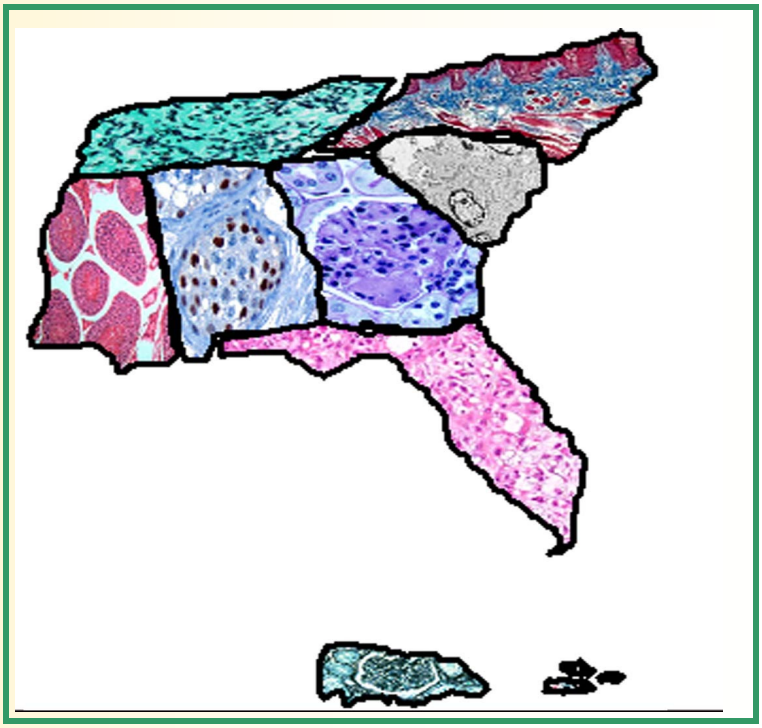
MICROTIME

The Georgia Society for Histotechnology

Inside.....



GSH Hosting Region III MEETING!

At Beautiful Callaway Gardens!

President Letter....

President's Message Spring 2012

Dear Members,

I am beginning this message with a lot of emotion, since it will be my last as your President. According to the bylaws, I cannot run again. A lot has changed in the last few years as I have now retired from my profession. Yes there are days I actually do miss the work. I do keep up through journals and articles, even though I know I can never work around the chemicals again.

When I became your President, there were only 6 attendees at the annual meeting the previous year and the membership was almost non-existent. With the help of some dedicated people like Chris Coley, Shirley Powell, Anne Taylor, Harriett Baker, Wanda Simons, and so, so many others, we were able to turn this around, with a membership currently almost 150 and of course I want 300, but it will come. The GSH annual meeting has drawn just over a hundred participants each year, and over 20 Vendors.

This year we are hosting Region III and it appears we will far exceed that number, with over 90 rooms already taken so far. I am excited to see the growth and all the new people who have stepped up to assume vital roles in the society, providing new ideas and enthusiasm, which is critical for GSH to continue into the future as the leader it has always been since 1973. Next year is the 40th anniversary of GSH and I encourage each of you to come up with ideas on how to celebrate it.

Vital to the success of any society, and especially to our meetings, are the Vendors who support us with their continued presence and financial support for our awards and so much more. We have 25 Vendors so far exhibiting this year and I hope you will take the time to visit each one and give them a "**personal thank you**" for all they do for us as a profession.

New officers will be taking over at this meeting and I am confident that the society is in good and capable hands. I hope you will give them your support, your ideas; your vote of confidence, to continue the mission of this society and this profession, for without you they cannot do their job. As I close this message, let me end it with the words of Bob Hope, "thanks for the memories "**it's been a heck of a ride!"**

Mike Ayers

President

Georgia Society For Histotechnology

Save the Dates

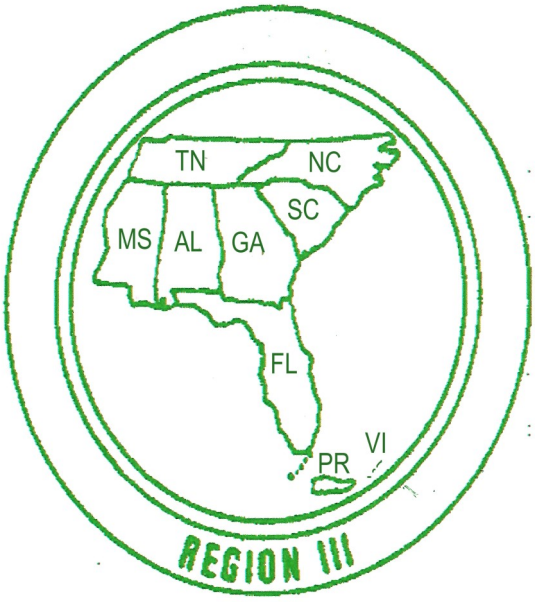
GSH Annual Meeting

See page 12 for Workshops!

April 13-15, 2012

Come join Your Friends and Fellow Histo Techs for a Great Weekend!

Callaway Gardens.



