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Proper Tissue Embedding Practices

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It was brought to my attention by one of my graduate students that nothing has ever been published on the proper placement of the tissue specimen in an embedding mold, so the tissue can be sectioned more easily and with less cutting artifact. I have been teaching these tissue placement techniques for so many years that it had not occurred to me that students could not refer to published material to justify the usefulness of these techniques.

There are a few basic rules related to embedding practices which should be followed to avoid streaking or lines caused by hard particles in tissue; to avoid compression artifact; to compensate for the different spreading properties of both paraffins and tissues; and to avoid artifactual elements in the many different types of tissues from interfering with the quality of the section. Following are a few examples of proper tissue placement techniques.

1. The specimen should be embedded with the longest side parallel to the knife edge (Fig. 1). If a long specimen is placed perpendicular to the knife (Fig. 1A), the tissue has a tendency to jam or compress. Microscopically the tissue will be distorted.

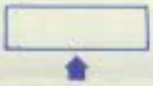
Fig. 1 

Fig. 1A 

2. The tissue specimen should be placed in the mold so that capsules, skin surface with hairs or keratin, or indications of slight calcification are at the top when placed on the microtome (Fig. 2). This allows the knife to pass over this area last. This prevents marring of the tissue section by the dragging effects of harder areas through the tissue.

Fig. 2 

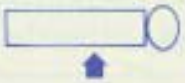
3. If two or more specimens are placed in one mold, the tissues should be in contact with one another (Fig. 3). If there is paraffin between specimens embedded together, the tissue will pucker during microtomy. These puckers eventually result in tissue wrinkles which form while the tissue sections are drying.

Fig. 3 

4. When it is desirable to place two specimens in the same mold, they should be of similar consistency so the knife will encounter similar resistance as it sections the tissue. For example, a piece of fat and uterus should not be embedded together.
5. Any circular specimen which contains a lumen should be placed on end (Fig. 4). This is true of tissue orbs, vessels, vas deferens, etc.

Fig. 4 

6. Muscle biopsies should be grossed and embedded to obtain a longitudinal and a cross section on the same slide (Fig. 5).

Fig. 5 

Recommendation for Kristensen's Decal Solution

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I am often surprised in talking with colleagues and reading procedures in journals that Kristensen's decalcifying solution is not known and/or rarely cited as the chosen method of demineralization.

I have been using Kristensen's decalcifying solution routinely for eight years and highly recommend it. Shrinkage is minimal, speed of decalcification is moderate (about 1 week for an adult human molar), final basophilic staining properties are excellent, overexposure (within reasonable limits) does not damage the tissue excessively, and it is one of the few demineralizing solutions that isn't particularly harsh to the soft tissue components.

My own high opinion of this solution was reinforced by the support given it in "The Preparation of Decalcified Sections" by Edward Brain.[1]
The formula is simple:

STOCK SOLUTIONS:	A 8N Formic Acid
	B 1N Sodium Formate
WORKING SOLUTION:	Mix equal volumes A & B[2]

References

1. Brain, Edward B.: The Preparation of Decalcified Sections, Charles C. Thomas, Publisher.
2. Humeson, Gretchen L.: Animal Tissue Techniques, 2nd ed., W. H. Freeman & Company, Publisher.

Stability of More Commonly Used Special Staining Solutions

An Editorial

The information contained in this handout concerning the stability of solutions used in the more common special stains is presented in answer to the many requests made by technicians throughout the country.

An attempt was made to include information for the more commonly used special stains. The staining procedures can be found in: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. (Luna, L.G., Ed), New York, McGraw-Hill, Blakiston Division, 1968.

General Remarks

1. Check stock solutions periodically for signs of precipitate. This can be done by holding the bottle in front of a strong light; for example, a gooseneck lamp.
2. All solutions should be labeled with date solution is prepared and initialed by histotechnologist.
3. Prevent cross solution contamination.
4. Use distilled water for making solutions.
5. Keep solutions well stoppered at all times.
6. Refrigerated solutions should be poured into staining dish, and the bottled solution returned to the refrigerator before it warms to room temperature.
7. Return all staining solutions to shelves soon after use to prevent mix-up.
8. Always use clean glassware.
9. Insure that scales, pH meter, etc., are in good working order.

Note **An Asterisk Preceding Solution Indicates Solution Is Used Once and Discarded.**

Hematoxylin and Eosin

1. Mayer's hematoxylin	stock	2 months
	working	2 wk/or 500 slides, whichever comes first.
2. Harris' hematoxylin	stock	6 months
	working	2 wk/or 500 slides, whichever comes first.
3. Alcoholic eosin	stock	4 months
4. Aqueous Phloxine B	stock	4 months
5. Eosin-Phloxine B	working	2 weeks

Remarks
Microscopic evaluation of H&E is essential to determine hematoxylin breakdown. This determination cannot be made, however, unless the technician is well experienced in recognizing good quality hematoxylin and eosin stained preparations.

McManus (PAS)

1. *Periodic acid		2 months
2. Coleman's or Schiff's	(refrigerate)	3 months
3. Mayer's hematoxylin		6 months
4. Light green		1 month
5. *Diastase of malt	(refrigerate)	1 week

Remarks
Discard light green solution if there is some evidence of mold growth. Mold strands will be deposited on microscopic slide if solutions containing them are used for staining.

Gridley (fungi)

1. Chromic acid		2 months
2. Coleman's	(refrigerate)	3 months
3. Aldehyde fuchsin	(refrigerate)	2 months
4. Metanil yellow		2 months

Remarks
Aldehyde fuchsin stains strongly when new, but weakens and deteriorates with age. Weakened or deteriorated solution can be recognized by poor staining of elastic fibers and mast cells. Fresh aldehyde fuchsin solution is a must for good demonstration of some substances such as pancreatic islet cells or sulfated acid mucosaccharides.

Grocott (fungi)

1. Chromic acid		2 months
2. *Sodium bisulfite		4 months
3. Silver nitrate	(refrigerate)	2 weeks
4. Methenamine	(refrigerate)	1 month
5. Borax		6 months
6. *Working solution (consists of solutions 3, 4 & 5		24 hours
7. Gold chloride		6 months
8. *Sodium thiosulfate		6 months
9. Light green		2 months

Remarks
Every effort should be made to prevent contamination of silver nitrate solution with any type of metal. Gold chloride solution should not be reused more than 3 times.

Ziehl-Neelsen (bacteria)

1. Carbol fuchsin	1 month
2. *Acid alcohol	6 months
3. Methylene blue	3 months

Remarks
Carbol fuchsin solution should be discarded if a precipitate is noticed adhering to the sides of the bottle.

Brown and Brenn (bacteria)

1. Crystal violet	3 months
2. Sodium bicarbonate	6 months
3. Gram's iodine	2 months
4. Ethyl ether-acetone	1 month
5. Basic fuchsin	1 month
6. Picric acid-acetone	1 month

Remarks
All solutions are discarded after use. Ethyl ether is extremely volatile and flammable. Be sure all open flames and cigarettes are extinguished when using this solution.

MacCallum-Goodpasture (bacteria)

1. Goodpasture's	(refrigerate)	4 months
2. *Formalin		6 months
3. *Saturated aqueous picric acid		6 months
4. *Stirling's gentian violet		2 months
5. *Gram's iodine		6 months
6. *Aniline-xylene		24 hours

Giemsa

1. *Giemsa's solution	working	3 months
2. *Rosin alcohol	stock	3 months
	working	24 hours

Mowry Colloidal Iron (mucopolysaccharides)

1. *Glacial acetic acid, 3%		6 months
2. *Colloidal iron	working	24 hours
3. *HCl + $K_4Fe(CN)_6 3H_2O$		24 hours
4. Van Gieson's		4 months
5. *Hyaluronidase	(refrigerate)	2 weeks

Remarks
The hydrochloric acid potassium ferrocyanide solution must not be contaminated by introduction of any metal substance. Hyaluronidase crystals should be stored in the refrigerator.

Alcian Blue (acid mucopolysaccharides)

1. *Glacial acetic acid, 3%	4 months
2. Alcian blue	2 months
3. Kernechtrot	2 months

Manuel (reticulum)

1. *Uranium nitrate		1 month
2. *Fontana's silver	(refrigerate)	1 month
3. *Formalin		6 months
4. Gold chloride		6 months
5. *Sodium thiosulfate		6 months
6. Kernechtrot		2 months

Mallory (iron)

1. *Hydrochloric acid	stock	1 month
2. *Potassium ferrocyanide	stock	1 month
	working	24 hours
3. Kernechtrot		2 months

Remarks
Potassium ferrocyanide solution must not come in contact with metal of any kind. Most frequent problem arises when metal capped containers are used for storage of this solution.

Fontana Masson

1. *Fontana's silver	(refrigerate)	1 month
2. Gold chloride		6 months
3. *Sodium thiosulfate		6 months
4. Kernechtrot		2 months

Mayer (mucin)

1. Weigert's hematoxylin	stock	4 months
	working	1 week
2. Mucicarmine	stock	4 months
	working	1 month
3. Metanil yellow		2 months

Remarks
Weigert's hematoxylin (working) can be reused at least 3 times with satisfactory results. Mucicarmine solution is very stable but on occasion mold will form in the bottom of the container. This mold will deposit on stained microscopic slides.

Mallory (PTAH)

1. Zenker's	stock	2 months
	working	2 weeks
2. Alcoholic iodine		2 weeks
3. *Sodium thiosulfate		6 months
4. Phosphotungstic acid hematoxylin (PTAH) solution		Indefinite

Remarks
PTAH solutions are generally considered to have a long shelf life, but one should always keep close check on the solution. The best way to do this is to use a piece of cerebrum as a control. Nerve trunks, axons, dendrites, etc., stain deep bluish-purple. Other tissue elements stain salmon color and light purple.

Masson (trichrome)

1. Bouin's fixative		2 months
2. Weigert's hematoxylin	stock	4 months
	working	1 week
3. Biebrich scarlet-acid-fuchsin		2 months
4. *Phosphomolybdic-phosphotungstic	working	1 month
5. Aniline blue		2 months
6. *Glacial acetic acid		6 months

Additional Information for Prestaining Small Specimens

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The recent edition of HISTO-LOGIC (January, 1975) emphasizes the need for more frequent and continuing communication between histotechnologists.

We have been using a prestaining method for small tissue specimens for several years. For the past year we have gone one step further. Once a month we put 0.5 grams Eosin Y in the last dehydrant and found that specimen gross structural details show up much better in the paraffin block and subsequent ribbon. This simple step has eliminated the problem of cutting incomplete tissue sections since one can readily see the tissue margins. This suggestion also helps to locate small fragments of tissue during the embedding process.

Utilizing this procedure we discovered a serious problem: The embedding center used in our laboratory incorporates a vacuum system within the paraffin dispenser. We have actually seen small pieces of stained tissue come out of the spigot, contaminating the paraffin in the embedding mold. We have corrected this problem by inserting a polyfoam pad, covered with filter paper, on the bottom of the reservoir.

Editor's Corner

Did You Know

. . . that both sides of a microtome knife can be used for sectioning. Ed. Note: This is in reply to a question sent by Linda M. Clark, High Point, North Carolina.

. . . that Hexamethylenetetramine $(CH_2)_6N_4$, which is used in combination with silver nitrate in Gomori's methenamine silver, is also known as:

Methenamine
Hexamine
Hexamethyleneamine
Formin
Aminoform
Urotropine

. . . that better cell block preparations can be obtained by (1) spinning fluid, (2) pouring off supernatant, and (3) adding a small amount of melted agar to the sediment. At this point mix preparation, and allow to harden. Process specimen in conventional manner. Suggestion submitted by Gordon Mann, Victoria General Hospital, Winnipeg, Manitoba R3T 2E8.

. . . that it is necessary to neutralize free aldehyde groups after glutaraldehyde fixation, to produce positive mucosaccharide staining.

Tissue specimens fixed in glutaraldehyde produce two distinct problems when mucosaccharide procedures are performed: The mucosaccharides are less vividly stained with the alcian blue and colloidal iron staining procedure. Secondly, the periodic acid Schiff reaction produces a more intense, generalized diffuse staining of all tissue structures. Application of Schiff's reagent without oxidation with periodic acid yields similar intense staining, indicating that the glutaraldehyde treated specimens provide free aldehyde groups to react with the Schiff's reagent. Glutaraldehyde produced reactivity may be neutralized by the use of the following method.

Solution

88% Aniline Oil

Aniline oils	88.0 ml
Acetic acid, glacial	12.0 ml

Neutralizing Procedure

1. Decerate slides and run through absolute alcohol, 95% alcohol, 3 changes each.
2. Place slides in aniline oil solution for 1 hour.
3. Rinse slides quickly in 2 changes of 95% alcohol.
4. Wash slides in running tap water for 10 minutes.
5. Perform periodic acid Schiff procedure in the usual manner.

Remarks
Sections should be celloidinized if glycogen digestion with diastase of malt is to be performed. Celloidin (0.5 gm celloidin in 50 ml ether and 50 ml absolute alcohol), can be applied after the absolute alcohol used in step one. Slides are dipped in celloidin solution and allowed to dry on a clean dry surface for 45 minutes. Slides are then dipped in 80% alcohol and transferred to the aniline oil solution. Continue with procedure outlined above.

Reference
Janoff, M., et al: Am. J. Clin. Path., 44: No. 2, 167-171, 1965.

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The Publication That's Written by Its Readers

Now in its fifth year of publication, HISTO-LOGIC remains unique in its field. With the exception of material contributed by its Editor, Lee G. Luna, HISTO-LOGIC is entirely written by its readers. We know of no other news publication that can say the same.

The success of HISTO-LOGIC has always depended on its readers in two ways. First, they keep us informed about new and improved techniques they have developed in the laboratory. And second, they keep us informed about their particular reading interests in this highly specialized field.

HISTO-LOGIC has carried information about new procedures, changes in old procedures, improvements in staining or block preparation, new and more economical methods and techniques. It also published announcements of symposiums, workshops, and elections of officers in the various local societies. It pointed out the availability of special educational material — films, books, audio-visual aids. All have been widely read. All have been helpful in generating closer lines of communication throughout the profession.

Now once again, we want to remind you that your manuscripts are not only welcome, but essential for the continued success of the journal that serves your needs. Submit them to: Lee G. Luna, Editor, HISTO-LOGIC, P.O. Box 36, Lanham, Maryland 20801. Unless accompanied by a written request when submitted, no articles, photographs, etc., will be returned.

HISTO-LOGIC

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