7/28

HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor   A Technical Bulletin for Histotechnology   Vol II No. 4 - Oct., 1972

Preliminary Embedding in Agar-Agar

Herbert Z. Lund, M.D.,
Linda Marshall, HT (ASCP)
The Moses H. Cone Memorial Hospital
Greensboro, North Carolina 27405

In 1961, Lund, et al., reported that preliminary embedding in agar-agar is helpful in safely carrying small biopsies through the process of dehydration and embedding, in orientating small specimens correctly, and in combining multiple small biopsies into a single block for individual identification. This article describes the technique used currently.

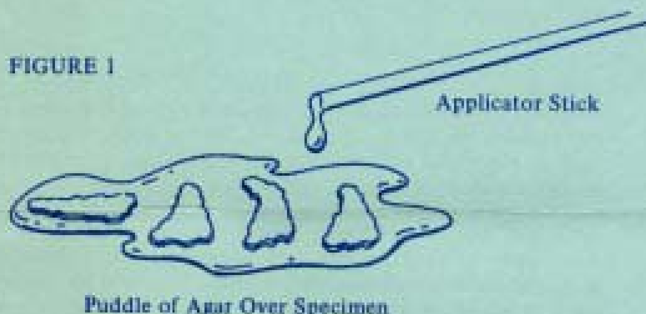
Agar-Agar Solution

Agar-agar	4.0 gm
Distilled water	96.0 ml

Dissolve by heating on a boiling water bath. Add 2 crystals of thymol to prevent fungal and bacterial growths.

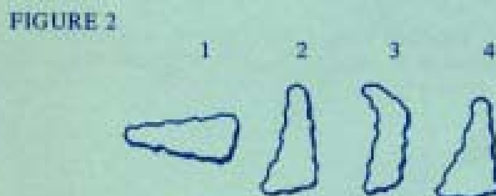
The agar is dispensed as needed in test tubes, kept at 56° or 60° C. We use a constant temperature test tube holder (Thermolyne Dri-Bath Model DB5925E, Dubuque, Iowa). Tubes of agar are kept where gross tissue is examined and cut.

FIGURE 1



Puddle of Agar Over Specimen

FIGURE 2



Preliminary Embedding

Small specimens are properly orientated on a flat, clean surface. It is helpful to have small solidified particles of agar on hand to prop up thin or limp pieces of tissue and maintain them on edge. Using an applicator stick, hot agar is dropped upon the tissue to form a small pool (Fig. 1). This is allowed to solidify and is trimmed into a small block. The block of agar is processed as a biopsy specimen, and embedded in paraffin in the desired position. (Agar tends to harden during dehydration and embedding.)

Combining several small biopsies into one block is accomplished by arranging them in a pattern which makes the first of the series distinct. The first of the series is placed at right angles. Subsequent biopsies are orientated on a vertical plane (See Fig. 2). In our laboratory the agar block is trimmed to a point on the side where the right angled specimen is embedded.

Advantages

Small biopsies are always carried through the embedding process safely. Orientation of very small biopsies can be done easily at the time of paraffin embedding. Multiple individually identified biopsies can be embedded in one block and mounted on one slide.

Disadvantages

Agar stains faintly with hematoxylin, and may stain intensely if a slide is "heavy on the hematoxylin." The authors find that agar-agar boiled on a direct flame becomes more basophilic than agar dissolved carefully in a hot water bath. Agar also stains with orcein, methenamine silver and other special stains, making an unattractive slide. However, the agar surrounds the tissue and does not permeate it, so the staining of the tissue itself is not altered.

Precautions

A minute specimen embedded with a large one may be cut through when a paraffin block is trimmed to obtain a full cross section of the large peice. Large specimens should be embedded separately.

Fine fragmented tissue specimens may spread in liquid agar and merge with particles from a second biopsy. To avoid this, small blocks of solidified agar are inserted between fragmented specimens before hot liquid agar is applied. In many cases it is best to process fragmented material in separate blocks.

Test tubes of agar used daily may become contaminated. Congealed agar at the upper end of the test tube projecting above the water level can be at an optimum temperature for bacterial growth. A fresh preparation should be made if this occurs.