Northside Hospital-Atlanta is looking for PRN Histotechs for

Saturdays & Sundays and evenings or early mornings for embedding and cutting help.
Go on our website @

northsidehospital.com atlanta

and fill out an application

GSH NEWSLETTER RATES

Business Card - $50

Half Page - $85

Full Page or Insert - $125

Make check payable to :

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%Ann Taylor
6645 Goodall Mill Rd.
Macon, GA 31216

Histotechnology - Southern Style

Histotechnology

Join us and discover new techniques and technologies to improve patient care. Challenge yourself to learn from Colleagues from across the country. Experience lectures and workshops in a relaxed learning environment, while earning CEU's. Enjoy the company of like minded professionals, and share challenges and experience. Vendors will be near to display and explain new technologies.

Southern Style

When our classes are done, escape the stress of everyday life and embrace the serenity that *Callaway Gardens* offers. You can stroll through over 13,000 acres of meticulously maintained gardens. Take your family on a bike ride to several attractions. Enjoy fishing, golf, spa treatments or exploration. If you prefer, just relax in the lounge with friends. Don't miss the experience of "Southern Style".

Callaway Gardens.

REGION III NSH

THE GEORGIA SOCIETY FOR HISTOTECHNOLOGY IS PROUD TO HOST THE 2012 REGION III NSH MEETING. IT WILL BE HELD IN THE MOUNTAIN CREEK INN CONVENTION CENTER AT CALLAWAY GARDENS IN PINE MOUNTAIN, GEORGIA APRIL 13-15, 2012.

Hotel information:

To make reservations please call1800-225-5292 and use the GSH group # 78K711 to get the discounted room rate of $109. I urge you to make your reservations now, the sooner the better. Spring is a popular time for golfing at Callaway and the rooms fill up fast. The price of your room includes access to the gardens. Your credit card will NOT be billed until you register at the hotel in April 2012. There are only a certain number of rooms blocked so don't delay. Visit their website at http://www.callawaygardens.com/resort/things-to-do/georgia-fun.aspx for more things to do and directions to the Mountain Creek Inn.

Check-in Location:

Mountain Creek Inn and Villa guests check in at the Mountain Creek Inn on Highway 27. The Mountain Creek Inn is open 24 hours.

Cottage guests check in at The Southern Pines Cottages Check-In Center. The hours of operation are Monday-Thursday from 9 a.m. to 7 p.m. and Friday-Sunday from 7 a.m. to 7 p.m. During non-operating hours, guests will check in at the Mountain Creek Inn.

Room	Single Rate	Double Rate
Mountain Creek Inn	$109.00	$109.00
Southern Pine One Bedroom Cottage	$170.00	$170.00
Southern Pine Two Bedroom Cottage	$330.00	$330.00
Callaway Gardens Villa Bedroom	$179.00	$179.00

Speaker roster for the meeting:

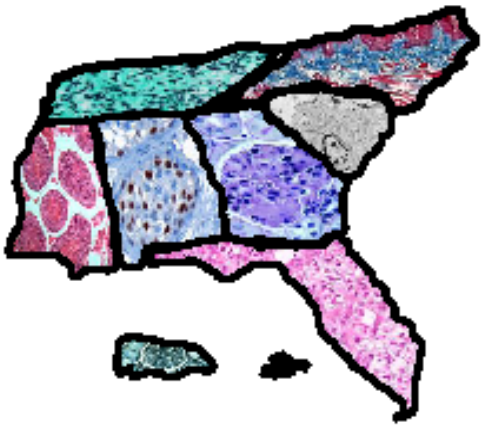
Taiquanda Winbush - HT/HTL Review – Instructor, Darton College Online Histology Program
Carl Sagasser, HT/HTL Review – Coordinator, Darton College Online Histology Program
Adrianna Eaton - Antibody Challenge 2012 – Cell Marque Corporation
Claudia Lawson - Advances in Molecular Testing – Clarient, Inc.
Joyce Weems - CPT Coding - Sweet Dream or Nightmare? – St. Joseph's Hospital, Atlanta
Marvin Hanna, MS,MBA - The Molecular Pathology Lab of the Future - Imeb
Ada Feldman - Troubleshooting Hematoxylin and Eosin Staining – Anatech LTD
Wanda Jones, HT(ASCP) - IHC Controls—The Good, The Bad and The Ugly! – Emory University Hospital
Ada Feldman - The Joy of Histology or What We Can Learn from the Kitchen – Anatech LTD
Steven Westra - Get the most out of your Immunohistochemistry: A Balance between Convenience, Cost, Education and Flexibility – Leica Microsystems
Lamar Jones,HT(ASCP) - Real Time *Rapid* Tissue Processing – Emory University Hospital
Ely Klar - Human Tissues: Histological Identification of the Different Types – Columbus State University

2012 Symposium Schedule at a Glance

Meeting Registration will be held Friday 10:00-12.00, Saturday 7:30-8:30, and Sunday 7:30-8:30.

