Histotechnology Group of Queensland

Tissue Paper

http://www.hgq.org.au

March 2012 – Volume 31

From the President

As I get older I struggle with how fast time is moving. We are now well into 2012 even though the scare of the millennium bug seems not that far ago. Well maybe this is the case for some of us of a certain vintage.

In 2011 there was a well organised National Conference in NSW and already this year I am looking forward to our State Conference to be held at Broadbeach on the Gold Coast in May. The venue looks great and it is difficult not to want to spend some time on the Gold Coast at that time of the year. The conference is getting closer by the day and I am very pleased with the program especially with the variety of topics and it is my desire that we will get a good number of registrations.

The committee have put a lot of hard work into the organisation of this conference and I would like to thank them personally for their endeavours. I would ask all members to please get your registrations in as early as possible as it makes life much better for the organising committee. Just remember if paying directly in to our account to make sure that your personal details are attached.

As well as our conference this year we are planning to have 2-3 scientific meetings which will be announced at a later date. Following the success of our social event in 2011 the committee are planning to hold another event this year and already we are tossing a few ideas about. There is a committee member from nearly every laboratory in Brisbane so if you have an idea for a social outing please pass it on to your local committee person or contact our Secretary Jerres via email or the website with your suggestion.

I hope to see you at Broadbeach in May.

Regards, Tony Reilly

Inside this Issue:

"Bridging Histology Laboratories since 1982"

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Publication Guidelines

Newsletter Correspondence

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Guidelines to Contributors

Please forward submissions in Microsoft Word or compatible program either via email and/or CD & DVD. For any attached photos, please also include these in a separate file. Include your name and address if required. Submissions can be in the form of a brief note, letter or as a complete article.

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This embedding centre is a modular tissue embedding system consisting of two components, a cold plate and a heated embedding module. The system has a large working platform with automatic recycling of wax residue. The temperature range of the paraffin reservoir, working surfaces and warming trays is between ambient and 99°C. The cold plate module can be adjusted from ambient to -30°C.

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12 treatment phases, 9 x 1200mL glass jars, 3 x 1000mL paraffin jars.
The temperature is adjustable from 45 to 85°C.
Net weight of this tissue processor is 60kg.

Secretarial Report – Jerres Alcober

Hello to all our readers. I hope everyone is having a fantastic start to 2012.

The Histotechnology Group of Queensland is looking at organizing 2 or 3 scientific meetings this year in conjunction with the State Histotechnology Conference in May. An April meeting hosted by the Mater Hospital prior to the conference has been proposed. Please log on to www.hgq.org.au for more information & to stay updated.

The HGQ executive committee has been very busy & on track with 2 months until the 2012 State Histotechnology Conference on Fri 4th – 6th May at the Sofitel Hotel - Broadbeach, Gold Coast. Delegate & Trade Registration, Accommodation, Deadline & Program details have now been released. Early bird rates for delegates will end on **Saturday 31 March**. All delegate registrations & payments will close on **Friday 20th April.** To register or to get more details and updates go to our official website.

As anticipated, we have seen a dramatic rise in membership with the upcoming State Conference being a contributing factor to this trend. We would love to welcome back 2011 members and prospective members for this year.

If you haven't become a member of the HGQ, it's not too late to join. HGQ membership covers the calendar year: 1st January – 31st December. Full membership is $25 & Student membership is $10. Being a financial member includes "Tissue Paper" subscriptions; website access; social event discounts; eligibility to vote; beverages & dinner covered at AGM.

Members will also receive a discount on delegate registration for the upcoming State Histotechnology Conference. Renewals and new memberships can be completed online. Check it all out at www.hgq.org.au.

I would like to take this opportunity once again to thank everyone for reading the "Tissue Paper" and to all that contribute to its success.

Hope to catch up with you at the conference.

