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Tissue-Tek® at 25 . . . Meeting the Needs of Histologists Worldwide

This year marks the 25th anniversary of the Miles Division that supplies you with well-known Tissue-Tek® Histology Systems and Lab-Tek® products. It has been through your constant support and confidence in these brands that we at Miles Scientific are able to celebrate this proud occasion.

Twenty-five years ago, the Lab-Tek Plastics Company was founded by J. B. McCormick, M.D., pathologist and inventor. Then in 1966, Miles acquired Lab-Tek from Dr. McCormick, and in doing so, obtained much more than a building and inventory. We acquired a tradition — the plans, ideas, and a commitment to serve histologists around the world and the creative thrust to innovate and modernize the histology lab with products to make work easier and more efficient.

Today, we still maintain this mission. The Tissue-Tek name can be found on products used in every area of histology . . . specimen collecting, processing, embedding, sectioning, mounting, staining, and storing. From the first Tissue-Tek Embedding System invented by Dr. McCormick to the newest Vacuum Infiltration Processor, your needs, as histologists, are our primary consideration.

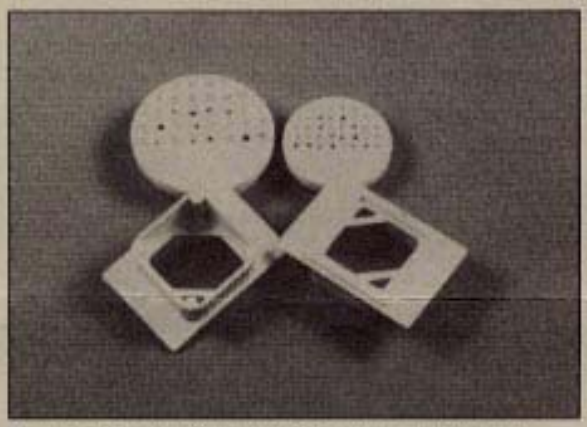
Inside This Issue

Tissue-Tek® instruments introduced in the 1960's filled a variety of needs in the histology laboratory.

The Tissue-Tek Embedding Ring System was introduced in 1958—perhaps the most significant advancement in embedding methods since the advent of Naples bars prior to 1883. The ring system was the first embedding technique to combine the heat exchange properties of metal and the convenience of disposables. Stainless steel Tissue-Tek base molds provided good cooling properties for proper paraffin casting while permitting a selection of block sizes. Disposable rings made it easy to remove the block from the mold and provided a firm base for

clamping. Furthermore, the Tissue-Tek Embedding Ring System allowed recutting of specimens in the same plane as the previous section.

In the early 60's, several Tissue-Tek instruments were added to the product line. These included Tissue-Tek Paraffin Dispenser, Tissue-Tek Vacuum Infiltrator, and Tissue-Tek Microtome/Cryostat. The next major innovation came in 1968 when another of Dr. McCormick's inventions was introduced as part of the Tissue-Tek II Embedding System. This invention was the Tissue-Tek II Process/Embedding Cassette. Here, for the first time, a single product safely held a specimen through processing and embedding to storage, so specimen identification remained with the tissue, reducing the possibility of misidentification.



Tissue-Tek® Embedding Rings revolutionized specimen handling and processing.

The Tissue-Tek II Tissue Embedding Center, introduced at the same time, was the first all-in-one instrument for embedding. It incorporated a paraffin dispenser, a cold plate, a warming drawer for base molds, plus a cold drawer for embedded specimens.

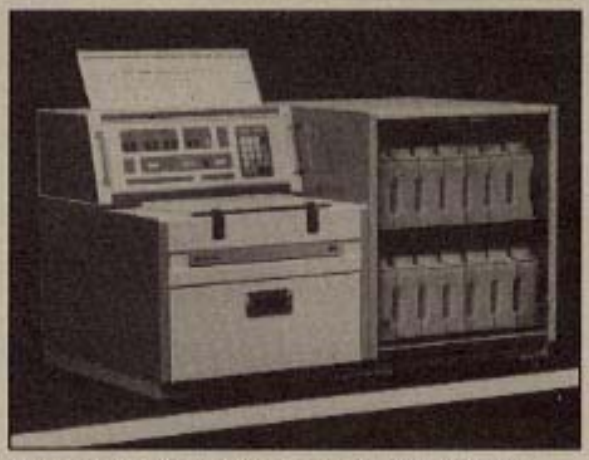
The Tissue-Tek III Uni-Cassette™ System was introduced in the late 70's. The one-piece molded design eliminated the stainless steel, reusable covers of the earlier Tissue-Tek II Cassette System. The line was refined to include biopsy cassettes, mega-cassettes, and cassettes in colors to permit convenient color-coding of specimens.