Work Shop #	RM		Time	-	- Description
Registration		Fri	10:00-12:00		
WS-1	A	Fri	1:30-5:30		HT/HTL Review
WS-2	B	Fri	1:30-5:00		Antibody Challenge 2012
Break		Fri	3:00-3:30		
WS-3	A	Fri	5:00-7:00		Advances in molecular testing
WS-4	B	Fri	5:00-6:00		CPT Coding - Sweet Dream or Nightmare?
WS-5	B	Fri	6:00-7:00		Molecular Pathology- The lab of the future
Vendor Reception 7:00-9:00 In the Exhibit Hall					
Registration		Sat	7:30-8:30		
WS-6	A	Sat	8:30-12:00		Troubleshooting H&E stains
WS-7	B	Sat	8:30-12:00		Real Time Rapid Tissue Processing
Break	Exhibit	Sat	10:00-10:30		
12:00-1:00 Awards Luncheon					
WS-8	A	Sat	1:30-5:00		The Joy of Histology
WS-9	B	Sat	1:30-5:00		Get the most out of your IHC
Break	Exhibit	Sat	3:00-3:30		
5:00 GSH General Membership Meeting / NSH Region III meeting					
Registration		Sun	7:30-8:30		
WS-10	A	Sun	8:30-10:00		IHC Controls: the Good, the Bad, and the ugly.
WS-11	B	Sun	8:30-12:00		Human Tissues: Histological identification
Break	Exhibit	Sun	10:00-10:30		

Georgia Society For Histotechnology



The 2012 NSH Region III Symposium Descriptions of Workshops April 13-15, 2012

Friday April 13, 2012

WS#1 1:30-5:00 RoomA

HT/HTL Review Are you preparing to take the HT/HTL exam and you are nervous about it? In this review class you will learn how to study and what to study. The class will consist of 3-4 hours of Q&A along with presented slides. A handout will be included as part of the program. Topics included in this workshop will include discussion on fixation, microtomy, staining, laboratory operations, safety and processing/embedding. Also included will be general discussion of histology at the microscopic level including images of representative tissues. Students taking this course should have a general knowledge of histological technique and will be taking the National Registry exam in the next few months.

Taiquanda Winbush, HT(ASCP)
Carl Sagasser, HT(ASCP)

WS#2 1:30-5:00 RoomB

Antibody Challenge 2012 Get a glimpse at how IHC comes into play for a patient. This workshop will be a fun, interactive look at how IHC can be used by pathologists. The discussion will be geared for an audience with a basic to intermediate level of IHC understanding. First we will take a brief look at how to interpret a panel of antibodies and review the significance of their use. We will move on to explore some of the basic panels and the markers that are involved. After we have established a solid understanding of basic panels, we will widen our exploration to include some of the more advanced algorithms as well. At this point we will begin the best part, with interactive participation of the audience. We will present real life scenarios accompanied with various clues and staining results, and allow the audience to use their newly acquired knowledge of panel usage to decide what markers might be helpful. At the conclusion of this talk, the audience will have seen for themselves how important IHC can be, and also how panels can be a great tool to assist pathologists with finding an accurate diagnosis.

Adrianna Eaton
Cell Marque Corporation

WS#3 5:00-7:00 RoomA

Advances in Molecular Testing This workshop will discuss the advances being seen in molecular testing for cancer diagnostics. Attendees should leave with a better understanding of the different methodologies being offered at different labs and how they may affect the outcome of these tests. They should also gain an understanding of what pathways these different drugs target and what future drugs and tests are on the horizon. This workshop will help those techs involved in ordering and discussing cases with both anatomical pathologists and the treating oncologists.

Claudia Lawson
Clarient Inc.

WS#4 5:00-6:00 RoomB

CPT CODING - SWEET DREAM OR NIGHTMARE? CPT codes for Anatomic Pathology have changed very little over the years. But they continue to be an area of mystery for many of us. This session will offer an opportunity for discussion to unravel the mysteries and introduce the few new codes that have recently appeared.

Joyce Weems
St. Josephs Hospital

WS#5 6:00-7:00 RoomB

The Molecular Pathology lab of the Future Pathology labs are a fast growing area of Pathology and are providing significantly better information to us on the genetic basis for cancer . This has been a major reason for the significant increases in survival rates for cancer patients we've seen over the last few years. A number of experienced histologists and pathologists have provided their opinions on what advances they see in the future for molecular pathology labs. This lecture will provide an overview of what these experts envision the molecular pathology lab will be in 10 years, including new tests, procedures and specific therapies based on the individualized genetics of cancer.

Marvin Hanna, BS, MBA
IMEB

Vendor Reception 7:00 - 9:00 In exhibit hall

Saturday April 14, 2012

WS#6 8:30-12:00 RoomA

Trouble shooting H&E Staining Each step in the handling of a tissue specimen has the potential of altering the final H&E result. This workshop will discuss the steps in tissue processing (excision through slide drying) and H&E staining (deparaffinization through coverslipping) that can modify the stained appearance of the tissue. Problems discussed include smudgy nuclei, aberrant staining, "soap suds", haziness and more. The workshop shows "problem" slides, their respective processing and/or staining schedules and the steps necessary to correct the problem. Traditional reagents (formalin, alcohol, xylene) as well as substitute reagents (xylene substitutes, special fixatives, formalin-free fixatives) are discussed.

Ada Feldman, MS, HT/HTL (ASCP)
Anatech LTD

WS#7 8:30-12:00 RoomB

Real Time Rapid Tissue Processing In pathology today the demands for rapid tissue fixation and processing has increased. The request to have tissues processed and slides prepared for diagnosis has matched the demand for TAT and quality management. This workshop will demonstrate the methods for proper tissue grossing and dissection, fixation, rapid tissue processing and validation. The affect on special stains, IHC and some molecular techniques will also be highlighted.

