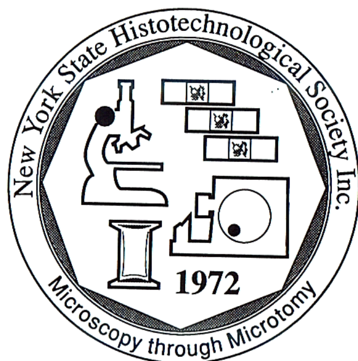

New York State Histotechnological Society

On Stage

Volume 31, Issue 4 Fall 2012

Decalcification Fact Sheet:

Sarah Mack
University of Rochester

In specimens that require decalcification the following elements should be considered. A decalcifier is used after the fixation process so it is important to find the correct decalcifying agent to use. Careful consideration must be given before you choose your decalcifying agent. Formic acid solutions work well with the common skeletal stain, such as Alcian Blue/Hematoxylin/Orange G, which is used for growth plate and articular cartilage staining, and Safaranin O/Fast Green, used for growth plate and articular cartilage staining. Formic acid allows for a good cellular detail.

Formic Acid, Immunocal, Decal Chemical Corporation catalog# 1454

Immunocal TM, Decal Chemical Corporation, it is a commercially available 5% formic Acid.
Immunocal is a gentle, quicker way to decalcify tissue. In our laboratory we use Immunocal decalcification for tissue that will be used for in situ hybridization, immunohistochemistry as well as basic staining.
DO NOT use Immunocal if you plan to do Tartrate-resistant acid phosphate (TRAP) staining.

KEY POINTS TO DECALCIFYING WITH IMMUNOCAL

- If your samples were not properly fixed, they will not be properly decalcified. Harvest tissue and strip as much soft tissue away from bone as possible without disrupting or distorting specimen. This allows for better penetration during fixation and decalcification. Fix tissue with appropriate fixative. Most of the tissue in our center is fixed for 3 days. Rinse tissue in 1X PBS 3 times for 5 minutes each, followed by 3 rinses of DI water, then into Immunocal.
- Decalcification needs to complete quickly, preserving tissue quality.
- Make sure that your specimens are in enough decal agent at least a 1:8 ratio.
- All of our samples are placed on a stir plate with a stir bar, stirring lightly or on a rocker, rocking gently.
- Large samples may take longer to decal. Always manually check your samples to ensure they are properly decaled.
- If the samples are not thoroughly decaled, they will not cut well and samples may be brittle.
- Samples decaled in an acid solution need to be neutralized; we use Cal-Arrest (Decal Chemical Corporation). Before using Cal-Arrest, rinse your specimens in1X PBS 3 times for 5 minutes each. Rinse in Cal-Arrest for 20 minutes.
- Samples should be rinsed in 1X PBS 3 times for 5 minutes each, followed by 3 rinses of DI water, 50% ethanol one time for 5 minutes, and 70% ethanol for 5 minutes to thoroughly remove any salts or acids before processing.
- After tissue is completely rinsed it may be kept in 70% ethanol and placed in 4 degree C for immediate processing. Do not store tissue in 70% ethanol for an extended time.
- Surface decal can also be used during sectioning, when mineral deposits/calcifications are found. The block can be placed face down in 5-10% Formic acid or 1% HCL. Make sure all acid solutions are rinsed off.

On Stage is published quarterly by the New York State Histotechnological Society for its membership. Contributions, suggestions and advertisements are welcome. Please visit the NYSHS website for submission information and guidance. Permission to reprint is granted as long as source and author are acknowledged and a copy of the reprint is sent to the editors. Articles without bylines are written by the editors. Please submit manuscripts to the editor-in –chief.

Deadlines for Submission are:

September 1 - Fall
December 1 – Winter
March 1 - Spring
June 1 - Summer

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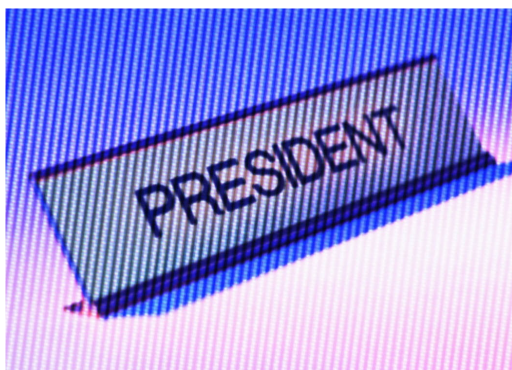


Where did the summer go….It seems like just yesterday we were talking about the spring meeting and elections! We hope you all had a great summer and that you are well rested for what I am sure will be a busy fall.