A major introduction in 1979 was the Tissue-Tek III Vacuum Infiltration Processor. This innovative design is still our largest tissue processor, holding 300+ specimens in a stationary processing chamber. The fully enclosed system improved the safety of processing for both specimens and operators.

Product development continued into the 80's with the introduction of Tissue-Tek III Tissue Embedding Console System, Tissue-Tek III Accu-Edge® Disposable Microtome Blade System and Tissue-Tek III Accu-Cut™ Rotary Microtome.

Three Tissue-Tek® Cassette Embedding Systems provide convenient, specimen identification and handling.

This year, 25 years after introducing the first Tissue-Tek Embedding Rings, we are still expanding the system to help you keep your histology lab well organized. Tissue-Tek Embedding Rings are now being offered in five colors: white, yellow, pink, green, and blue. Ten colors are offered in both Tissue-Tek II Process/Embedding Cassettes and Tissue-Tek III Uni-Cassette™ products: tan, orange, lilac, gold, aqua, yellow, pink, green, blue, and white.



New Tissue-Tek® VIP™ Tissue Processor is the latest advancement in computer controlled automatic processing.

In addition, two new Tissue-Tek VIP™ Tissue Processors have been introduced. These are VIP 1000 and VIP 2000 which have specimen capacities of 100 and 200 cassettes, respectively. These new processors are computer controlled and packed with features to give you the best possible processing with continous, reliable service.

As for the years ahead, one thing is clear: our future is built upon your continued confidence and support. We shall maintain our commitment to improve and develop products for histology as we begin our second quarter century as your histology systems source.

Incompatible Chemicals — An Issue to Consider

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In a previous issue,[1] Tamela Bird reported a chemical fire accident which occurred during preparation of a reagent for softening keratin and chitin, as outlined in a staining manual.[2] In reaction to Ms. Bird's report, the following note on incompatible chemicals is presented. Hopefully, a general familiarization with some of the more hazardous chemicals's routine use in the laboratory will aid in prevention of reactive chemical hazards in the work place.

The constituents of the reagent in the Bird report are mercuric chloride, chromic acid, concentrated nitric acid, 95% ethanol, and distilled water. The accident occurred when 95% ethanol, a flammable liquid, spilled over the work area and ignited, as it was added to a container of mercuric chloride and chromic acid. An editorial note appended to the report suggested that a safe way to prepare the reagent was to first dilute the 95% ethanol with distilled water; each of the other constituents was then to be added, separately and slowly, to the diluted ethanol.

Chromic acid, concentrated nitric acide, and 95% ethanol are of the class of chemicals described as "incompatible" because they react with each other to create a flammable and/or explosive condition.[3] The fire accident described by Ms. Bird would seem to be the result of the reaction between chromic acid and ethanol. Conceivably, a more hazardous reaction would have resulted if the concentrated nitric acid had been exposed to the burning reaction mixture. Some attention should be given to the general properties, handling, use and storage of potentially hazardous "incompatible" and "reative" chemicals used in staining. Particularly useful in this regard is a Safety Handbook incorporated into the catalog of the MC/B Manufacturing Chemists.[4] With the kind permission of the MC/B Company, the lists of "Incompatible" (Table I) and "Reactive" (Table II) chemicals presented here are reproduced from the MC/B Handbook (p. 339, and Table V, p. 359, respectively).