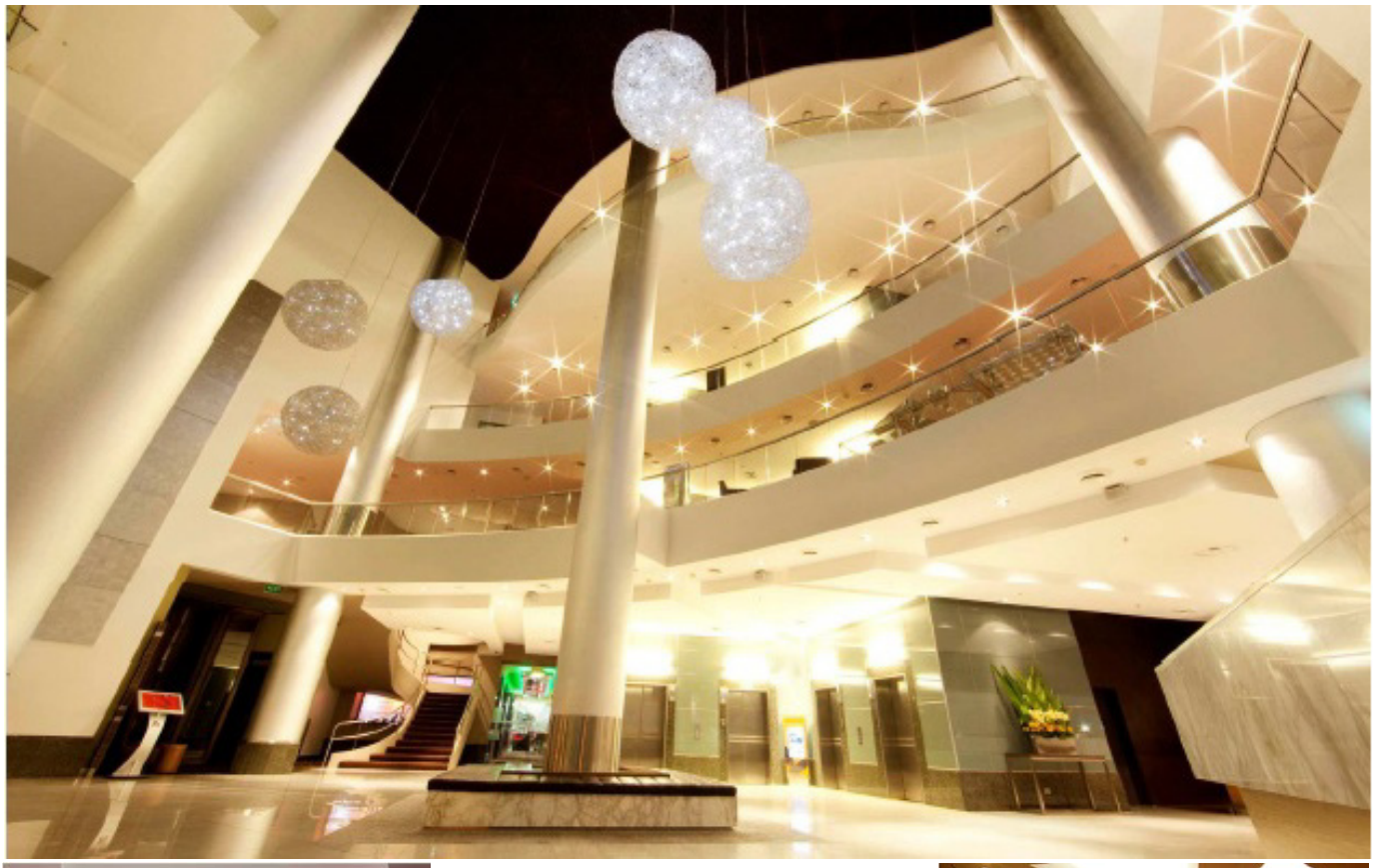
Enjoy!! – *Jerres Alcober*

Editor's Note – Anthony Van Zwieten

2012 is upon us already with all eyes firmly on the State conference at Broadbeach in May. Jerres and the fellow committee members are working very hard to ensure that it's one to remember, with the program close to completion whilst putting the finishing touches on what promises to be a fantastic Saturday night's entertainment. The last 12 months have been great for the HGQ, with memberships increasing and the social aspect going to another level. Keep an eye out for an October social gathering. As you'll see again from this edition, the trade companies are recognising the HGQ as an important avenue for advertising.

Thanks to Tennille and the ever-reliable Gan Man for their contributions.

Happy Reading! – *Anthony Van Zwieten*



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2012 State Histotechnology Conference

Program, Registration and Accommodation details NOW available on the official HGQ website

EARLY BIRD rates end on Saturday 31st March. Registration & Payment closes on Friday 20th April

Register NOW to book your spot at the "Not-To-Be-Missed" event on the 2012 Calendar

Check out www.hgq.org.au for more information, updates and membership

Friday 4th – Sunday 6th May 2012

Sofitel Broadbeach – 81 Surf Parade, Broadbeach QLD 4218

Histotechnology Group of Queensland

www.hgq.org.au admin@hgq.org.au

Conference Program

Friday 4th May 2012

5:30pm	Registration Desk Opens – Level 1 Foyer
6:00pm – 9:00pm	Welcome Function & Trade Exhibition – Level 1 Foyer

Saturday 5th May 2012

8:00am	Registration Desk Opens – Level 1 Foyer
	1st Plenary Session - Grand Ballroom
9:00am – 9:30am	**Dr Andrew Dettrick**
	"C4D and Antibody Mediated Rejection in the Heart"
9:30am – 10:00am	**Bruce Corney & Amanda De Jong**
	"Hendra, Horses & Hysteria"
10:00am – 10:30am	**Christopher Schmidt**
	"Melanoma Vaccines: Can they work?
10:30am – 11:00am	Morning Tea Break
	2nd Plenary Session - Grand Ballroom
11:00am – 11:45am	**Damien Cass**
	"Disaster Victim Identification: QLD & Off-Shore Operations"
11:45am – 12:30pm	**Naomi McCallum & Joshua Masterson**
	"Digital Imaging Demystified: From Pixels to Pathological Diagnosis"
12:30pm – 1:30pm	Lunch Break
	3rd Plenary Session - Grand Ballroom
1:30pm – 2:00pm	**A/Prof Damien Harkin**
	"Histology of the Corneal Limbus & Cultivated Tissue Substitutes"
2:00pm – 2:30pm	**Dr Peter Hopkins**
	"Lung Transplantation Overview: Covering important aspects of Immunology & Histology"
2:30pm – 3:00pm	**Susan Branford**
	Topic To Be Confirmed
3:00pm – 3:30pm	Afternoon Tea Break
	4th Plenary Session - Grand Ballroom
3:30pm – 4:00pm	**Emma Raymond**
	"An overview of the Mincom Wesley Research Institute Tissue Bank"
4:00pm – 4:30pm	**David Gan**
	Topic To Be Confirmed
5:00pm	Trade Exhibition Area closes

