. Specimens are often needed for further testing. For example, a pleural fluid may be required for a screening test of high concentrations of hyaluronic acid in a suspected diffuse malignant mesothelioma.

Conclusion

The pathologist and the patient rely heavily on the histotechnologist in this most responsible area of cyto-preparation. He can only call it as he sees it, and for this he depends on your technique.

H&E Staining With Paper Strips

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It is not uncommon to have to do a frozen section and find the hematoxylin and other solutions evaporated and/or precipitated. The histotech must then rush to change the solutions.

To prevent this, we find that small strips of bibulous paper soaked with hematoxylin and eosin work very well for STAT H&E's. These squares of solution soaked papers are easily prepared. Coverslip size, or 24 x 40 mm, strips of bibulous paper are dipped in hematoxylin, placed on a microscopic slide and dried in a slide dryer. Eosin strips are prepared in the same manner.

To use, a frozen section is cut, picked-up on a slide and immediately dipped in 95% alcohol to fix. Drying of the section is avoided. Rinse slide in tap water, cover section with hematoxylin impregnated strip, moisten with a few drops of tap water and allow to stain for 2-5 minutes. Rinse strip and excess stain from slide with tap water, which will also blue the section. The eosin impregnated strip is applied in the same manner, moistened with tap water and stained for 15-30 seconds. Rinse slide with tap water, dehydrate with alcohols, clear in xylene and mount. Coverslip with a resinous media.

Pathology Through the Ages

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Going back through time, archeologists have found that prehistoric people made gross observations of external disease. Some of these archeological discoveries include marble representation of hernias, breast tumors, varicose veins, and ulcers. The major healing art of this period was bound with theological practices, by praying to the gods to remove the pain.

Ancient Egyptians believed that demons caused disease. As early as 4,000 B.C., Egyptian medical students and priests had written anatomy books. These were laid out in an anatomical systematization starting at the head and working down through the body (this systematization of the body was the one enduring contribution of the ancient Egyptians). The Papyrus Ebers (approximately 1,550 B.C.) reveal the most important information on Egyptian medicine.

During the Hippocratic period (460-370 B.C.) the Greeks developed "humoral pathology." This type of pathology attributed disease to anomalies of body fluids or humors of the body. For example, a boil was believed to be caused by an effort of the blood to rid itself of impurities, while a fever would be a result of the body trying to cook the altered body fluids.

Some human dissection was being practiced throughout the previous periods, but Erasistratos (310-250 B.C.) deliberately dissected for the purpose of explaining disease. Therefore, he is considered one of the founders of pathology.

Galen is perhaps one of the greatest medical figures during the Middle Ages. His works were considered **the** medical authority in Europe for over thirteen centuries! Many of Galen's works were faulty due to the fact that human dissection ceased during this period. Thus, all of Galen's observations were based on the living patient and knowledge of ape and swine anatomy.

The return of human dissection in the 13th century brought great developments in pathological anatomy, but it also caused a theological dispute. The theologians felt that dissecting a body was desecration and should result in the excommunication of the people involved. This conflict was not completely resolved until 1556.

With the invention of the compound microscope by Hans Janssen, a new era in pathology was opened up. Malpighi introduced the microscope to medicine by his discovery of capillaries and red blood corpuscles. He is also considered one of the founders of histology.

The word "histology" is derived from the Greek words "histo" (meaning tissue) and "logia" (meaning the study of). The first recorded tissue staining was done in 1719 by Leeuwenhoek.

There was a slowdown in microscopic anatomy in the 18th century (an age of theory and system). The 19th century brought extensive histopathological advances and is considered an age of organized advancement of medicine. Schleiden and Schwann introduced the "cell theory" — nucleated cells are the basis of animal and plant formation. In 1841, Henli developed a new direction in pathology by publishing a comprehensive account of human histology.

The use of dyes (known as biological dyes) in tissue staining was well on its way by 1848. This led to an explosion of study of histopathology in the last half of the 19th century. Increased technology led to improved microscopes, fixation and stain methodology. Rotary microtomes were developed in 1883 which resulted in serial sectioning of tissues.

The 20th century is considered the period of organized preventative medicine. Histology has continued to progress through the invention of the electron microscope, frozen tissue techniques, and in the broadening field of histochemistry.

Histopathology will be an ever-increasing integral part of the medical field in the future.

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Solutions to Amylase Problems

Editor's Note: The following articles are in response to an inquiry which appeared in Vol. X, No. 1, January 1980, issue of **Histo-Logic.**

Bill Barlow
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I have a possible solution to the amylase problem that Paulyne Lawton referred to in **Histo-Logic.** 50 mg amylase (A-amylase #A-6505 from Sigma Chemical Co. seems to be very specific) was dissolved in 50 ml of Luna-Parker Giemsa buffer (pH 6.0) with no filtering required. Complete digestion of glycogen takes place in 20 minutes or less at 37°C, or preferably at room temperature in 1 hour.

S. A. Gourley
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We have solved the problem of "commercial malt diastase" in the following manner. Remove slide from distilled water; place on rack or jar. Expectorate saliva plus a few drops of water and a sprinkling of diastase. Let stand on the slide for 15 to 30 minutes. Wash in running water for 20 minutes and stain. We have experienced good results regardless of the diastase used.

Can You Help?

Brenda Collins
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I am having a problem obtaining reliable results with silver impregnation techniques for axons in the central nervous systems of large and small animals. I have experimented with both the Bodian and Holmes methods and obtained variable results. Axons may stain well in small animals and faintly with large animals with the same technique. Our tissues are perfused-fixed or immersion-fixed with 10% BNF, but the fixation method has not consistently been associated with either faint or deep staining.

Is there any technique or modification of the above methods for axons which works well with large and small animal central nervous systems?

Any help and advice may be forwarded directly to Ms. Collins with a carbon copy of suggestion to the Editor of **Histo-Logic.**

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Journals and Publications

This is part two of a four-part article listing various educational aids applicable to the field of histotechnology. Part one — "Text Books" — appeared in Vol X, No. 2, April 1980, **Histo-Logic.**

Journal of Histotechnology
Published quarterly: March, June, Sept., Dec.
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American Journal of Medical Technology
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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.