TABLE I
Example of Incompatible Chemicals

Chemical	Keep Out of Contact With:
Acetic acid	Chromic acid, nitric acid, hydroxyl compounds, ethylene glycol, perchloric acid, peroxides, permanganates
Acetic anhydride*	Hydroxyl-containing compounds, ethylene glycol, perchloric acid
Acetone*	Concentrated nitric and sulphuric acid mixtures
Acetylene	Chlorine, bromine, copper, fluorine, silver, mercury
Alkaline metals, such as powdered aluminum or magnesium, sodium, potassium	Water, Carbon tetrachloride or other chlorinated hydrocarbons, carbon dioxide, the halogens
Ammonia, anhydrous	Mercury (in manometers, for instance), chlorine, calcium hypochlorite, iodine, bromine, hydrofluoric acid (anhydrous)
Ammonium nitrate	Acids, metals powders, flammable liquids, chlorates, nitrates, sulfur, finely divided organic or combustible materials
Aniline	Nitric acid, hydrogen peroxide
Bromine	Same as for chlorine
Calcium oxide*	Water
Carbon, activated	Calcium hypoclorite, all oxidizing agents
Chlorates	Ammonium salts, acids, metals powders, sulfur, finely divided organic or combustible materials
Chromic acid and chromium trioxide	Acetic acid, naphthalene, camphor, glycerin, turpentine, alcohol, flammable liquids in general
Chlorine	Ammonia, acetylene, butadiene, butane, methane, propane (or other petroleum gases), hydrogen, sodium carbide, turpentine, benzene, finely divided metals
Chlorine dioxide	Ammonia, methane, phospine, hydrogen sulfide
Copper	Acetylene, hydrogen peroxide
Cumene hydroperoxide	Acids, organic or inorganic
Flammable liquids	Ammonium nitrate, chromic acid, hydrogen peroxide, nitric acid, sodium peroxide, nitric acid, sodium peroxide, the halogens
Fluorine	Isolated from everything

TABLE I (continued)
Example of Incompatible Chemicals

Hydrazine	Hydrogen peroxide, nitric acid, any other oxidant
Hydrocarbons (butane, propane, benzene, gasoline turpentine, etc.)	Fluorine, chlorine, bromine, chromic acid, sodium peroxide
Hydrocyanic acid	Nitric acid, alkali
Hydrofluoric acid anhydrous	Ammonia, aqueous or anhydrous
Hydrogen peroxide	Copper, chromium, iron, most metals or their salts, alcohols, acetone, organic materials, aniline, nitromethane, flammable liquids, combustible materials
Hydrogen sulfide	Fuming nitric acid, oxidizing gases
Iodine	Acetylene, ammonia (aqueous or anhydrous), hydrogen
Mercury	Acetylene, fulminic acid, ammonia
Nitric acid (concentrated)	Acetic acid, aniline chromic acid, hydrocyanic acid, hydrogen sulfide, flammable liquids, flammable gases
Nitroparaffins*	Inorganic bases, amines
Oxalic acid	Silver, mercury
Perchloric acid	Acetic anhydride, bismuth and its alloys, alcohol, paper, wood
Peroxides, organic*	Acids (organic or material), avoid friction, store cold
Potassium	Glycerine, ethylene glycol, benzaldehyde, sulfuric acid, carbon tetrachloride, carbon dioxide, water
Potassium chlorate	Sulfuric and other acids
Potassium perchlorate (see also chlorates)	Sulfuric and other acids
Potassium permanganate	Glycerin, ethylene glycol, benzaldehyde, sulfuric acid
Silver	Acetylene, oxalic acid, tartaric acid, ammonium compounds
Sodium	Carbon tetrachloride, carbon dioxide, water
Sodium nitrite*	Ammonium nitrate and other ammonium salts
Sodium peroxide	Ethyl or methyl alcohol, glacial acetic acid, acetic anhydride, benzaldehyde, carbon disulfide, glycerin, ethylene glycol, ethyl acetate, methyl acetate, furfural
Sulfuric acid	Potassium chlorate, potassium perchlorate, potassium permanganate (or compounds with similar light metals, such as sodium, lithium)

*Additions culled from a similarly useful list from Reference 2.

The following is a list of chemicals in which fire and/or explosion is a hazard.

NOTE: It is recommended that these chemicals be separated from other chemicals in storage. Fire and/or explosion is a possible hazard when reactive chemicals combine.

TABLE II
Chemicals with which Fire or Explosion is a Hazard

Reactive Chemicals

Ammonium Nitrate	Methyl Chloroformate	Sodium Chlorite
Ammonium Perchlorate	o-Nitrotoluene	Sodium Chromate
Calcium Hydride	p-Nitrotoluene	Sodium Dithionite
Chromium Nitrate	Picric Acid	Sodium Ethoxide
Chromium Trioxide	Potassium Metal	Sodium Lead
m-Dinitrobenzene	Potassium Nitrate	Sodium Methoxide
p-Dinitrobenzene	Potassium Perchlorate	Sodium Methoxide 25% in Methanol
Hydrazine	Sodium Dispersion	Sodium Nitrate
Hydrazine Hydrate	Sodium	Sodium Peroxide
Hydrogen Peroxide	Sodium Azide	Urea, Compound with Hydrogen Peroxide
Magnesium Perchlorate	Sodium Chlorate	
Mercurous Perchlorate		

Another class of incompatible chemicals comprise those reagents which, upon reaction with each other, produce highly toxic compounds, rather than a fire and/or explosion hazard. These are listed in Table III.