Conclusion

After a decade of experience with this technique, in a service where small skin biopsies are predominant, the authors have found it time saving and economical.

Reference

Lund, H.Z., et al.: Preliminary embedding in agar-agar. *Amer. J. Clin. Path., 36:* No. 6, 562-564, 1961.

COPYRIGHT © 1971, LAB-TEK PRODUCTS, DIVISION MILES LABORATORIES INC.

Burned Tissue Artifact and Staining Technique

An Editorial

The artifact presented herein was submitted by Mr. Julian M. Slaughter, Veterans Administration Hospital, Topeka, Kansas, 66622.

The problem concerns the causative agent for vacuolization and the highly eosinophilic staining reaction on the peripheral margins of the tissue (Figure 1A). Microscopic examination of the slide strongly suggested burning or drying. Subsequent written communication with Mr. Slaughter confirmed that the specimen was surgically removed with a thermal knife. The burned areas were microscopically identified with a modified Verhoeff stain (Figure 1B). The method is one of three published in 1956 by Hinshaw and Pearse.[1] It has been used advantageously in the AFIP Laboratory for differentiating burned from normal tissue. If extensive work is contemplated in staining burned tissue, consult the original article since another method may prove more desirable for a particular need.

Fixation
Phosphate-buffered 10% neutral formalin.

Microtomy
Paraffin sections at 6 micra.

Solutions

Verhoeff's Stock Solution

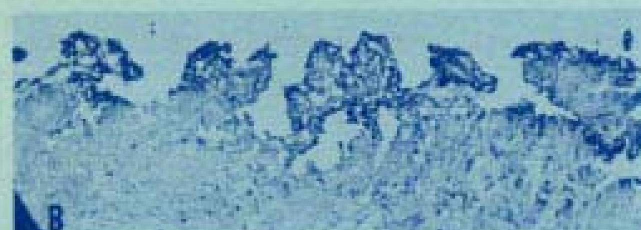
Solution A

Hematoxylin . . . . 5.0 gm
Ethyl alcohol, 100% . . . . 95.0 ml
Dissolve with the aid of heat.

Solution B

Ferric chloride. . . . . 10.0 gm
Distilled water . . . . 90.0 ml

H & E stained section demonstrating vacuolar surfaces and highly acidophilic peripheral margin. The vacuoles are also characteristic of an artifact produced during tissue freezing. Burned tissue artifacts can be differentiated by the procedure presented herein. X70.



The dark area shows the staining reaction produced by the modified Verhoeff procedure. Notice the dark area on the surface of the tissue. The reaction is better illustrated microscopically than photographically. X70.

Solution C

Iodine . . . . 2.0 gm
Potassium iodide . . . . 4.0 gm
Distilled water. . . . . 94.0 ml

Verhoeff's Working Solution

Immediately before use, combine above solutions in the following order:

Solution A . . . . 40.0 ml
Solution B . . . . 16.0 ml
Solution C . . . . 16.0 ml

2% Ferric Chloride

Ferric chloride. . . . . 2.0 gm
Distilled water. . . . . 98.0 ml

Van Gieson

Acid fuchsin, 1% aqueous . . . . . . 2.5 ml
Picric acid, saturated aqueous. . . . 97.5 ml

Results

Normal epidermis. . . . . Gray
Burned epidermis. . . . . Pale yellow
Normal tissue . . . . Yellow to brown
Burned tissue. . . . . Black

Staining Procedure

1. Decerate and hydrate to distilled water.
2. Stain in working Verhoeff's solution for 30 minutes.
3. Differentiate in 2% ferric chloride solution by dipping slide in this solution 20 times in 30 seconds.
4. Wash in running tap water for 3 minutes.
5. Place in 95% ethyl alcohol for 1 minute.
6. Wash in running tap water for 5 minutes.
7. Counterstain in Van Gieson's solution for 15 seconds.
8. Dip slides once in tap water.
9. Dehydrate in 95%, 100% ethyl alcohol, 2 changes each.
10. Clear in xylene, 3 changes.
11. Coverslip.