Our elections results have been posted and I would like to take a moment to recognize the changes. Thanks to Amy Farnan, who has stepped down from her role as membership secretary, for her years of dedicated service. Sarah Mack has take on this position and I am sure she will do an excellent job. Linda Chen was elected as corresponding secretary taking over for Emily Ash. We welcome two new members to the Board of Directors, Nathan Jentsch (Buffalo) and Sara Laviska (Albany). We will be bringing them up to speed quickly as we are already in the planning phases of our upcoming spring meeting. As always, visit our website where contact information for the officers and board of directors is posted should you have any questions comments or concerns.

Speaking of websites, check out the NSH Histology Career Video posted on our home page. It took a while but NSH did a great job. If you are involved with HS school or college age kids who are looking for a promising career, be sure and steer them to our direction. There is a wealth of information about Histotechnology on our website. A special thanks to Angela Fogg for helping get NSH video up on our site and webmastering. After a few bumps and hiccups, the yahoo message board calendar is finally back up and running. Yahoo has streamlined and enhanced many features so we will be uploading dates as reminders for upcoming seminars and meetings so that you can stay abreast of what's gong on in NYS and the region.

I have also been receiving many inquiries from individuals around the region and country who are seeking employment in NY and are struggling to get their license. Many of these individuals already have advanced education, work experience or have met and exceeded the state requirements for licensing (individuals with HTL but not HT for example) but are forced to backtrack, or take lower paying positions because they must hold the limited license and take the time to meet NYSED criteria for licensing. This is a significant problem and I have been approached by a number of lab supervisors and directors who have also commented on the difficulty of getting people licensed in order to work in NYS. As a reminder, the "Limited License", the last remaining "grandfathering" mechanism is set to expire September 1st of 2013. We are currently formulating a plan to address this issue and hope to be able to engage in discussion with NYSED. Please stay tuned as it will take all of us to help resolve this issue.

As we move forward into the fall, we will be updating you on our progress with our spring meeting so please be sure to check the website for updates. We will of course post updates on the message board as well. In the meantime I hope that many of you are able to attend the NSH meeting in Vancouver. If so, I hope to see you there. If not, have a safe and happy fall!

All the best

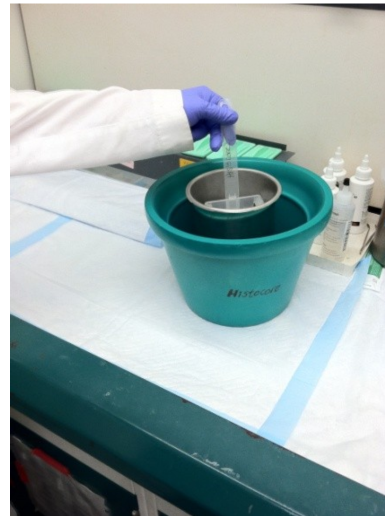
Preparation of Frozen specimens

Sarah Mack
University of Rochester Medical Center

In the HBMI Core at the University of Rochester Medical Center, we use the method of snap freezing our tissue. Snap freezing refers to the ultra-low temperature freezing method used to prepare high-quality cryo-sections. Ice-crystals that form during a slow freezing process cause distortion in tissue morphology and can lead to more difficult sectioning. If the specimen is cooled rapidly, the crystals that form are much smaller and fewer which leads to much better morphology. Dry ice (-80°C) can cool a standard sized specimen submersed in O.C.T. within 3 minutes typically, but is still not cold enough to eliminate crystal formation. The method below, uses a super-cooled bath of 2-methyl butane (-150°C) that can freeze a standard specimen within 1 minute, thus greatly reducing crystal formation.

Materials:

- Ice bucket
- Stainless steel bucket
- Liquid nitrogen
- 2-methyl butane (Isopentane) (CAS# 78-78-4)
- Plastic staining rack with long handle
- O.C.T. Compound (Tissue Tek, cat# 4583)
- Standard Cryomolds (Tissue Tek, cat# 4557)
- A marking pen to identify specimen on Cryomold.
- Timer
- Aluminum foil



Procedure:

1. Specimen must already have been fixed in PFA or 10% NBF, decalcified. Times will vary depending on the results you are looking for. Specimens are then run through 10%, 20%, and 30% sucrose gradients each for 24 hours.
2. Rinse samples in PBS and infiltrate in O.C.T. for 30 minutes prior to snap freezing.
3. When snap freezing, all steps should be done under a fume hood!
4. Place each specimen into cryomolds and fully cover with O.C.T. Specimens may be oriented once sub-mersed in O.C.T. and will usually stay in place due to the viscosity of the medium.
5. Fill a cryobucket with liquid nitrogen, approximately 3cm deep. *****Take serious care using liquid nitrogen and wear proper PPE!! Transport bucket and liquid with a cover and on a cart for extra stability.*****