TABLE III
Incompatible Chemicals
Producing Toxic Compounds

Chemical	Incompatible With	Toxic Compound Produced
Azides	Any reducing agent	Hydrogen azide
Cyanides	Acids	Hydrogen cyanide
Hypochlorites	Acids	Chlorine or hypochlorous acid
Nitrates	Sulfuric acid	Nitrogen dioxide
Nitric acid	Copper, heavy metals	Nitrogen dioxide
Nitrites	Acids	Nitrous fumes
Sulfides	Acids	Hydrogen sulfide

The warning label on a reagent bottle or container often provides essential information for avoiding the more serious hazards associated with the chemical. In case of an accidental swallowing of the chemical, or of an accidental contamination by it, instructions for antidote/first aid procedures are also given on the warning label. Thus, a brief familarization with the warning label, before a chemical is used or put away for storage, is the first important step in the prevention of chemical hazards in the laboratory.

References:

1. Bird, T. A. Word of Caution. *Histo-Logic* XI, No. 4: 189, 1981
2. "Keratin and Chitin Softening Procedures". In: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, p.11 (Lee G. Luna, Ed.) McGraw-Hill Book Company, N.Y. 1968
3. Bretherick, L.: "Reactive Chemical Hazards". In: Hazards in the Chemical Laboratory, Chp. 5, pp.65-80 (L. Bretherick, Ed.). The Royal Society of Chemistry, Lon., 1981
4. Safety Handbook, in Stain and Dye Catalog, pp. 318-330, MC/B Manufacturing Chemists, Ohio and California

Removing Mercuric Chloride Precipitate from Tissue

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The classical method of removing mercuric chloride precipitate from tissue may be shortened and made somewhat easier if the following steps are performed.

Solutions:

1% Iodine Solution

Iodine ..... 1.0 gm
Absolute ethyl alcohol ..... 100.0 ml

5% Sodium Thiosulfate

Sodium thiosulfate ..... 5.0 gm
95% Ethyl alcohol ..... 100.0 ml

NOTE: In the above solutions iodine is completely dissolved, but the sodium thiosulfate in 95% alcohol will appear to be saturated.

Procedure:

1. Deparaffinize slides with 3 changes of xylene.
2. Place slides in 2 changes of 100% alcohol, 2 changes of 95% alcohol.
3. Place slides in 1% alcoholic iodine solution for 10-15 minutes.
4. Rinse in 95% alcohol.
5. Place in 5% alcoholic sodium thiosulfate UNTIL COLORLESS (30 seconds to 2 minutes).
6. Wash in water 2-5 minutes.
7. Proceed with routine hemotoxylin and eosin (H&E) procedure.

Removal of mercuric chloride precipitate takes place more rapidly and more thoroughly in alcoholic iodine solvent. Removal of iodine is quickly accomplished with alcoholic sodium thiosulfate.

A Spinal Fluid Stain for Cryptococcal Meningitis

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Although a hemotoxylin and eosin (H&E) stain is done on all spinal fluids brought to our laboratory for membrane filtration, we have found in cases of suspected cryptococcal meningitis, it is very beneficial to the pathologist to stain the membrane filter with the following periodic acid-Schiff (PAS) procedure. It has been very helpful in distinguishing "ghost" erythrocytes from cryptococci. On occasions, it has been helpful to decolorize an H&E preparation and restain it with the PAS technique.

Procedure:

1. After the spinal fluid has been put through the membrane filter (we use a filter with a pore size of .045 um), fix in 95% ethyl alcohol for 10 to 15 minutes.
2. Wash in distilled water for 5 minutes.
3. Oxidize in 0.05% periodic acid for 5 minutes.
4. Wash in distilled water for 5 minutes.
5. Stain in Schiff's solution at room temperature for 15 minutes.
6. Wash in running water for 10 minutes.
7. Stain in Gill's hematoxylin (formula #2) for 30 seconds.
8. Wash in distilled water for 5 minutes.
9. Blue in Scott's water, 12 dips.
10. Wash in distilled water for 5 minutes.
11. Dehydrate in 95% ethyl alcohol, 10 dips.
12. Dehydrate in 100% ethyl alcohol, 5 minutes.
13. Clear in xylene for 5 minutes and mount coverslip with resinous media.
14. For a more permanent slide, seal around coverslip with clear fingernail polish.