M. Lamar Jones, BS, HT (ASCP)
Emory University Hospital

12:00 - 1:00 Awards Luncheon

WS#8 1:30-5:00 RoomA

The Joy of Histology or What we can Learn From the Kitchen How does the water temperature for brewing tea have anything to do with slide drying? What does cheese have in common with tissue fixation? Whether it is a fried egg or a properly stained gastric biopsy, the disciplines of cooking and histotechnology both rely on chemistry to acquire proper end results. This workshop will be an enlightening discussion of seemingly different yet similar disciplines. Fixation, processing, quality control, recycling and enzyme histochemistry are a sample of topics discussed.

Ada Feldman, MS, HT/HTL (ASCP)
Anatech LTD

WS#9 1:30-5:00 RoomB

Get the most out of your Immunohistochemistry: A Balance between Convenience, Cost, Education and Flexibility In this workshop we will present the key benefits and disadvantages of using either predilutes or antibody concentrates both in a manual and automated setting. We will then review the steps required in order to effectively optimize an antibody concentrate covering topics such as: datasheet interpretation, choice of diluent, detection, suitable storage conditions, pipetting techniques and antibody titration protocols and the use of controls.
In today's IHC laboratory finding a balance between convenience and cost will only help laboratories retain their flexibility but most importantly help their histotechnicians retain valuable skill.

Steven Westra
Leica Microsystems

5:00 GSH Membership Meeting followed by Region III meeting

Join us on Saturday night at *"Carriage & Horses"* for a fun dining experience. *Dagher,* our chef & host, will welcome us with a cocktail hour of appetizers and wine, followed by a delicious meal of filet mignon with vegetables, salad, & dessert. (tea, coffee & entertainment included!) Please take advantage of this special event hosted by GSH. More details to follow ~ www.cometodagher.com

Sunday April 15, 2012

WS#10 8:30-12:00 RoomA

IHC Controls-The Good, The Bad and The Ugly! The proper selection and use of controls for IHC is critical to the assay, test, and patient diagnosis. Controls must be carefully selected, tested and validated before use. Antibody specification sheets provide helpful guidance in the selection process of a positive control. The fixation, processing and even microtomy of tissue for IHC controls play a great role in the IHC assay. Negative controls are just as crucial as a known positive control and must be evaluated equally. This workshop will provide information on the selection, fixation, processing, testing, and review of both true positive and negative controls for immunohistochemistry.

Wanda Grace – Jones, HT(ASCP)
Emory University Hospital

WS#11 8:30-12:00 RoomB

Human Tissues: Histological Identification of the Different Types In this session, the histological features of the different types and categories of tissues will be described to facilitate identification of tissues. Specific characteristics such as cell types, membranes, fibers and extracellular matrixes will be discussed as well as how these tissues function in their respective organs. Knowledge of the structural and histological differences between the tissues allows for a better understanding of the individual functions of these tissues in the human body.

Elizabeth (Ely) Klar

GSH REGISTRATION FOR 2012 MEETING
APRIL 13-15, 2012

Mountain Creek Inn, Callaway Gardens, Pine Mountain, Georgia
Please fill out a form completely for each attendee mail along with check to the address below. When paying by PayPal you need to mail the form at the same time payment is submitted to the address below or you will not be registered.

NAME_____

HOME STREET ADDRESS: _____

HOME CITY: _____**STATE:**_____**ZIP:**_____

HOME PHONE: _____**PREFERED EMAIL ADDRESS:** _____

EMPLOYER: _____

WORK STREET ADDRESS: _____

WORK CITY: _____**WORK STATE:** _____**ZIP:**_____

WORK PHONE: _____**FAX:**_____

NONREFUNDABLE REGISTRATION FEE: $35 (includes the Awards Luncheon on Saturday and attending vendor reception only)

FEES: Friday, Saturday, Sunday $100 plus non-refundable $35 registration fee $135.00

Students $50 plus $35 registration fee... $ 85.00

STUDENTS: Your instructor must sign here to be eligible for student rates

Name: _____**School Name:** _____

YOU MUST CIRCLE WORKSHOPS DESIRED: <u>You can only attend a total of 6 workshops.</u>

Friday: Workshop #1 Workshop #2 Workshop #3 Workshop #4 Workshop #5

Saturday: Workshop #6 Workshop #7 Workshop #8 Workshop #9

Sunday: Workshop #10 Workshop #11

PLEASE TOTAL	**Registration fee for all attendees**		**$ 35.00**
	Extra Luncheon tickets	**#__**	**_____ $ 35.00**
	Workshop Fee		**_____ $100.00**
	Student Workshop Fee		**_____ $ 50.00**
	Dinner at Carriage and Horse Saturday Night		
			_____ $15.00 each for attendees
			_____ Extra non-attendee tickets $30.00
	Total		**$ _____**

Paid by(please check) _____ **check or** _____ **Credit Card via PayPal**

MAKE CHECK PAYABLE TO GSH

NOTE: IF PAYING WITH CREDIT CARD USING PAYPAL PLEASE BE SURE TO MAIL REGISTRATION FORM AT THE TIME OF PAYING ONLINE IN ORDER TO BE REGISTERED.