***Saturday 5th May 2012* (cont)**

6:15pm – 7:00pm	Pre-Dinner Function – Level 1 Foyer
7:00pm – 11:30pm	Conference Dinner – Grand Ballroom

Sunday 6th May 2012

10:30am – 11:00am	Morning Tea Break
	1st Plenary Session - Grand Ballroom
11:00am – 11:30am	**Anthony Van Zwieten**
	"The use of Tissue Microarray Technology (TMA) in the Diagnostic Immunohistochemistry Laboratory"
11:30am – 12:00pm	**Dr Brian Miller**
	Topic To Be Confirmed
12:00pm – 12:30pm	**Dr Robin Cooke**
	Topic To Be Confirmed
12:30pm – 1:30pm	Lunch Break
	2nd Plenary Session - Grand Ballroom
1:30pm – 2:00pm	**Emma Hughes**
	"Handling Breast and Sentinel Lymph Node specimens at Sullivan Nicolaides Pathology"
2:00pm – 2:30pm	**Dr Eugene Petcu**
	Topic To Be Confirmed
2:30pm – 3:00pm	**A/Prof Anthony Woods**
	Topic To Be Confirmed
3:00pm – 3:10pm	**HGQ Executive Committee**
	"Final Presentations & Conference Close"
3:10pm – 3:40pm	Afternoon Tea Break
4:30pm	Trade Exhibition Area closes

Disclaimer: The information specified in the conference program is accurate & true at the time of printing. The Histotechnology Group of Queensland reserves the right to amend the program as required.

Cytology — Pathology Sector

Original Cytofunnels® — Thermo

The original Cytofunnel® is still the most trusted clipped funnel available today.

- Disposable sample chamber with white filter card and cap
- Pre-attached filter card
- Available in both single and double
- Cytoclips® easily unlock to dispose of the sample chamber and to remove the slide

Code	Description	Unit	Price/Unit
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ALPS9910052	Cytoclip Stainless Steel Slide Clip	Pk/6	$459.00

EZ Cytofunnel® Sample Chambers — Thermo

- Optimise cell yield, and reduce the risk of exposure to pathogenic samples
- Disposable sample chamber with white filter card and cap
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- Available in both single and double
- Produce more cost-effective thin layer preparations

Code	Description	Unit	Price/Unit
ALPA78710020	EZ Single Cytofunnel with White Filter Card	Pk/200	$647.00
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Code	Description	Unit	Price/Unit
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FNNII003	Harris Haematoxylin	5L	$135.00
FNNII002IMP	Harris Haematoxylin Mercury Free	2.5L	$83.00
FNNII003IMP	Harris Haematoxylin Mercury Free	5L	$148.00
FNNII035	Papanicolaou EA50	5L	$109.00
FNNII038	Papanicolaou EA65	5L	$109.00
FNNII041	Papanicolaou OG6	5L	$109.00
FNNGG020	Quick Dip I	500ml	$16.75
FNNGG022	Quick Dip I	5L	$75.00
FNNGG023	Quick Dip II	500ml	$16.75
FNNGG025	Quick Dip II	5L	$75.00
FNNGG017	Quick Dip Fixative	500ml	$15.50
FNNGG019	Quick Dip Fixative	5L	$59.00
FNNFF008	Safety Spray Fixative	200ml	$5.55

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The Shandon Cytospin was the first device to use carefully controlled centrifugation to separate and deposit a thin layer of cells on slides while maintaining cellular integrity. It produces better cell capture and good representation of all cell types present in homogeneous liquid samples.

- Processes 12 specimens at the same time.
- One-handed opening and closing of outer lid
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Description	Code
Cytospin 4 Centrifuge	ASHA78300002

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Description	Code
CytoFuge 2 Centrifuge	IRICF02

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There's more to me than Histology!

Thanks to Tennille Griffin from SNP for giving us an insight into her working holiday in Europe, and for being the first person to agree to an interview with me ☺

When did you work overseas?
From October 2009 until September 2011 in London, UK

How long for?
The entire two-and-a-bit years I was in the UK

Why did you choose to work abroad?
I knew that there was demand for contract lab workers & it was good money (compared to bar work, hospitality, etc) & that it would allow me the flexibility to travel while I worked

Where did you work?
In London, at St Mary's Hospital in Paddington & The Royal London Hospital in Whitechapel

What were that differences between the lab there and at SNP?
The labs are not 24 hours which made the working day less extreme (as I'd been working shift work/early morning shifts for the previous 4 years); machines are basically the same; some microtomes were semiautomatic; one lab had ice bricks that you had to get in & out of the freezer; hot plates to heat slides before oven/stainer; biggest difference was training/compulsory further education to progress through the levels; governing bodies (HPC & IBMS)