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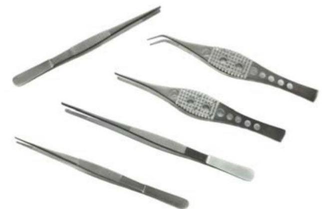

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6. Fill stainless steel bucket with 2-methyl butane so that a thin layer of liquid covers the bottom of the container. The level of the liquid should just reach the top of the cryomold so that the blocks are never fully submersed during the freezing process, as the 2-methyl butane should never touch the O.C.T. Ideal depth should be approximately 1cm across the bottom, or ~150ml in a 1.5L container.
7. Carefully place steel bucket into the liquid nitrogen. Vapors forms at first as the 2-methyl butane rapidly cools. After 3 minutes, solid white ice is visible at the bottom of the steel bucket as the 2-methyl butane freezes. This is indication that liquid has reached its freezing point of -150°C.
8. One specimen at a time, carefully dip block into 2-methyl butane with long-handled staining rack. Complete freeze is achieved at ~60 seconds (90 seconds maximum for larger specimens).
9. Remove block from 2-methyl butane and examine for characteristic bump that forms on the top center of the block. Wrap specimen in aluminum foil immediately and store in dry ice or -80°C freezer. This should be done within a minute of snap-freeze so as to prevent any thawing.
10. Liquid nitrogen quickly boils away at room temperature and may need to be replaced if preparing many samples. Indication that the 2-methyl butane is warming up again is when the white layer of ice begins to disappear.
11. Remove steel bucket from liquid nitrogen and allow all materials to warm to room temperature under fume hood. Dispose of 2-methyl butane in hazardous waste container and label properly. All liquid nitrogen will boil and evaporate away.

"Keep this In Your Site"

Histology Lab Quiz Flash Cards

The flashcard set features a photo review of human tissue histology. You may find it a helpful resource as you study the tissues for your lab practical exam.

http://quizlet.com/2621787/histology-lab-photo-quiz-flash-cards/

Update on SUNY Cobleskill Histotechnology Program
By: Pamela Colony, PhD

The year 2012 has been one of improved excellence for the Anne Marie Behling Histotechnology lab at SUNY Cobleskill. The SUNY construction fund plan included not only a new building addition to the original Wheeler Hall, but also renovations of the original building. This included extensive renovations of the Histotechnology lab. Aesthetic changes included new floors, ceilings and lights as well as a new paint job. The cabinetry was also replaced and new locks installed. Equally exciting was the fact that there was some money for the purchase of new equipment. One of the first orders was for a Millipore water system to replace the iron rich local water. This has arrived and we are awaiting the installation. We have also received a new Tissue Tec embedding station and two Thermo Microm HM 3235 microtomes and we purchased an additional 8 Olympus CX31 student microscopes. We now have a high quality microscope for every student in the program. We also have an outstanding order for a new Olympus DP 26 digital camera to use with the video-microscope. This unit is invaluable in teaching and I anticipate upgrading a couple of the objective lenses in the near future.

Naturally there are glitches in the changes and we are working with the contractors to resolve the problems. We anticipate the connection of our computer port (hopefully in the near future) and the installation of electricity on the student desk so we can use our microscopes in the Histology class! In the interim we have your basic extension cord(s) for the latter and we scout out empty classrooms to use for the lab Power-Points of tissues they will be studying in the lab for the former.

In addition to the lab renovations I am pleased to report that we have a full second year class of 16 students. This should benefit the labs in need of qualified personnel. I would encourage labs that have openings to contact me in late spring and I will post the information for the graduating class. Each student is required to complete a 400 hour clinical rotation so they are typically seeking a job during the summer months. Also, if you are interested in serving as an Affiliate teaching site, please contact me by phone (518-255-5417) or e-mail (colony@cobleskill.edu).

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(Continued from page 1)

14% EDTA

When performing enzymatic stains like Tartrate-resistant acid phosphatase (TRAP) stain to identify osteoclasts EDTA should be used. Similar issues affect beta-galactosidase staining. EDTA is a chelating agent that aids in the removal of calcium and mineral from cartilage of bone. However if it is used for extended amounts of time it can have adverse affects on proteoglycans found in the extracellular matrix of cartilage and bone. EDTA should be at a pH of 7.3 and changed daily.

KEY POINTS TO DECALCIFYING WITH 14% EDTA

- If your samples were not properly fixed, they will not be properly decalcified. Harvest tissue and strip as much soft tissue away from bone as possible without disrupting or distorting specimen. This allows for better penetration during fixation and decalcification. Fix tissue with appropriate fixative. Most of the tissue in our center is fixed for 3 days. Rinse tissue in 1X PBS 3 times for 5 minutes each, followed by 3 rinses of DI water, then into EDTA.

- Decalcification needs to complete quickly, preserving tissue quality.