Localization of Prostatic Acid Phosphatase by Immunoperoxidase Staining

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Indirect immunoperoxidase technique has been employed to demonstrate prostatic acid phosphatase in human prostate. In this procedure, unlabeled rabbit anti-human prostatic acid phosphatase is reacted and bound with the antigen. Next, the bound rabbit antibody is reacted with peroxidase-labeled goat anti-rabbit IgG, heavy and light chain (H+L). Finally, the resulting complex is demonstrated with amino ethylcarbazole (chromogen).

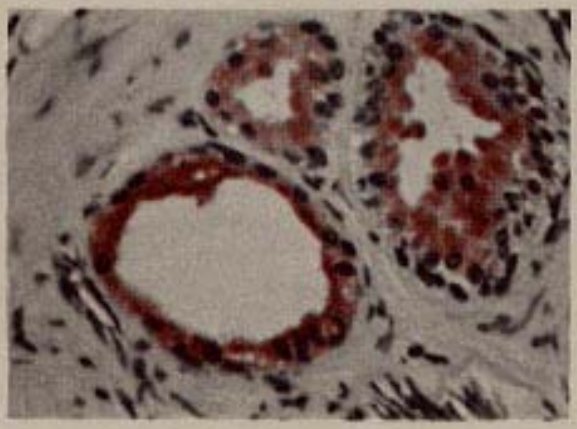
Materials

1. 2.3% periodic acid
2. 0.02% potassium borohydride
3. Rabbit Anti-Human Prostatic Acid Phosphatase, Miles code no. 65-304-1
4. Goat Anti-Rabbit IgG (H+L)), Peroxidase, Miles code no. 61-202-3
5. Ovalbumin, Miles code no. 95-051-2
6. 3 amino-9 ethylcarbazole (Polysciences, Inc.)
7. 30% hydrogen peroxide
8. Dimethyl sulfoxide (DMSO)
9. 0.2M acetate buffer, pH 5.0
10. Phosphate buffered saline, pH 7.2 (PBS)
11. BRIJ 96®

Substrate Solution

The substrate is prepared as follows:

1. Dissolve 10 mg of 3 amino-9 ethylcarbazole in 6 ml of DMSO.
2. Add 50 ml of acetate buffer.
3. Add 0.1 ml of a 0.5% solution of hydrogen peroxide (0.5 ml of 30% H_2O_2 in 30 ml water).



Method

1. Dewax paraffin section to water.
2. Treat with 2.3% periodic acid for 5 minutes at room temperature to block endogenous tissue peroxidase.
3. Wash in tap water.
4. Block aldehyde groups with 0.02% potassium borohydride for 2 minutes at room temperature.
5. Wash in tap water.
6. Rinse in PBS.
7. Incubate in a moist chamber with a 1:500 dilution (e.g., 10μ 1 in 5 ml) of rabbit anti-human prostatic acid phosphatase diluted in 1% ovalbumin in PBS for 30 minutes.
8. Wash off with PBS.
9. Wash in two changes of PBS containing four drops of detergent (1% BRIJ 96) for 5 minutes each.
10. Incubate in a moist chamber wth a 1:500 dilution of goat anti-rabbit IgG (H+L) peroxidase diluted in 1% ovalbumin in PBS for 30 minutes.
11. Wash in two changes of PBS containing four drops of detergent (1% BRIJ 96) for 5 minutes each.
12. Incubate in the 3 amino-9 ethylcarbazole substrate solution and leave for 3 minutes at room temperature.
13. Wash in acetate buffer followed by running tap water for 5 minutes.
14. Counterstain lightly with Mayers' hematoxylin.
15. Blue in tap water.
16. Mount in an aqueous mountant (the final reaction product is alcohol soluble).

NOTE: Some workers may wish to reduce non-specific staining by blocking Fc receptors before reacting the primary antibody. Normal goat serum, Miles code no. 64-292, is useful for this purpose (approximately a 1:5 dilution for > 10 minutes).