Anne Taylor, GSH Treasurer
6645 Goodnall Mill Road
Macon, GA 31216

Credit Card payments are made by going to www.paypal.com
And send funds to the email address ataylor1286@bellsouth.net

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GSH VENDOR LIAISON
Scott Brown, Biocare

HOW I STUMBLED INTO MY CAREER

By Shirley Powell, HT(ASCP)HTL

After my first year at the University of Georgia, I obtained a job as a bookkeeper at a furniture store to help my Dad pay for my next year's tuition. Needless to say, bookkeeping and desk work was not my cup of tea. A high school friend came by to visit and told that one of the histotechs at the Macon Hospital Pathology lab had not come in for two weeks and they were looking for someone to fill her position. I asked her what histology was and after a brief explanation, I decided to try it. I was willing to try anything but bookkeeping. I applied the next day. On July 18, 1962, I began working in the histology lab. It was there that I obtained my OJT. I took and passed my HT in 1964. The exam was given only once a year then and I had not worked a full year when it was given in 1963 so I was not eligible. I had to wait until 1964 to take the exam.

I continued to work at the Macon Hospital, now the Medical Center of Central Georgia, for another year and became supervisor by default under duress. The chief pathologist left to create his own lab and took 2 other histotechs with him, one was the supervisor. I was the only registered tech at that time and did not want the supervisor position but had no choice in the matter. I was young and inexperienced lacking the supervisory skills. Since then I fortunately learned a lot of people skills. Over the years I have had to apologize to the techs who worked under me during that preliminary period of time, I was a micromanager and not very flexible. I would have fired me.

After the major pathologist turnover in the lab, Dr. Joseph W. Eversole, from Jacksonville, FL, arrived as the director of the laboratory. He began encouraging us histotechs to form a professional society for histologists in Georgia. He fostered a sense of professionalism in all the lab employees. It was with his help and financial support that Shirley Justice, a co-worker, and I began to locate Georgia histotechs who were interested in supporting the effort. In 1972 we sent out postcards asking if there was interest in participating in such a society. The response was overwhelming. We had sent out 50 cards and got 33 positive responses. We had our first organizational meeting in Macon and all of those 33 attended. Thus emerged the birth of the Georgia Society for Histotechnology. I served as Co-Chairman, President, Vice president, and currently Secretary. GSH was incorporated as a nonprofit organization in 1973, one year before NSH. I was also fortunate to be one of the founding members for NSH as board of director, and witnessed the birth of that organization as well. I served on the bylaws and symposium committees and was also NSH Region III Treasurer for a number of years after its formation.

In 1970 my husband at that time got an opportunity to manage a restaurant in Daytona Beach, FL and we moved there in May. I was 5 months pregnant with my son and could not get a histology job at the Fairfax Hospital. I spent the rest of my pregnancy at home until his early delivery the first of September. The restaurant venture did not pan out so we prepared to move back to Macon around the end of December. A MCCG Pathologist, Dr. John G. Etheridge, was to be the director of the new HCA Coliseum Park Hospital Laboratory just opening in January of 1971. I was hired as supervisor at that hospital, now Coliseum Medical Centers, and worked as supervisor there for the next 15 years.

In the early 80's the importance of having Immunohistochemistry procedures on board had grown. I agreed to take on the task of performing Immunos at the Coliseum for all the Macon area hospitals. In time the Medical Center hired someone to run the IHC lab and for a few months relieved me from that job. Soon all the pathologists were sending their cases back to me as the IHC tech quit. I continued to do Immunos as an add-on to all my other duties.

It soon was decided by the Macon pathology group to create an IHC reference laboratory to handle all the IHC cases at Mercer University School of Medicine. They had just begun accepting students a few years earlier. After learning of this position, I applied for it and was hired in October of 1985. This began a new learning experience for me here at Mercer. I had performed the immunos but never had the time to really know what I was doing, why I was doing it and how to troubleshoot the procedures. All I had was kits and followed the directions. I was processing 60 slides at a time by hand at the sink until the automated IHC stainers came on the scene. My first Immuno machine was the IL Code-On. I made it work for me until Ventana came out with their first machine, the Ventana ES in 1991, which was a perfect answer for me as the only tech in my lab. Around that time I begun to take care of my disabled Father and my Mom in my home. I needed flexibility and dependability to do my job and take care of my family. This machine and my lab director, Dr. Anna Walker, made it possible for me to care for my parents. The next machine I got was the NexES. Then Mercer decided in 2001 to shut my reference lab down, reasons still not clear. This left me with redefining my position here at Mercer; it was quite terrifying at the time. My job description was rewritten and my duties were reassessed. My job title changed to Technical Director of the Histology Curricular Support Laboratory. My duties now are to provide support histology technical and supervisory services for Mercer researchers. I managed plastination resources for the medical students, provided learning tours/classes for area students, and developed the Medical Students MDE exam image sheets. If that wasn't enough I also provided histology support for our sister medical school on the Savannah campus as well as histology service for the Bibb County Medical Examiner for the GBI. I wore a lot of hats then and still today have my job, thankfully.

After my parents passed away around 2005 I began teaching the technical laboratory portion for an online histology course at Gordon College and did so until 2010 when Gordon decided to take back the laboratory space we were using. That helped me to realize my passion for teaching. I loved working with the students and helping them to reach their goal of becoming a histotechnologist. I hope to be able to do that again someday, maybe when I retire, OH NO, wait, I don't plan to retire. On July 18, 2012 I will celebrate my 50th year in histology, how is that for stumbling, and hope to keep on practicing my profession many more years. I will just have to make teaching a second job again.

Shirley Powell

GSH Secretary

Histotechnology Professionals Day

Touching Lives, One Slide at a Time

Georgia Governor Deal signed a proclamation declaring March 10, 2012

National Histotechnology Professionals Day in Georgia.