The Histo lab at St Mary's Hospital - small! 2 embedders, 6 microtomes & 3 VIP's

What was your favourite destination overseas and why?
So many, didn't dislike anywhere that I travelled; best holiday was La Tomatina (tomato throwing festival) in Spain; also really loved Italy & Croatia

English Beer or Aussie Beer?
Not a huge beer drinker but I didn't find English beer horrible. They do seem to think that VB is awesome & that every Aussie drinks Fosters which is wildly untrue! Cider definitely became my drink of choice

On Westminster Bridge in front of Big Ben & Houses of Parliament

How many other Aussies did you find working in labs?
None. I knew of some through other contract workers but never actually met any. This made my accent a novelty

Would you recommend working In the UK to other histology staff?
Yes definitely (I don't know if there are many jobs available right now due to their struggling economy) but it was a great experience & one that I'd gladly do again if I had the chance

How did you organise employment before you left Australia?
I used a company based in Australia who signed me up with a UK Agency (they got me my first contract) but I was signed up to three agencies in the time I was there whom I also got work through. It's easy enough to join an agency to find yourself work. Most places won't hire you unless you're through an agency so this is the best way to go

Was there enough sunlight to perform Von Kossa stains?
Hahaha... nice one. Umm no, not really. But I don't actually remember one being done while I was around.

Do you have any other comments?
Brilliant experience; great travels; awesome new friends; best thing I've ever done!

The view over Dubrovnik (Croatia) from the city walls at sunset

Do you miss working with Jerres, Jason and me?
Yes... well 2 of you at least ☺

Where was the best place in London to hit the dancefloor and why?
If dancing was involved, it was usually after many drinks at one of the huge amount of pubs in London. There are many clubs but I generally found myself hanging out in the pubs more.

Manual or semi-automatic microtome?
Semi-automatic

How old do you think Jerres really is? I do know but I won't lie. I'll just keep that to myself ☺

After La Tomatina (in Brunol, Spain)- covered in tomatoes!

If you are or know of someone worth reading about, then it needs to be in the HGQ's "Tissue Paper"
Without any other suggestions, The State Conference 2012 Edition will have an interview with a Pathologist / Pilot / Adventurer who will reveal all!

Histotechnology Group of Queensland

Membership Application Form

To complete membership online go to: http://www.hgq.org.au

Please indicate by ☑:

☐ New Membership ☐ Renewal of Membership ☐ Change of Details

Name: _____

Employer: _____

Mailing Address: (Work Address Preferred)

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Class of Membership:

☐ Full - $25 per year

☐ Student (Full-Time Only) - $10 per year with proof required

For New Members Only: New members must be proposed and seconded by current financial members. Should you not know of any members, the executive committee can purpose and second a new member's application.

Proposed by: _____ Signature: _____

Seconded by: _____ Signature: _____

Applicant's signature: _____ Date: _____

Please return completed form via (options below):

Postal Address: Jerres Alcober
Anatomical Pathology Department,
Pathology Queensland
The Prince Charles Hospital
Level 2 – Clinical Sciences Building
Rode Road, CHERMSIDE, QLD 4032
Email Address: admin@hgq.org.au
Fax number: (07) 3139 4546

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Cash: In person - Stephen Riley/Jerres Alcober

Toluidine Blue
A comparison of dye brands

Anthony van Zwieten

Toluidine Blue staining is a metachromatic dye used in the demonstration of mast cells. *(Metachromasia = stains different colour to the dye used, compared to orthochromasia = stains same colour as dye used)*

Metachromasia is attributed to the cationic or basic dye and is dependant on pH, dye concentration and temperature. For example, blue or violet dyes turn towards red, while red dyes show a yellow colour

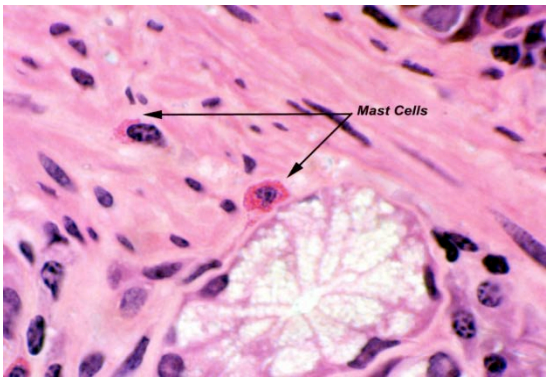
Mast cells compared to basophils

Mast cells
- Localised within connective tissue

Basophils
- Low numbers present in circulation

Mast cells are found on the mucosal surfaces of the lung, gastrointestinal tract, and in the dermis. They contain IgE receptors and are associated with vasoactive mediators.

Antigenic stimulation results in a release of dense cytoplasmic granules, including histamine, heparin and other acid mucopolysaccharides.



Images: www.vetmed.vt.edu

The stain is typically used in the lab as a 1% aqueous solution. This can be modified, with a lowered pH of around 2.5 improving staining contrast. Our laboratory follows this method:

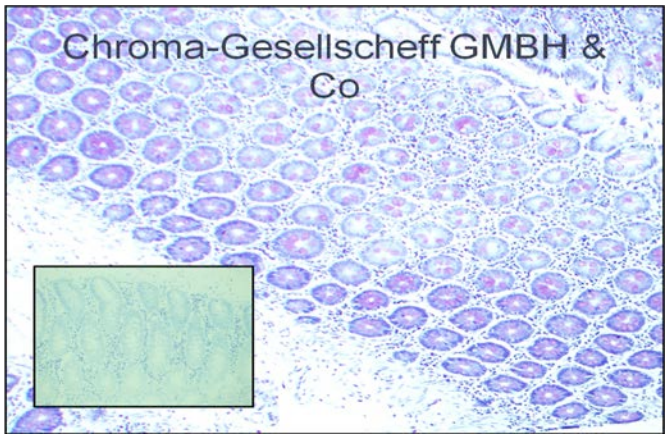
- 1% tol blue for 5 minutes, then blot dry (no dehydation) before clearing and mounting

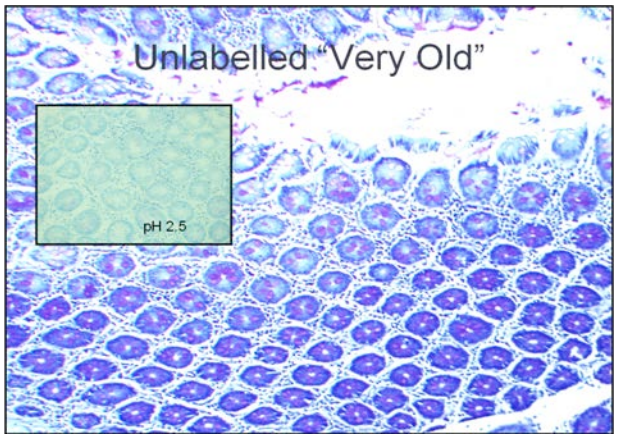
To improve contrast by lowering pH, this method can also be adopted:

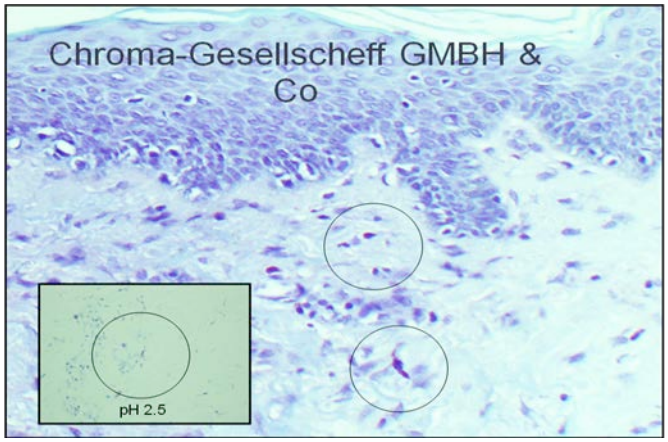
- 0.5ml of 1% tol blue in 4.5 ml of 1% NaCl (ph2.0-2.5 with HCl)
  - Stain in this fresh solution for 3 minutes
  - rinse in distilled water then rapidly dehydrated, cleared and mounted.

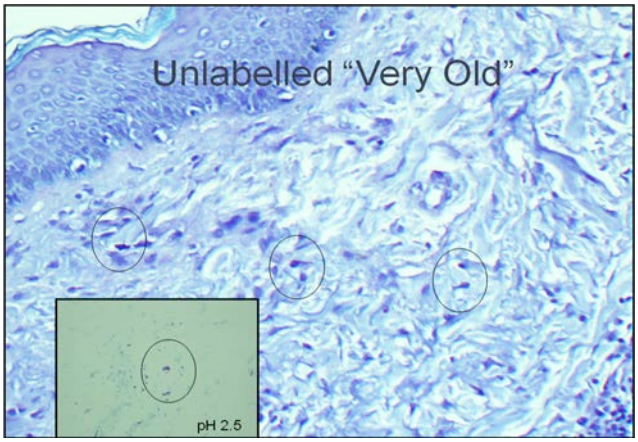
A comparison of toluidine blue dyes was conducted using both the standard and low pH methods on sections of normal colon and skin (Results on the next two pages, with mast cells circled and low pH staining results as the smaller inserted image).

As you can see from the images below, there was a large difference in age of the dyes tested (Thanks to David Gan for supplying the dye powders).