Remarks
Verhoeff's staining solution is stable for 24 hours. Fresh staining solution should be used after every 75 slides. Differentiation in 20% ferric chloride is accomplished more rapidly than in the standard Verhoeff elastic stain. Until exact timing is established, a single slide may be examined in water to determine the degree of differentiation. The last washing in tap water (step 8) must be thorough, particularly when staining a group of slides simultaneously. A short exposure to Van Gieson's solution yields slides which may be interpreted very rapidly. The more brilliant colors produced by a longer counterstaining time are less easy to read.

Reference

1. Hinshaw, J.R. and Pearse, H.E.: Histologic Techniques for the Differential Staining of Burned and Normal Tissue. *Surg. Gyn. Obstet., 103:* No. 6, 726-730, 1956.

Modified Gomori's Method for Staining Reticulum and Collagen

Charles J. Churukian
The University of Rochester
School of Medicine and Dentistry
Rochester, New York 14620

Gomori's[1] method, for many years, has been a preferred technique for demonstrating reticulum fibers. It is consistent, relatively easy to perform, gives good results, and tissue sections rarely fall off the slides. Background staining must be considered. Gomori's method demonstrates reticulum fibers as black, collagen fibers appear rose to brick red, nuclei are rusty brown to deep red, and other tissue elements stain grey to brownish red. Background shades can vary considerably and sometimes are so intense they have a masking effect on the reticulum fibers.

The present modification of Gomori's method for reticulum was developed to reduce the background staining. A relatively clear background accentuates reticulum fibers and various counterstains for collagen can be employed. It is useful in differentiating reticulum and collagen and especially valuable in characterizing connective tissue elements in pathological conditions of the liver and lymph nodes.

Fixation
10% phosphate-buffered neutral formalin

Microtomy
Cut paraffin sections at 6 micra.

Solutions

Acidified Potassium Permanganate

Potassium permanganate	0.25 gm
Distilled water	100.00 ml
Sulfuric acid	0.25 ml

Potassium Metabisulfite

Potassium metabisulfite	2.0 gm
Distilled water	98.0 ml

10% Silver Nitrate

Silver nitrate, ACS	10.0 gm
Distilled water	90.0 ml

10% Potassium Hydroxide

Potassium hydroxide	10.0 gm
Distilled water	90.0 ml

20% Formalin

Formaldehyde 38-40%	20.0 ml
Distilled water	80.0 ml

0.2% Gold Chloride, Brown*

Gold chloride, brown	0.2 gm
Distilled water	100.0 ml

*Gold trichloride acid: $HAuCL_4$

(Ed. Note: Gold chloride, brown, is caustic, detrimental to the skin and should be used with extreme care.)

2% Sodium Thiosulfate

Sodium thiosulfate	2.0 gm
Distilled water	100.0 ml

Ammoniacal Silver

To 10 ml of 10% aqueous silver nitrate add 2.5 ml of 10% potassium hydroxide. Add concentrated ammonium hydroxide drop by drop with constant shaking, until the precipitate dissolves. Then add 10% silver nitrate drop by drop with shaking, until a few granules of precipitate reappear. This may require 10 or more drops. Make up the solution to 45 ml with distilled water.

Staining Procedure

1. Decerate and hydrate slides to distilled water.
2. Oxidize with acidified potassium permanganate for 1 minute.
3. Wash well with tap water.
4. Reduce with 2% potassium metabisulfite for 1 minute.
5. Wash well with tap water followed with 3 changes of distilled water.
6. Place in ammoniacal silver solution for 1 minute.
7. Rinse with 3 changes of distilled water for a total of 10 seconds.
8. Reduce in 20% formalin for 3 minutes.
9. Wash with tap water for 3 minutes.
10. Tone in 0.2% aqueous gold chloride, brown, for 3 minutes.
11. Wash briefly with tap water.
12. Fix in 2% aqueous sodium thiosulfate for 1 minute.
13. Wash well with tap water and rinse in distilled water.
14. Counterstain with one of the following collagen stains.