From left to right Wanda Simons, Nancy Crane, Gov. Nathan Deal and Shirley Powell

BY THE GOVERNOR OF THE STATE OF GEORGIA

A PROCLAMATION
HISTOTECHNOLOGY PROFESSIONALS DAY

WHEREAS: The health and well-being of Georgia's residents is a major concern and responsibility for the health professionals serving them; and

WHEREAS: Histotechnologists are formally educated to perform cutting edge procedures to enhance that quality of care. Through skillful application of sophisticated laboratory techniques, professionals in clinical histotechnology enable the seemingly invisible world of tissue structures to become visible under the microscope; and

WHEREAS: The search to unlock the secrets held by tissue structures reaches into many fields. Histotechnologists work as medical and veterinary professionals, pharmaceutical and industry research scientists, anatomists, and chemists; and

WHEREAS: Professionals in histotechnological sciences are dedicated to the highest standards of professionalism and are committed to maintaining and improving this through education, lifelong learning, credentialing, and personal commitment; now

THEREFORE: I, NATHAN DEAL, Governor of the State of Georgia, do hereby proclaim March 10, 2012, as HISTOTECHNOLOGY PROFESSIONALS DAY in Georgia.

In witness thereof, I have hereunto set my hand and caused the Seal of the Executive Department to be affixed this 27th day of February in the year of our Lord two thousand twelve.

Nathan Deal
GOVERNOR

ATTEST

CHIEF OF STAFF

Histotechnology Professionals Day in Georgia.

Go to www.histosearch.com/gsh<http://www.histosearch.com/gsh to see a great photo of this memorable occasion for the histology professionals of Georgia on our home page. I hope everyone will seize the opportunity to inform our communities of our contribution to their tissue diagnoses and treatment.

Your Path to Professional Development

nsh

GEORGIA SOCIETY FOR HISTOTECHNOLOGY 1973

April 13-15, 2012

The Georgia Society for Histotechnology hosts:

The 2012 NSH Region III Symposium

At Callaway Gardens

Pine Mountain, GA

Jones Rapid Method for Demonstration of Chromoblastomycosis

M. Lamar Jones, BS, HT(ASCP), Emory University Hospital, Department of Pathology, Atlanta, GA

Microorganisms are distinct, many times very beautifully demonstrated with special stains but often deadly. Those microorganisms of medical importance usually fall into the following groups: bacteria, fungi, viruses and protozoa. One of the most interesting microorganims is the fungi. Fungi are primitive plants that possess no roots, stems, leaves or chlorophyll. They are also a large and varied group. *Mycology* is the study of fungi and *mycoses* is the disease produced by fungi. The identification of fungi usually depends upon culture appearance and microscopic morphology. Most fungi of medical importance are divided into four (4) groups: filamentous fungi, yeasts, "yeast-like" and dimorphic. One of the most interesting fungal microorganisms of study is *"chromoblastomycosis"*.

Chromoblastomycosis is multinucleated cells with pigmented fungal cells, somewhat muriform shaped, Figure 1. It is also known as "chromomycosis, Cladosporium carrionii, Fonsecaea compacta, F. pedrosoi or Rhinocladiella cerophilum and was first noted in 1915. These cells multiply by elongation, septation and delayed separation of cells (fission), no budding or hyphae. The conidiophores vary in length and have tree-like branching which are pigmented dark- brown, about 5 – 12 microns in diameter. Chromoblastomycosis is world wide, occurring most commonly in tropical or sub-tropical rural climates. Age and sex are not important. Laborers working with wood are often infected. It is found in wood, soil and decaying vegetation and can be caused by many different types of fungi that becomes implanted under the skin often by thorns or splinters.

Chromoblastomycosis is a disease of humans and the initial incident causing the infection is often not noticed. The infection can build over a period of years spreading by the lymphatic ducts to establish small lesions usually affecting the arms, legs, hands, face or chest. The infection builds at the site later forming a pinkish-red verrucous lesion. Very few symptoms are noticed and no pain is associated with the lesion.

The diagnosis of Chromoblastomycosis is often confirmed by "*scrapings*" of the lesion, then cultured. The lesion can be excised, grossed and dissected then processed for microscopic preparation. The tissue sections stained with the H and E stain reveal brown pigmented yeasts that are similar to "copper pennies". The PAS and GMS special stain can be used to demonstrate the microorganism but not always needed.

Chromoblastomycosis is difficult to cure usually by either a fungal medication or surgical excision. The prognosis is good although sever cases are more difficult to cure and is rarely fatal.

The special stains that are commonly used to aid in demonstrating the microorganisms are Giemsa, Periodic Acid Schiff (PAS) and the Gomori or Grocott's Methenamine Silver (GMS). The Giemsa stain will stain a brownish – green tint, the PAS a pink ot magenta color and the GMS a brown to black.

A simple rapid method for demonstrating Chromoblastomycosis fungal cells was developed by M. Lamar Jones in 2009 and can be used rather quickly.

Jones Rapid Method for Demonstration of Chromoblastomycosis

Purpose: Demonstration of Chromoblastomycosis

Principle: The Chromoblastomycosis fungal organism if a muriform sclerotic body that has brown pigment. The organism can often be seen very readily visible on a lightly stained H and E stain. This rapid method allows the brown pigmented fungal organism to be seen more readily with a light green background.

