Histotechnology Group of Queensland

Tissue Paper

http://www.hgq.org.au

May 2012 – Volume 32

From the President

Our first Scientific Meeting for the year was held at the Mater Hospital with 2 presentations on Her2 including some of the latest updates from Roche. This was well attended and I am looking forward to the next 2 meetings which will be organised by the Pathology Queensland Central laboratory at the Royal Brisbane Hospital campus and the Pathology Queensland Prince Charles laboratory. I would like to thank the staff of the Mater Anatomical Pathology for organising the speakers and venue. So far the rotation system for our Scientific Meetings has worked well for us with 6 laboratories included on the roster. I would like to extend an invitation to all of the other Histology laboratories to be involved. With the 6 laboratories at present it only requires the organisation of 1 meeting every 2 years and the inclusion of more labs would reduce this even further.

The thoughts and actions of the committee are now firmly focused on our upcoming State Conference on the 4-6th May at the Sofitel Broadbeach. With over 100 delegates and 15 trade booths I feel that it will be a very successful and enjoyable meeting. I would like to thank the committee for all of the effort that has gone in to the planning and execution of this conference but particularly to our Secretary Jerres Alcober. As anybody who has been involved in any type of committee the secretary takes on the largest workload of all committee members and this is particularly exaggerated leading up to a conference. However Jerres is not only the Secretary but also our website administrator and regular designer for any artwork or photography.

I hope to see you in the near future on the Gold Coast.

Tony Reilly

President

HGQ

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Secretarial Report – Jerres Alcober

Hello to all our readers. This year has been action packed at HGQ headquarters with the half way mark fast approaching

Shortly following the Easter Break, we had the first scientific meeting of 2012. This meeting was hosted by the Mater Pathology at the Mater Adult Hospital. It was well attended with around 20 people making the journey to the South Brisbane venue. Amy Broomfield from Mater Pathology & Janet Brown from Roche Diagnostic held a presentation on current developments in HER-2 testing with particular focus on the evolution from the CISH to DDISH technique. Thank you to the speakers, guests & Mater Pathology for another prosperous scientific meeting.

Two more scientific meetings are scheduled for the year with the last one coinciding with the AGM/Christmas Party. A social event or two is also scheduled in the latter part of 2012. