# Histo-Logic®

Miles Scientific
Vol. XV, No. 2, 1985
Technical Bulletin for Histotechnology • Editor, Lee G. Luna, D. Lit., H.T. (ASCP)

## Floatation Receptacle for Collecting Histologic Material*

**Peter J. Tsaknis, DDS, MS, MEd**
**Ida L. Hayden, AMT**
**Howard University, College of Dentistry**
**Washington, D.C. 20059**

**Virginia J. Lux, HT (ASCP)**
**Formerly Supervisor, Histopathology Laboratory**
**U.S. Army Institute of Dental Research**
**Washington, D.C. 20012**

* U.S. Government Patent No. 4210099 issued by the U.S. Government Patent Office, Washington, D.C.

The floatation receptacle was fabricated to replace the difficult method now being used to recover plastic tissue sections 2 mm or less in diameter. Prior to the development of the floatation receptacle, the method used for recovery of tissue specimens involved dipping a slide into container of water with floating tissue sections and manually picking the sections up onto the glass slide.

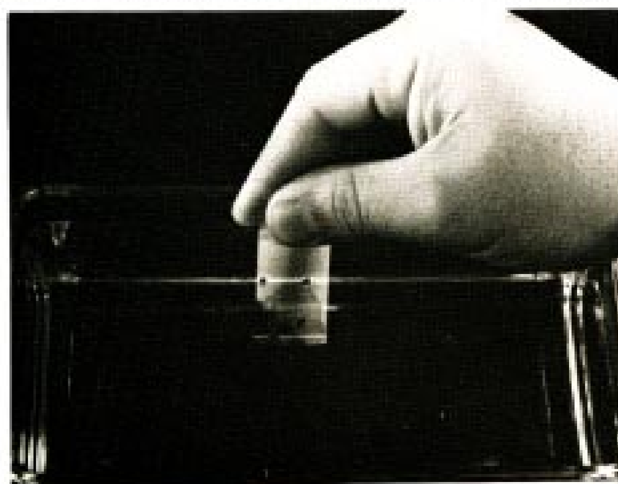

Figure 1 – Attempting to manually recover floating tissue sections.

The size of the tissue sections are often compared to the point of a pencil, movement of the water caused by the slide coming up under the section for recovery resulted in the section moving away from the slide. To recover even a few specimens the technician had to employ both speed and agility. The traumatic effect on the tissue section and the stress on the technician often caused folds and tears in the tissue. These folds trap stain within the tissue as well as chemicals which could adversely affect the final tissue stain. This resulted in many sections falling off the slides during the staining procedure. The folded sections remaining on the slide caused research projects to be significantly affected and in the case of surgical pathologic specimens the quality was sufficiently affected that the pathologist's diagnosis was in jeopardy.

continued on page 241

## INSIDE THIS ISSUE

Floatation Receptacle for Collecting Histologic Material 239
Attend the 1985 NSH Symposium/Convention 240
Introduction to Immunochemical Staining 241
Tissue-Pigments and Artifacts — An Update 242
The Immunohistology Spotlight New Immunohistology Grade Antisera 243
Tissue Section Transfer and Slide Repair 244
Update on Educational Programs 245

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.

COPYRIGHT ©1985, MILES SCIENTIFIC, DIVISION OF MILES LABORATORIES, INC., NAPERVILLE, ILLINOIS 60566

# Attend the 1985 NSH Symposium/Convention Washington, D.C. "Capitalize on Monumental Ideas"

**Capitalize on Monumental Ideas** is the theme for the Eleventh Annual Symposium/Convention of the National Society for Histotechnology, scheduled October 20–25, 1985, in the Washington, D.C. area. The meeting will be hosted by the Hyatt Crystal City Hotel, located directly across the Potomac River from Washington National Airport.

The symposium/convention program includes 37 NSH-CEU approved workshops, presented Sunday through Tuesday, followed by 2½ days of concurrent scientific and veterinary histology lecture sessions. The program offers something for everyone; topics range from basic to advance histotechnology.

The meeting will host two days of scientific exhibits, with more than 40 companies displaying the latest laboratory equipment and supplies. Scientific exhibits will be open: Tuesday, October 22nd, 7–9 p.m.; Wednesday, 9:30 a.m.–4 p.m.; Thursday, 9:30 a.m.–3 p.m. Everyone is welcome to visit this comprehensive scientific exhibition for the histotechnology community.

The 1985 "Professor C.F.A. Culling Memorial Lecture" will be presented by JOHN D. BANCROFT, Senior Chief Medical Laboratory Scientific Officer from the University Hospital, Queens Medical Center of Nottingham, United Kingdom. NSH is delighted to have this world renowned author open the Scientific Session program on Wednesday, October 23rd at 8:30 a.m. His keynote address is entitled "The Evolution of Histotechnology: The Merging of Art and Science".

The 1985 NSH Symposium/Convention will continue the Health Hazard Study conducted by Dr. Kaye Kilburn, Professor of Medicine at the University of Southern California. This program is examining toxic effects of formaldehyde and solvents on the histology technician. Neurobehavioral and pulmonary function testing will be accomplished. It is especially important for those previously tested to be re-evaluated. If attending the meeting, take advantage of this testing program and aid in the endeavor to answer many questions facing the histotechnologist and provide a cleaner environment for all laboratory professionals. Testing will be available Sunday through Thursday, any time of day.

The NSH External Degree Program has workshops scheduled during the symposium/convention and will administer course examinations throughout the week. This program is in conjunction with Thomas Edison College of New Jersey and is a "home study program", whereby histotechnologists earn college credits towards an A.A. Degree with emphasis in histotechnology. NSH is in the process of completing the baccalaureate level program, which will aid those desiring HTL certification. A representative from Thomas Edison College will be available to answer questions relative to the program.

NSH awards will be presented during the banquet activities, Thursday evening, October 24th. The Society offers its members a number of awards and educational scholarships, through the sponsorship of various companies. For award criteria and nomination form, please write the NSH office.

In addition to the educational process afforded by the NSH Symposium/Convention, the Society's official meetings are conducted during the week. Scheduled meetings include the Board of Directors, Regional Directors with state members, State Presidents, Standing Committees, House of Delegates and the annual NSH Membership Meeting. All are "open meetings". Everyone is invited to attend and take part in your Society.

For your evening pleasure, there will be many outstanding hospitality functions throughout the week. The cocktail reception sponsored by Miles Scientific will highlight the banquet activities and bring another year of festivities to a close.

For complete Symposium/Convention program/registration information, write:

**Registrar**
**National Society for Histotechnology**
**5900 Princess Garden Pkwy, -805**
**Lanham, MD 20706**
**(301) 577-4907**

**Power Processing**

**Tissue-Tek® V.I.P.**
**Vacuum Infiltration Processor Series**

- Microprocessor controlled — retains 10 user-defined processing programs and monitors the processing sequence.
- Fail-safe system protects specimens from drying if processing is interrupted.
- 3 processing variables — pressure, vacuum and heat.
- "Sealed System" fume control.
- 10 reagent and 4 paraffin reservoirs.
- Operator training at Miles Scientific.
- Choose from 100 or 200 specimen capacity units in floor or bench-top configuration.

**Miles Scientific**, Division of Miles Laboratories,
2000 North Aurora Road, Naperville, IL 60566

**Miles Scientific**

continued from page 239

The floatation receptacle eliminates the technician having to chase and manually pick up the tissue sections. The receptacle can be fabricated to hold any number of glass slides.

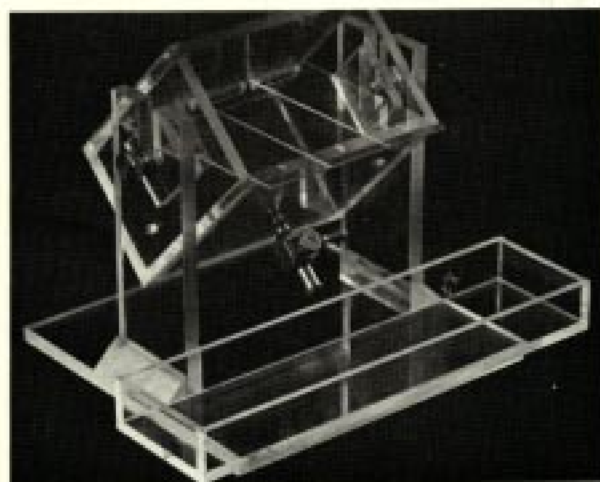

Figure 2 – Floatation receptacle with glass slides in receiving position.

Cut a sufficient quantity of sections and float them in the receptacle water. The receptacle is then angled so that the slides are positioned parallel to the table top. The metal stopcock is opened and the water level lowers causing the tissue sections to gravitationally float downward and settle on the glass slides. When the water level falls to just below the slides, close the stopcock and then manually remove the slides from the floatation receptacle. Drain and place on a warming plate to dry.

The time and stress involved in retrieving the plastic tissue specimens is eliminated, resulting in a superior quality slide. Valuable time is saved by eliminating the need to recut sections for staining. Pathologists and researchers are freed from diagnosing and evaluating specimens of less than excellent quality.

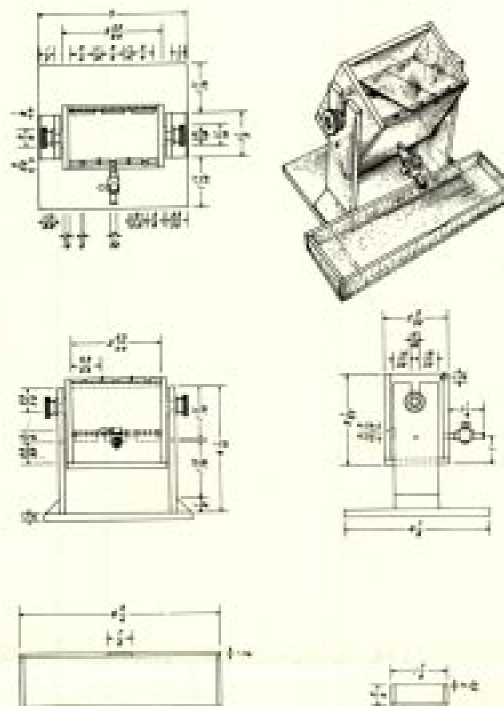

Figure 3 – Detailed plans for fabrication of the floatation receptacle.


# Introduction to Immunochemical Staining

## November 16-17, 1985

**Presented by:**
**Lee G. Luna**
**Center for Histotechnology Training**

This 1½ day course will deal with the basics of immunochemical staining. It is intended for individuals who want to know what is involved in the methodologies dealing with this new up-and-coming technology and persons who expect to be involved in doing immunochemical staining. The course will cover:

1. The Immune System–how it works, to include definition of terms.
2. Immunochemical Staining–how it works, to include definition of terms.
3. Discussion on various methods of staining.
4. The last half day will be devoted to the practical aspects where several staining procedures will be performed by participants.

The course will be presented with the aid of 2x2 photomicrographs and staining kits, furnished by Miles Scientific, Naperville, Illinois.

This course is very basic.

For further information, contact:

**Registrar**
**P.O. Box 2453**
**Rockville, MD 20852**
**(301) 468-6552**

**Note:** NSH-CEU's will be awarded participants.

# Tissue Pigments and Artifacts — An Update*

**Anthony F. Henwood, Concord Hospital**
**Concord, N.S.W. 2139, Australia**

Histotechnologists are required to identify many pigments such as melanin, carbon and bile, etc. Most pigments can be demonstrated by various histochemical techniques but some remain an enigma even with today's technology. Presented below are some examples of pigments and artifacts which can be encountered in histological preparations.

**Melanosis Duodeni:** This is a brown pigment sometimes seen in duodenal biopsies. It is deposited in the tips of villi and is not seen below the level of the Brunner's glands.[1] There have been suggestions as to the composition of this pigment. Some authors believe that this pigment is melanin, rather like that contained in nevus cells.[1] It shows positive staining after Fontana's silver technique and is bleached out by hydrogen peroxide. It is Oil Red O and Periodic Acid Schiff (PAS) positive, while negative for bile. As such, it mimicks the pigment present in Melanosis coli. Melanosis duodeni has been found negative for iron, though in a case seen at Concord Hospital, there seemed to be iron pigment associated with the larger granules. On the basis of electron probe x-ray analytical findings, the pigment is essentially iron sulfide and not melanin or a melanin-like compound.[2] It is believed that this pigment is derived from hemorrhage in the gastrointestinal tract.[2] This could possibly be caused by the consumption of iron tablets after donating blood or during pregnancy.

**Melanosis Coli:** This is a golden brown pigment occurring in the colon and rectum. It is related to chronic constipation and to the consumption of purgatives of the anthracane group. This pigment is present in macrophages within the lamina propria of the muscularis mucosae. Histochemically, the pigment is argentophilic and reacts positive with Schmorl's reaction. As such, it is similar to melanin. It is PAS positive. There is little demonstrable iron associated with this pigment.[3]

**Russell Bodies:** Russell bodies are bright eosinophilic bodies that are found in association with plasma cells. They are present either in the cytoplasm of plasma cells, or as free extracellular bodies. They are best demonstrated by PAS. Russell bodies are not specific to any particular disease or lesion, but are seen in general plasma cell proliferations such as chronic inflammatory conditions or rheumatoid arthritis.[4] Bright eosinophilic bodies may also be seen in hepatocellular carcinomas and after PAS staining, may appear quite similar to Russell bodies. Since liver carcinomas often produce $\alpha_1$-antitrypsin, an immunochemical technique can be employed to demonstrate this antigen and therefore differentiate it from Russell bodies.

**Crooke's Hyaline Change:** Crooke first described a peculiar condition that sometimes occurs in the pituitary.[5] This condition was associated with increased serum adrenocorticotropic hormone (ACTH) levels, either due to a basophil pituitary adenoma, resulting in Cushing's disease, or ectopic ACTH production from such tumors as small cell carcinomas of the lung and prostate, resulting in Cushing's syndrome. Crooke noted that the basophils in the pituitary showed a peculiar replacement of the granular material of their cytoplasm by a homogeneous hyaline material. Using a PAS stain, the hyaline material first accumulated in the zone surrounding the nucleus and then spread gradually throughout the cytoplasm, replacing the PAS granular material before it. ACTH was not demonstrated in Crooke's hyaline change, using immunohistochemistry. The functional significance of this change is unknown.

**Peripheral Orceinophilia:** Artifactual peripheral staining of needle biopsies of the liver has been documented with several stains.[6] This is particularly a problem when the orcein stain is used, since this artifact could be confused with hepatocytes containing hepatitis B surface antigen.[7] This artifact occurs in a significantly larger proportion of needle biopsies, as compared to wedge biopsies. It has been suggested that this staining is caused by the denaturation of the biopsy edges due to the physical force of the needle entering the liver. If this artifact causes diagnostic concern, then the use of an immunochemistry stain for hepatitis B surface antigen is suggested.

**Dubin-Johnson Pigment:** The Dubin-Johnson syndrome is described as a chronic idiopathic jaundice, with deposition in liver cells of a pigment of unknown constitution. Several tags have been applied to this pigment, such as lipomelanin, lipochrome or mesobilirubin.[8] The pigment somewhat resembles the normal lipofuscin pigment and occupies a similar pericanalicular site, but is darker, more abundant, larger and more variable in size. Dubin-Johnson pigment has few reliable constant features. It is argentophilic, as well as weakly PAS positive.[8] The distinction between lipofuscin and Dubin-Johnson pigment is usually clear morphologically.

continued on page 245

# New Immunohistology Grade Antisera

**For Research Use Only**

The following antisera are specifically characterized for immunohistology:

## Polyclonal Antiserum

| Code No. | Antiserum to: | Host Form | Size |
|---|---|---|---|
| 64-735-1 | Human $\alpha_1$-Antichymotrypsin | sh/ly | 1 ml |
| 64-731-1 | Human Cathepsin B | sh/ly | 1 ml |
| 64-732-1 | Human Cathepsin G | sh/ly | 1 ml |
| 64-733-1 | Human CEA (NCA II adsorbed) | sh/ly | 1 ml |
| 64-730-1 | Human Elastase | sh/ly | 1 ml |
| 64-734-1 | Human α-Fetoprotein | sh/ly | 1 ml |
| 64-713-1 | Serotonin-H | rb/ly | 0.25 ml |
| 64-720-1 | Vasointestinal Peptide-H | rb/ly | 0.25 ml |
| | **Cytoskeletal Related** | | |
| 65-093-1 | α-Actinin | rb/lq | 1ml |
| 65-797-1 | Spectrin | rb/lq | 1 ml |

H = Hemocyanin used as carrier protein for hapten antigen

## Monoclonal Antibodies

| Code No. | Monoclonal Antisera to: | Clone | Host Form | Size |
|---|---|---|---|---|
| 63-750-1 | Human Myoglobin | MG-1 | mo/lq | 0.5 ml |
| | **Extra-Cellular Matrix** | | | |
| 69-605-1 | Human Collagen Type IV | 117-3 | mo/lq | 0.2 ml |
| 69-625-1 | Keratan Sulfate | 1/20/5-D-4 | mo/lq | 0.2 ml |
| 69-610-1 | Laminin | LAM-1 | rt/lq | 0.2 ml |
| 69-620-1 | Proteoglycan ΔDi 0S | 1-B-5/C5 | mo/lq | 0.2 ml |
| 69-622-1 | Proteoglycan ΔDi 4S | 9-A-2/E9 | mo/lq | 0.2 ml |
| 69-621-1 | Proteoglycan ΔDi 6S | 3-B-3/C1 | mo/lq | 0.2 ml |

More information on each of these antisera, including typical working dilutions in various immunohistology assays, are available on request from our Customer/Technical Service office.

### Nomenclature

We have adopted several forms and abbreviations.

**mo** - mouse
**rb** - rabbit
**rt** - rat
**sh** - sheep

**lq** - liquid
**ly** - lyophilized

### Ordering Information

Current pricing and terms of sale can be found in the numerical price list published by the Miles Scientific Sales Office serving your country.

Inquiries about product availability or technical data should be made by telephone or letter to your nearest Miles Scientific Sales Office. Ask for Miles Scientific Biochemicals/Immunochemicals.

In countries where it is required, an import license must be secured by the customer prior to placing an order.

**Discounts:** Standing orders and bulk or multiple package quantities may be eligible for discounts. Contact your Miles Scientific Sales Office for a quotation.

**U.S.A.**

**Miles Scientific**
Division of Miles Laboratories, Inc.
2000 North Aurora Road
Naperville, IL 60566
Telephone: (800) 348-7465
in Illinois, call (312) 983-5700
Telex: 210197

**UNITED KINGDOM EIRE/SCANDINAVIA**

**Miles Scientific**
Miles Laboratories, Ltd.
Stoke Poges, Slough SL2 4LY
Buckinghamshire, England
Telephone: (2814) 5151
Telex: 848337 MILABS G

**WEST GERMANY**

**Miles Scientific**
Bayer Diagnostik, GmbH
Steinerstrasse 15
P.O. Box 701 360
D-8000 Munich 70
Telephone: 089-724030
Telex: 522406 MILES D

**AUSTRALIA NEW ZEALAND**

**Miles Scientific**
Miles Laboratories Australia (Pty), Ltd.
Private Bag 5
Mulgrave North 3170, Victoria
Telephone: (03) 560-0611
Telex: MILES AA31139

**JAPAN**

**Miles Scientific**
Miles Labs, Inc.-Japan Br.
Aoyama Bldg.
2-3, Kita-Aoyama 1-chome
Minato-ku, Tokyo 107
Telephone: (03) 403-9951
Telex: 242-3548

**CANADA**

**Miles Scientific**
Miles Laboratories, Ltd.
77 Belfield Road
Rexdale, Ontario M9W 1G6
Telephone: (416) 248-0771
Telex: 06-989399

**ITALY/SWITZERLAND GREECE**

**Miles Scientific**
Miles Italiana, S.p.A.
via F.L. Miles 10
20040 Cavenago Brianza
Milano
Telephone: 02-95019231
Telex: 332016 MILESLAB I

**FRANCE/BENELUX**

**Miles Scientific**
Laboratoires Miles S.A.
Tour Maine Montparnasse
33 avenue du Maine
75755 Paris — Cedex 15
Telephone: Paris 5385243
Telex: MILAB 204315 F

**SPAIN/PORTUGAL**

**Miles Scientific**
Miles-Martin Laboratories, S.A.E.
Plaza de Espana, 10
Madrid-13
Telephone: Madrid 242-5104
Telex: 22590 MILAB E

# Tissue Section Transfer and Slide Repair

**Jack B. Wenger**
**Armed Forces Institute of Pathology**
**Washington, D.C. 20306**

Slides are often submitted to the laboratory for section transfer or repair of microscopic slide. The slides are usually in one of the following conditions:

a. broken with coverslip intact;
b. broken with coverslip shattered or missing;
c. slide with multiple sections. (This generally occurs when no paraffin blocks are available and one or more sections need to be transferred to other slides so special staining can be performed; or to produce additional slides for study, file or when a contributor's slide must be returned.)

The following procedures provide a useful means of accomplishing and/or correcting all of the above situations.

1. a. Broken slide with coverslip intact: *Before removing* coverslip in xylene, attach a new slide to the back of the broken slide with a cement that *is insoluble in xylene or chloroform* (e.g., Duco cement) and allow to dry overnight.

Figure 1 – Apply Duco cement to new slide.

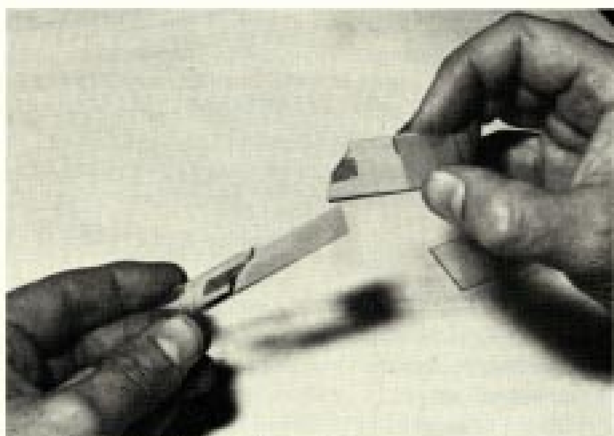

Figure 2 – Attach broken slide to Duco cement-coated slide.

   b. Both slide and coverslip are shattered: Arrange the pieces on a new slide and follow the cementing instructions in 1a.

   c. Slide with multiple sections: Remove coverslip with gentle heat, then remove mounting medium with xylene and proceed to *Step 3.*
   **Note:** If slide is an unstained paraffin section, deparaffinize in xylene and proceed to *Step 3.*

2. **Caution:** Use only with *Step 1a or 1b.*
   Soak coverslip off broken slides using warm chloroform or xylene. *Do not heat over flame.*

Figure 3 – Remove coverslip from broken slide.

3. Rinse slide in xylene. While slide is still wet from xylene rinse apply Diatex to sections. Cover only the sections not entire slide.
   **Note:** Diatex Liquid Cover Glass #M7638-Diatex is available from American Scientific Products in McGaw Park, IL. Other "liquid coverglass" type products may also be suitable for use with this method. The following characteristics are desirable:
   a. must be soluble in xylene, chloroform or toluene;
   b. must be flexible when dry, to prevent shattering as the section is removed from the slide;
   c. should be clear, allowing the technician to see the tissue section.

Figure 4 – Apply Diatex to the tissue section.

4. Allow slide to dry overnight at room temperature or on a 37° – 40°C warming table.
5. Soak slide for several hours in warm water.
6. Try to remove the slide and attempt to peel the section from the slide by gently and carefully teasing the edge of the section, being careful not to cause distortion. If the section peels away easily, proceed to *Step 7*. If section resists peeling, continue to soak in warm water until it peels easily.

Figure 5 – Tease the tissue section from the slide before remounting on unbroken microscopic slide.

7. Trim excess Diatex from around the tissue.
8. Immerse both the Diatex impregnated section and the fresh slide in warm tissue floatation bath containing ¼ teaspoon dissolved high bloom (250) gelatin and 3 ml undiluted formalin.
9. Mount Diatex strip to clean slide *with tissue side against the slide.*
10. Wipe off excess water from around the Diatex impregnated section and allow to dry overnight in a horizontal position, as you would a paraffin section (37° – 40°C warming table).
11. Dissolve the Diatex in xylene and mount coverslip with resinous media. If slide is unstained, hydrate in the normal manner and stain with desired procedure.

continued from page 242

## Conclusion

Some examples of unusual pigments and artifacts that can be encountered in histological sections have been described. They include Melanosis duodeni, Melanosis coli, Russell bodies, Crooke's hyaline change, Peripheral orceinophilia and Dubin-Johnson pigment. Current available techniques for their demonstration have been outlined, but they tend to lack specificity and further work is required to accurately identify them.

## Acknowledgement

I would like to thank Mr. Alan Smith for his advice and support in the preparation of this manuscript.

## References:

1. Breslaw, L. (1980) *Gastroint. Endosc.* *26*(2), 45.
2. Pounder, D.J., *et al.* (1982) *J. Submicrosc. Cytol.* *14*(2), 389.
3. Ghadially, F.N. and Parry, E.W. (1966) *J. Path. Bact.* *92*, 313.
4. Cook, H.C. (1974) *Manual of Histological Demonstration Techniques*, 250. Butterworths & Co.:London, England
5. Crooke, A.C. (1935) *J. Path. Bact.* *41*, 339.
6. Lyon, H. and Prento, P. (1973) *ACTA Path. Microb. Scand.* *81*, 9.
7. Henwood, A.F. (1983) *Aust. J. Med. Lab. Sc.* *4*(2), 76.
8. Dubin, I.N. (1958) *Am. J. Med.* *24*, 268.

* Presented at the Inaugural Joint New South Wales-Victoria Weekend Symposium of the Histotechnology Groups, Clyde Cameron College, Wodonga, June 18-19, 1983.

## CORRECTION

"Rapid Dieterle Stain with Microwave Heating", Histo-Logic, Vol XV, No. 1, 1985, pg 232. The distilled water, 60 ml, in the developing solution, should be omitted. The last addition to the developing solution should read:

10% Gum mastic solution . . . . . . . . . . . . . . 10.0 ml

# Update on Educational Programs

### Immunohistology: Principles and Technical Tips

Over 600 histotechnologists have attended this workshop on a local level since it was first offered in March 1985!

This workshop on immunoperoxidase techniques is of practical value to those histotechnologists with beginner-to-intermediate level experience.

Contact your Miles Scientific or American Scientific Products representative for information on workshops scheduled in your area.

### Immunohistology: Applications in Pathology

This popular seminar and workshop is intended primarily for pathologists. Histotechnologists with some experience in immunoperoxidase techniques will also benefit from this course.

Two sessions are scheduled for the near future:

Toronto, Ontario, Canada - August 28 & 29, 1985
Chicago, Illinois - September 26 & 27, 1985

Call or write the Educational Program Coordinator at Miles Scientific for registration brochures, or contact your Miles Scientific representative.

**Coming Soon! A new line of over 100 Avidin-Biotin and Monoclonal PAP kits. Please inquire.**

**Tissue-Tek® Immunohistology Kits**

- 38 PAP kits for enzymes, hormones, immunoglobulins, viral antigens, tissue specific markers and other cellular antigens.
- Demonstrate tissue antigens or disease status to supplement routine morphologic analysis.
- Visualize the antigen-antibody complex with a standard light microscope.
- Each kit contains reagents for 30 tests.
- Reliable results with paraffin sections. Useful with frozen sections, smears, imprints or cytospins.

**Stains You Can Identify With *Routinely!***

**Miles Scientific**
Division of Miles Laboratories
2000 North Aurora Road
Naperville, IL 60566

**Miles Scientific**

**Miles Scientific**
Division of Miles Laboratories, Inc.
2000 North Aurora Rd.
Naperville, IL 60566

Address Correction Requested

# Histo-Logic

A Technical Bulletin for Histotechnology

To receive your own copy of HISTO-LOGIC, or to have someone added to the mailing list, submit home address to: **Miles Scientific,** Division of Miles Laboratories, Inc. 2000 No. Aurora Rd., Naperville, IL 60566. 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 20706. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.