A. Modified Gomori's Trichrome[2,3]

1. Stain in Weigert's Iron Hematoxylin[3] for 3 minutes.
2. Wash well with tap water.
3. Differentiate in 0.5% HC1 in 70% alcohol for 1 minute.
4. Wash well with tap water.
5. Stain with Gomori's one step trichrome stain, made with aniline blue (C.I. 42755), for 5 minutes.
6. Wash with 0.5% acetic acid water.
7. Rinse in distilled water.

B. Van Gieson

Stain in Van Gieson's stain [3,4] for 2 minutes.
Formula: 10 ml of 1% aqueous acid fuchsin (National Aniline C.I. 42685). 100 ml of saturated aqueous picric acid.

15. Dehydrate in 95%, 100% ethyl alcohol, 2 changes each.
16. Clear in xylene, 3 changes.
17. Coverslip.

Results

Reticulum – black
Collagen – blue or red, depending upon the counterstain

References

1. Gomori, G.: Silver impregnation of reticulum in paraffin sections. *Amer. J. Clin. Path., 13:* 993-1002, 1937.
2. Gomori, G.: A rapid one step trichrome stain. *Amer. J. Clin. Path., 20:* 661-664, 1950.
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. Mallory, F.B.: Pathological Technique, New York, Hafner Publishing Co., 1968.

NOTE: ADDRESS CHANGE
Lee Luna, Editor
P.O. Box 36
Lanham, Maryland 20801

HISTO-LOGIC T.M.
A Technical Bulletin for Histotechnology Vol II No. 4

LTM 21-771 Printed in the U.S.A.

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

Address Correction Requested

BULK RATE
U.S. POSTAGE
PAID
PERMIT NO. 4954
Chicago, Illinois

It's easy to see...

why our newly improved TISSUE-TEK® II Cassette makes tissue processing and embedding more efficient:

- in keeping with the most advanced histologic techniques, the new style Cassette can be used with any processor
- the new transparency allows you to see the tissue through the Cassette for better positioning
- the see-through feature lets you detect air bubbles or debris which could cause confusing artifacts in the section

In ordering the new-style Cassette, use the same #4191.

The new Cassette utilizes the same metal cover #4194.

In order to write on the new Cassette, a soft-leaded art pencil is recommended.

NOTE: Tissue-Tek II Processing/Embedding Cassette is a plastic "disposable" designed for one-time use. Exposure to chloroform and/or molten paraffin contaminated with an excess of any clearing agent may cause Cassette distortion.

Editor's Corner

Let's Get Our Facts Straight

The *ARGENTAFFIN AND ARGYROPHIL REACTIONS.* The term *argentaffin* is of Latin origin: argentum, silver; affinis, having an affinity for. *Argyrophilia* is of Greek derivation: argyros, silver; and philein, to love. Despite their etymological similarity, the terms have acquired distinctly different meanings. *Argentaffin* means possessing the ability to reduce silver salts without light or the aid of any reducing agent. *Argyrophil*, on the other hand, indicates that the tissue element can be impregnated with silver, but that light or a reducing agent is required to produce the black deposit of metallic silver.

Examples		
Argentaffin	**Argyrophil**	
Fontana-Masson	Sevier-Munger	Nauta-Gygax
Gomori-Burtner	Warthin-Starry	Grocott
	Reticulum Reactions	Von Kossa

Reference: Lillie, R.D.: Histopathologic Technic and Practical Histochemistry. McGraw-Hill Book Co., Blakiston Division, New York, 3rd ed., 239, 1965.

ANNOUNCEMENT

GOLDEN FORCEPS AWARD

The editor and publisher of HISTO-LOGIC announce the first Golden Forceps Award to be presented to the author of the best article contributed to HISTO-LOGIC. All articles accepted from the first issue, July, 1971, through the March 1, 1973 issue will be considered. The winner will be notified by mail, and a set of golden forceps presented in person at the October, 1973 AFIP Histopathologic Symposium. The award recipient will also receive an expense paid trip to Washington, D.C. to attend the symposium.

Articles will be judged on originality, clarity, and scientific contribution. All articles become the property of HISTO-LOGIC.