Chroma-Gesellscheff GMBH & Co


Unlabelled "Very Old"
pH 2.5


Chroma-Gesellscheff GMBH & Co
pH 2.5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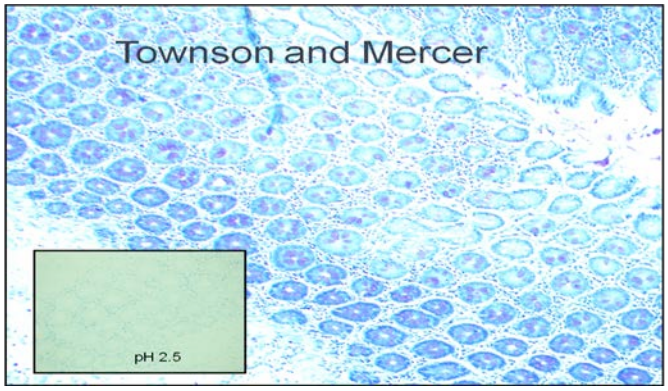
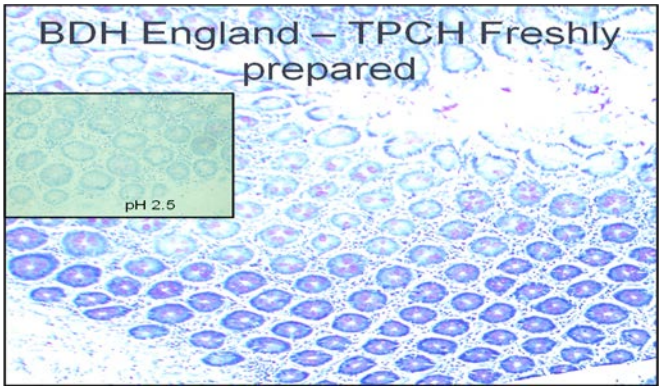
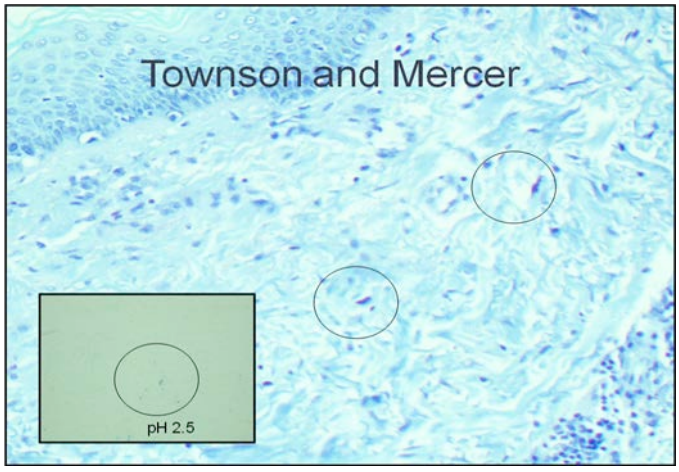
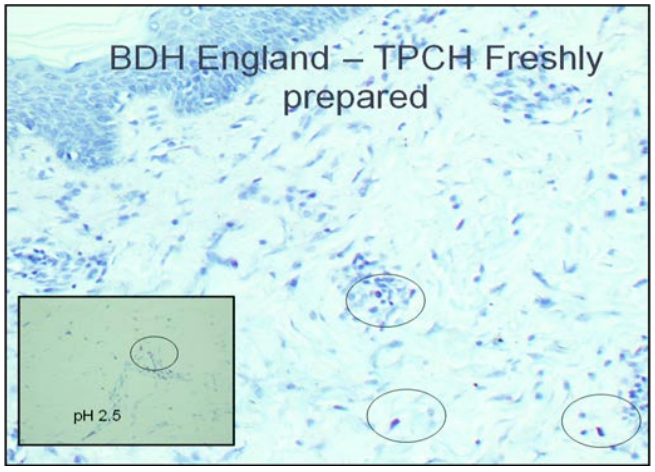
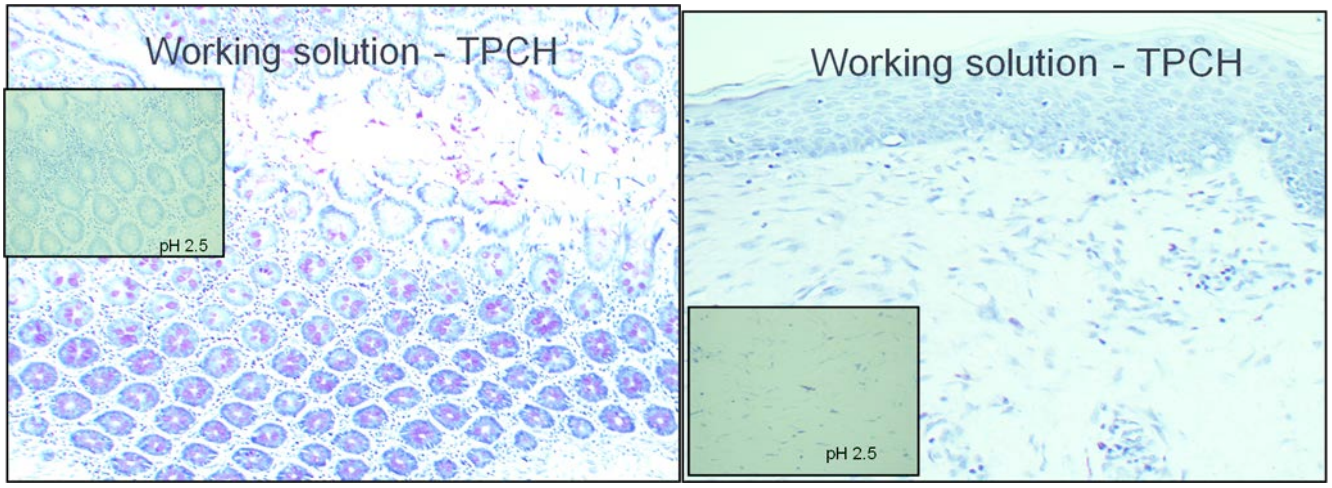

Townson and Mercer
pH 2.5


BDH England – TPCH Freshly prepared
pH 2.5


Townson and Mercer
pH 2.5


BDH England – TPCH Freshly prepared
pH 2.5



As the staining results have shown, not all Toluidine Blue dyes are created equally! The intensity of colonic mucin staining varies between dye brands, and mucin staining is completely removed when dehydrating. Alcohol cannot be used for dehydration in most metachromatic staining methods, but metachromasia of mast cell granules are stable in alcohol.

Decreasing the pH resulted in a clearer staining background and demonstrated good contrast for mast cells in the dermis.

It is important to continually validate the performance of dyes, especially those used sparingly.

*Alternative Rapid screen for mast cells – 1% methylene blue (as in ZN technique).

Reference: Carson and Hladik (2009) Histotechnology: A Self-Intructional Text p188-189

Does your lab have interesting cases?

Special techniques?

Does something different?

If so, put it in the HGQ's Tissue Paper

Histotechnology Group of Queensland

We connect to fight cancer

01
March 2012

A message from Jeremy Tyson, Country Manager

It's been a busy time for Dako in Queensland. We've welcomed back Paul Steward as our new Account Manager (see story below). We've been on the Gold Coast as a Major Sponsor for the 2nd International Diagnostic IHC Workshop and we're looking forward to being the Major Sponsor of the Histotechnology Group of Queensland Conference in May. We hope to see you there.

Our goal is "to provide excellent products, at a fair price, with terrific customer service". With our new pricing, new team and new products, I believe we have one of the best histo staining solutions on the market today. From our H&E to IHC and Special Stains, no one can offer a more integrated workflow solution for your lab.

We've also recently launched four new antibodies including a new Estrogen Receptor clone developed by Epitomics and Dako called EP1. If you think your current ER should be giving you a better result, please contact us regarding a trial.

The Dako Coverstainer is now available in Australia. Contact your Account Manager to book a trial.

Dako Never Sleeps - iStore open for business!

You can buy antibodies and other products, check prices and track your orders at your convenience. Register for your online account today and receive your login within 48 hours. Simply visit www.dako.com and click on "Register now".

Paul Steward, Account Manager, Queensland, WA and NT

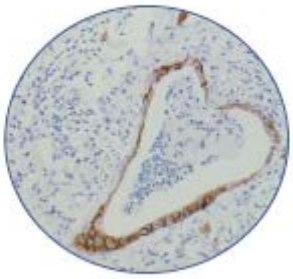
I have returned to Dako after a four year sojourn at Olympus Australia, a little older and wiser for the time. Much of my time at Olympus was spent calling on those same customers I had visited while representing Dako previously. When the opportunity arose to return to Dako I chose to grab it with both hands.

The Dako I return to is very different to the company I left four years ago and, from what I hear, it's very different to 12 months ago. Gone are all the 'bibs and bobs' we sold, gone are the old ways of doing business. Dako is focused on a single portfolio of reagents, kits and instruments delivering a complete solution to pathology laboratories which both delights me and, I know, will challenge me.

I am back to provide the service and support my customers expect of Dako, and to again develop and grow the Dako business.

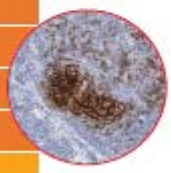
I believe in Dako, that is why I have returned!

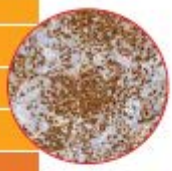
Paul Steward is back at Dako Australia after a four year sojourn with Olympus.



New Dako Monoclonal Mouse and Rabbit Antibodies

Antibody	Description	Platform	Order Number
Monoclonal Mouse Anti-Human CD5, Clone 4C7	Flex RTU 60 Tests 12 mL	Autostainer Link 48	IR08261
	FLEX RTU 30 tests, 6 mL	Dako Autostainer Plus	IS08230
	Concentrate 1 mL	-	M364101
Picture: Mantle cell lymphoma (FFPE) stained with FLEX Anti-CD5, on Autostainer Link 48, Code IR082	Concentrate 0.2 mL	-	M364129
Monoclonal Mouse Anti-Human CD23, Clone DAK-CD23	Flex RTU 60 Tests 12 mL	Autostainer Link 48	IR78161
	FLEX RTU 30 tests, 6 mL	Dako Autostainer Plus	IS78130
	Concentrate 1 mL	-	M731201
Picture: B-cell lymphoma (FFPE) stained with FLEX Anti-CD23, on Autostainer Link 48, Code IR781	Concentrate 0.2 mL	-	M731229
Monoclonal Rabbit Anti-Human Cyclin D1, Clone EP12	Flex RTU 60 Tests 12 mL	Autostainer Link 48	IR08361
	FLEX RTU 30 tests, 6 mL	Dako Autostainer Plus	IS08330
	Concentrate 1 mL	-	M364201
Picture: Mantle cell lymphoma (FFPE) stained with FLEX Anti-Cyclin D1, on Autostainer Link 48, Code IR083	Concentrate 0.2 mL	-	M364229
Monoclonal Rabbit Anti-Human Estrogen Receptor alpha Clone EP1	Flex RTU 60 Tests 12 mL	Autostainer Link 48	IR08461
	FLEX RTU 30 tests, 6 mL	Dako Autostainer Plus	IS08430
	Concentrate 1 mL	-	M364301
Picture: Breast carcinoma (FFPE stained with FLEX anti-Estrogen Receptor alpha, on Autostainer Link 48, Code IR084	Concentrate 0.2 mL	-	M364329