Fixative: 10% neutral buffered formalin

Equipment: Coplin jars, forceps, balance, weigh boats, spatula, magnetic stir plate and stir bar, graduated cylinder.

Technique: Cut paraffin sections at 4-5 microns

Control : A known positive tissue control.

Reagents:

Light Green SF (yellowish) Stock Solution

Light Green SF (yellowish)……..0.3g
Distilled Water……100 mL
Glacial Acetic Acid……0.2 mL

Light Green SF (yellowish) Working Solution

Light Green stock solution……10 mL
Distilled water……40 mL

Procedure:

1. Deparaffinize tissue sections in xylene I……5 minutes
2. Xylene II……5 minutes
3. Xylene III……5 minutes
4. 100% alcohol I……2 minutes
5. 100% alcohol II……2 minutes
6. 95% alcohol I……2 minutes
7. 95% alcohol II……2 minutes
8. Rinse well in running tap water
9. Rinse in distilled water
10. Place slides in Working Light Green solution……30 dips
11. Rinse in 95% alcohol , two (2) changes
12. 100% alcohol I……2 minutes
13. 100% alcohol II……2 minutes
14. Xylene I……2 minutes
15. Xylene II……2 minutes
16. Xylene III……2 minutes
17. Coverslip with permanent synthetic mounting medium

Results: Chromoblastomycosis cells – brown
Counterstain – light green
(Figure 2)

Notes: The counterstain should be a light green color and should not mast the organism.

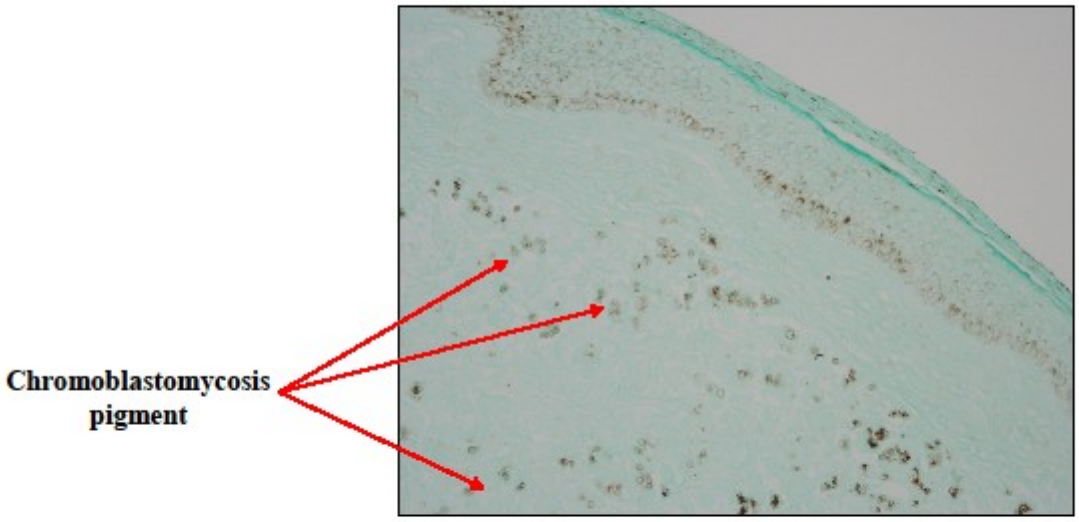

Fig. 2 Jones' Rapid Method for Chromoblastomycosis

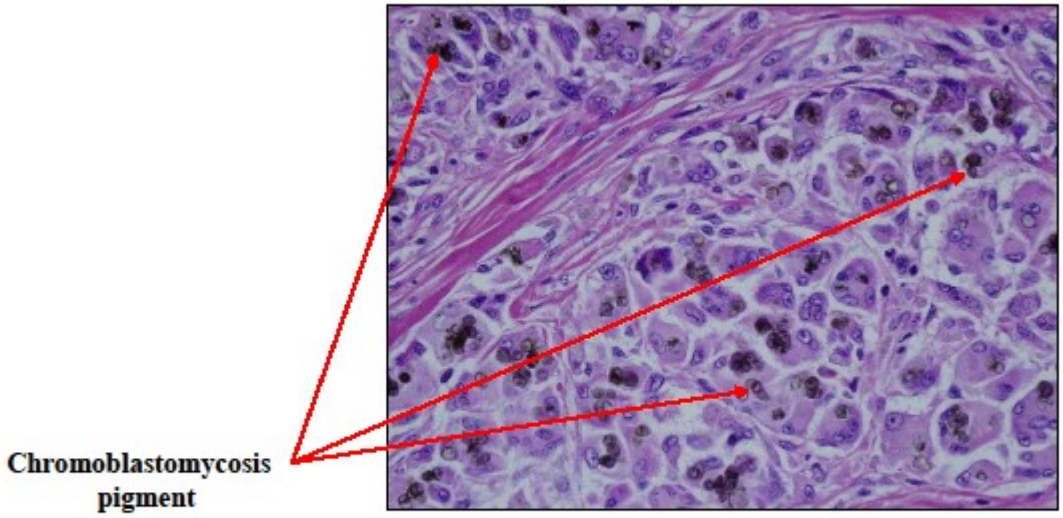

Fig. 1 Skin with Chromoblastomycosis pigment, 40X

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METHOD TO FACILITATE SECTIONING INCOMPLETELY DECALCIFIED BONE

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Bone biopsies are sometimes difficult to obtain and uncomfortable for the patient. Therefore, incomplete decalcification before processing can create problems if the bone sections cannot be achieved without loss of tissue, and require re-decalcification, which takes precious time.