Product	Code No	Size
Anti-Human Prostatic Acid Phosphatase (rabbit) liquid	65-304-1	1 ml
Anti-Rabbit IgG (Heavy+Light Chain) (goat) Peroxidase Conjugate	61-202-3	2 ml
Normal Goat Serum, lyophilized	64-292-1	50 ml
Ovalbumin, 5X crystallized	95-051-2	5 g

* BRIJ is a registered trademark of ICI America, Inc.

A Method for Making Gram Controls in Tissue

Ms. Masaye Tanaka, R.T. (CSLT), B.Sc., B.A. and Mr. Colin Henderson, B.Sc., R.T. (CSLT)
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The probability of obtaining a well-fixed piece of tissue containing both Gram-positive and Gram-negative bacteria in their exponential growth phase, uncompromised by antimicrobial therapy and therefore, displaying typical Gram staining, is remote. This lack of suitable Gram control material has encouraged many histotechnologists to make their own *in vitro* controls using suspensions of bacteria in agar.[1,2] Presumably growing bacteria in tissue, *in vitro*, has not been recommended because the resulting necrosis would be unacceptable. Masaye Tanaka decided to challenge this presumption, reasoning that by using a porous tissue, incubated with a heavy suspension of bacteria in a nutrient broth, an acceptable Gram control could be produced.

Materials and Method

1. Blood agar plates were heavily inoculated with pure cultures of a Gram-positive cocci (Staphylococcus) and a Gram-negative bacillus (Proteus or Klebsiella) and incubated overnight at 37 °C.
2. The next day the entire inoculum from each plate was suspended in 15 ml of Brain Heart Infusion broth.
3. Small 0.5 cm clippings of placenta were suspended in the inoculated broth and incubated at 37 °C for three hours. Placenta was chosen because it is porous and is available, fresh and unfixed, from the labour and delivery rooms of most hospitals.
4. After incubation, the clippings of placenta were fixed in 10% neutral buffered formalin overnight and processed in the usual manner.

Results and Discussion

Slides were stained using the Brown and Brenn method as outlined in the A.F.I.P. Manual[3] (the method of choice at the A.F.I.P. is now the Brown-Hopp's procedure).[4,5] The stained slides showed extracellular Gram-positive and Gram-negative bacteria throughout the tissue. The degree of necrosis was unremarkable.

The method described provides an easy and reliable way of making Gram controls in tissue with a minimum amount of necrosis. Bear in mind, however, that these controls are artificial and may exhibit much better Gram staining than your test slide.

The bacteria in the control slide are from an exponential growth phase population, uncompromised by antimicrobial therapy, and are not under attack from the immune system. They have also been properly fixed. For all these reasons they will display optimum Gram staining characteristics. The test bacteria, however, may not be from an exponential growth phase population and may have poorly formed cell walls, as a result of antibiotic therapy. They may also have cell wall damage due to enzyme degradation and complement binding and/or due to poor fixation. For these reasons the test bacteria may stain Gram variable.

In histotechnology the artificial Gram control tests your staining reagents but does not necessarily control for the staining of the test section, therefore, when there is any suspicion of infection a suitable piece of unfixed tissue should always be sent to the microbiology department for culture smear.

References:

1. Robinson, C.R., Methods for Obtaining Fungus, Bacteria and Acid-Fast Controls, *Histo-Logic*., 3(1):26, 1973.
2. England, A.M., Gram-positive and Gram-negative Controls, *Histo-Logic*., 8(1):107, 1978.
3. Luna, L.G., *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, Third Edition, McGraw-Hill Book Co., New York, p. 222, 1968.
4. Brown, R.C. and Hopps, H.C., Staining of Bacteria in Tissue Sections: A Reliable Stain Method, *Histo-Logic*., 7(1):108, 1978.
5. Luna, L.G., Editorial Comment, *Histo-Logic*., 11(2):161, 1981.

HT/HTL Examinations

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Beginning in 1984, the HTL certifying examination will be administered once each year in February, by the Board of Registry. The HT examination will continue to be administered in February and August. The deadline for receipt of applications is October 1 for the February examinations and April for the August examination.

Beginning with the February 1984, HT/HTL examination cycle, the practical component of the examination will vary with each cycle. Utilizing survey statistics from the field, different practicals of comparable difficulty have been developed and will be administered for HT and HTL examinations.

For a complete listing of stains and tissues, refer to the JOURNAL OF HISTOTECHNOLOGY, Vol. 6, No. 2, page 108-109, June, 1983.

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