- Make sure that your specimens are in enough decal agent at least a 1:8 ratio.

- All of our samples are placed on a stir plate with a stir bar, stirring lightly or on a rocker, rocking gently.

- Large samples may take longer to decal. Always manually check your samples to ensure they are properly decaled.
- If samples are not thoroughly decaled, they will not cut well and samples may be brittle.
- Samples should be rinsed in 1X PBS 3 times for 5 minutes each, followed by 3 rinses of DI water, 50% ethanol one time for 5 minutes, and 70% ethanol for 5 minutes to thoroughly remove any salts or acids before processing.
- After tissue is completely rinsed it may be kept in 70% ethanol and placed in 4 degree C for immediate processing. Do not store tissue in 70% ethanol for an extended time.

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2012 Award's Report
Mary Georger, Past President

The Region 1 Awards were presented on April 28th 2012 at the NYSHS hosted meeting held at the Islandia Marriott Long Island.

The Region 1 Histotech of the Year is given out every year and recognizes someone in the field who has shown exemplary qualities of dedication and service to the Histology Profession within their state or within the region. This years' award was given to Clare Thornton from Dahl Chase Pathology Associates in Bangor Maine. Clare has been an active member in the Maine Society of Histotechnology, playing an integral part in planning both the State and Regional meetings, as well as serving as an officer in the state society.
The Region 1 Scholarship is a financial award given to a histologist or supervisor who demonstrates a financial need and motivation for attending an annual meeting. This person must also demonstrate involvement in State, Regional or National activities promoting the field of Histotechnology. This years' recipient was Emily Hast from Yale University of Medicine.

The Charles Churukian Award is a financial award presented annually at a Region 1 meeting to a histotechnologist who demonstrates both financial need and motivation to attend a continuing education meeting. This person must also be active in State, Regional or National activities promoting the field. This Award is given in memory of Charles Churukian, a long time Histotechnologist at the University of Rochester, member of the Biological Stain Commission as well as a founding member of the NYSHS. The recipient of this years Award was Nicole Valade from St Peter's Health Partners/Albany Memorial Hospital.

NYSHS Fellowship Award
Awarded to Sandra Cummings

Sandra (Sandy) Cummings has been known as a force to reckon with in the histology world and has earned great respect from her peers for her continued dedication to the field of histology.

Sandy graduated from the SUNY Cobleskill Histology program in 1986. As a student at SUNY Cobleskill she attended an NYSHS symposium with her class mates and Professor Anne Marie Behling. During this meeting she quickly realized she wanted to become involved with the society in promoting the profession in any way possible. She volunteered to take the nomination chairperson position. In this roll she was able to see the inner workings of the society and earn a great respect for what the society had to offer her profession. From that time she stayed active in the society fulfilling the following rolls: 2 years as nominations chairperson, 4 years as treasurer, 3 years as Vice President, 5 years as president and 4 years as past president.

Sandy not only dedicated herself to the state society but she also fulfilled the position of an adjunct professor at SUNY Cobleskill and was an active member of the Histology Affiliate Board for many years. For her years of dedication to the field Sandy was presented with the Fellow to the Society Award at the Spring NSH Region 1 meeting in Long Island. Many congratulations to Sandy!

Answers to Last Issue's Crossword Puzzle
Back to The Basics:
Fixation, Pigments Metals

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2012 Histotechnology Student Awards and Scholarships

Siemens –ASCP Student Scholarship Award
Sarah Lamoureux ($1000.00)

Academic Achievement Award in Histotechnology
Alicia Reed

University Pathologist Scholarship From Upsate Medical Center
Dan Peyron ($6000.00)

Newcomer Supply Student Scholarship
Alicia Reed($500.00)

Sakura Fintek Student Scholarship
Sarah Lamoureux ($500.00)

Histotechnology Laboratory Excellence Award
Alicia Reed

Phi Theta Kappa
Alicia Reed
Catherine Repicky

Dean’s List
Taylor Clifford
Sarah Lamoureux
Alicia Reed
Catherine Repicky
Jonathan Wood

Dean’s List Honorable Mention
Anna Draus
Pamela Jones
Dan Peyron

CONGRATULATIONS TO ALL & CONTINUED SUCCESS !!

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Type of Membership: (check one)
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( ) Previous (date previous membership)
( ) Student (College name)

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Instructor's signature

National Information: (check one)
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( ) Non-Member
( ) Please send me an NSH application

Nature of Work: (check one)
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( ) Research
Education (highest level): ____________________
( ) HT
( ) MT
( ) HTL
( ) Other ____________________________

Referred by member: ________________________

Registration: (check one) Membership year runs from July 1 to June 30

( ) Education Annual Membership Fee (tax deductible): $20.00

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Membership will expire June 30th,

Please send applications & check payable to **NYSHS** to:

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