If you would like to see how good the new clones really are, contact the Customer Care Team and we will send you a sample slide stained with the new EP1 Clone.

Material Safety Data Sheets

Dako's MSDS's are now available to download from our website: www.dako.com.

Dako

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Demonstration of Equine Cytoplasmic Granules

David Gan

Mast cell tumours in humans are quite rare but can be quite nasty. Systemic mastocytosis can affect multiple organs and can act similarly to some leukaemias. The cells can rupture and release chemicals such as histamine, heparin and proteases, which can cause a variety of symptoms including anaphylaxis, nausea, edema and peptic ulcers. They are more common in dogs and are always malignant. We had a case of a mast cell tumour in a horse which is very uncommon. These tumours in horses are usually benign.

Horse eosinophils are unique in that the granules in their cytoplasms are huge (about 6 times the size of human eosinophil granules). I thought that it would be interesting to demonstrate the granules in both the mast cells and the eosinophils in the one stain (yes I don't get out much!). Initially I tried a routine Giemsa and although the mast cells stained up well, the granules in the eosinophils were extremely hard to differentiate properly, so looked quite smudgy. An H&E would stain the eosinophils up well but not the mast cells and the Toluidine blue stain wouldn't stain up the eosinophil granules.

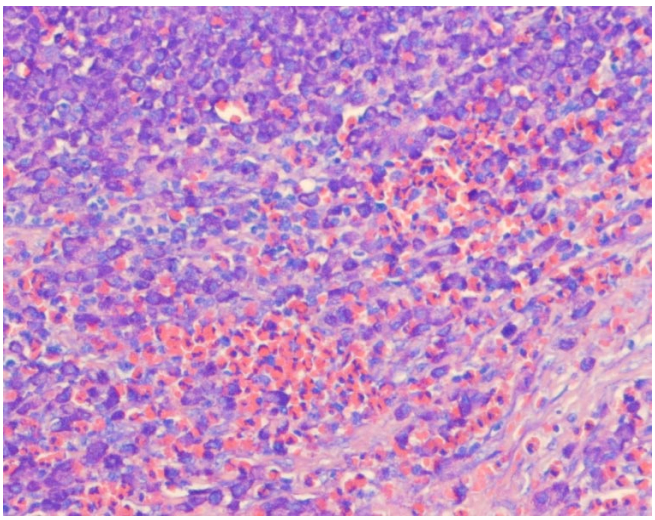
I thought that Toluidine Blue to stain the mast cells and eosin to stain the eosinophils may work. Toluidine Blue then eosin failed because the alcoholic eosin washed out the toluidine blue very quickly. I found that 1% alcoholic eosin for 1minute a quick wash in deionized water then 10 seconds in 1% aqueous Toluidine Blue worked well. There was still metachromasia in the mast cells (sorry, they didn't turn out very well in the pictures) and the eosinophil granules were still clean.

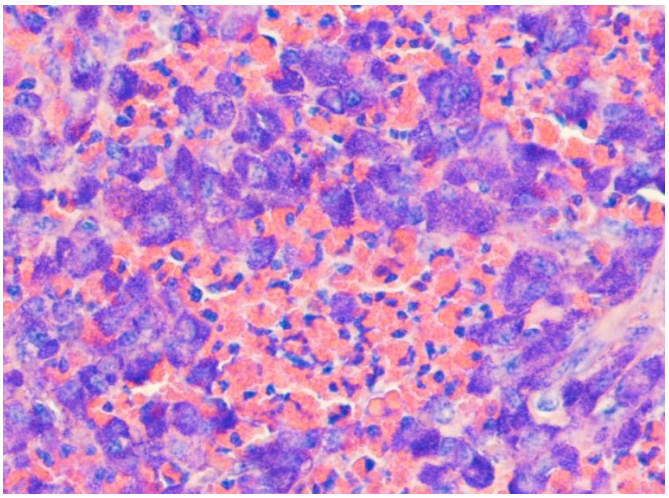
To celebrate the opening of our toll bridge and tunnels in Brisbane, I have decided to call this stain the E-Tol (Eosin Toluidine Blue). If you would like one, at the moment it costs $2.00 to stain and $2.00 for the return of the slide. The price is due to increase in 3 months time depending on usage!

As I said, for me this is quite useless (like the tunnels) but it still looks pretty. Let me know if you have a use for this and I will send you the method.

References:

Journal of Physiology (1995), 483.1





Images of mast cells (purple) and eosinophils (pink/red)
Left: Low power Right: High power