Ideally, the specimen should not be processed until decalcification is complete but as we know, many times the histologist is rushed by the pathologist or clinician to produce section in an inadequate period of time. Sections of bone that have been hurriedly processed and fail to section because of incomplete decalcification may be placed in a decal solution for a short period of time (this will vary with the density and size of the bone), washed in ammonia water, rechilled, and sectioned in the same day without having to remove from the block and reprocess. Small pieces of bone such as bone marrow biopsies take only a short soak of about 30 minutes and will section easily. Larger pieces also will section but take a longer soak. The depth of effectiveness is minimal and if levels are needed, the block may have to be reintroduced to the decal solution more than once.

RDO is one of the decal solutions, which produce the fastest results. There are other rapid decalcifier that are on the market and may work as well. This is a solution to an uncontrollable problem, but the best method is to do it right the first time. Necessity is the mother of invention but you cannot beat "the right way, baby." Most assuredly the patient will this, "uh huh!"

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Fixation - Part 3: How much formalin is enough to fix tissues?

by

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The volume of formalin needed to fix a specimen is not a settled issue. Histotechnology books and journals articles dealing with this aspect recommend formalin volume to tissue volume ratios ranging from 0.5:1 to 200:1 depending on the tissue involved, the proposed study and the authors' preference.

Out of 100 references 4 listed ratios of less than 10:1; 49 had ratios from 10:1 to less then 20:1; 39 preferred ratios of 15:1 to 20:1 and the remaining 8 reported ratios of more than 20:1 but none offered a scientific justification for any selection.

Some preferring 20:1 consider that fixatives, in general, are poor buffers, but that does not apply to 10% Neutral Buffered Formalin (NBF), in spite of which this argument still prevails. This and other large ratios are also favored because some argue that the fixing molecules can be "depleted" which again does not apply to formalin as its concentration is not a critical factor.

Other authors favor large fixative to tissue volume ratios arguing that the fixative can be diluted while fixing, something that is mostly applicable to fixatives that do not contain water, which does not apply to NBF. There are others that argue that NBF will "become acid" if not enough is used, something that is nonsensical because of the buffering intrinsic characteristics of NBF.

The fact remains that the ratio of NBF to tissue mostly results from personal preferences without specific scientific evidence. The qualification of "ideal amount" has been applied to ratios greater than 10:1; to 20:1 or from 20:1 to 50:1, along with others like "not to exceed 15:1"; or that "5:1 is enough". Such dispersion of favorably considered ratios shows how little is known about this important aspect of tissue fixation.

If, on the other hand, you ask any histotech how much formalin should be used to fix, the most likely answer will be a ratio of "at least 10:1" but if you ask

"why 10:1" no clear justification is ever provided, which demonstrated how deeply rooted this misconception is.

Since formalin is a very toxic substance, any effort to reduce its presence in the laboratory is a welcomed event. Consequently a study was carried out (1) using uterus, breast, skin, liver and subcutaneous fat fixed during 8; 24 and 48 hours at room temperature using 4 different "NBF-volume-to-tissue-volume" ratios (1:1; 2:1; 5:1 and 10:1). After fixation all samples were processed manually using isopropyl alcohol and mineral oil (2) and the sections were stained with hematoxylin and eosin along with some special procedures in uterus and liver. There were two quality indicators to evaluate the results: the quality of the paraffin infiltration and, more important, the usefulness of the sections for diagnostic purposes. The microtomy quality of the 60 blocks processed was the infiltration indicator with four levels: "Good" with a score of "3"; "Fair" with a "2"; "Bad" with a "1" and if no section was obtained the score was "0". All the evaluations were "blind" (the evaluator did not know the treatment conditions) and the results were statistically analyzed.

All the sections obtained, from a ratio of 1:1 fixed for 8 hours to a ratio of 10:1 fixed for 48 hours were equally valuable for diagnostic purposes meaning that once a section is obtained and stained it can be used for diagnosis regardless of the amount of formalin or the fixation time.

The differences were more noticeable in the microtomy where there were some combinations of fixative and time that did not produce sections in some tissues, especially fat and skin.

The microtomy quality differences between the samples was not statistically significant for the different fixation volume ratios tested, but the differences between fixation periods and tissues types were, with 48 hours being the optimum fixation period, with skin and fat the most difficult to infiltrate.

Neither the time or volume ratio combinations affected the pH of the NBF or the immunoreaction to vimentin in uterus or the histochemical periodic acid reaction or reticulin demonstration fibers in liver.

At the end it was possible to conclude that fixing tissues with a ratio of "NBF-volume-to-tissue-volume" of 2:1 for 48 hours at 20-22ºC was enough to assure a proper fixation and infiltration of the tested tissues and there is no objective reason to expect that other tissues will not behave similarly.
The ideal fixation time of 48 hours cannot be reduced by an increment of the NBF to tissue ratio, but it could be reduced to about 10 hours if the fixation with NBF at 2:1 takes place at 45ºC with pressure and agitation.
The key element to a good fixation leading to a good infiltration and better quality of sections resides in the amount of time the tissues are fixed, and not in how much formalin is used. Using less formalin should be a goal of any laboratory wanting to increase laboratory safety.

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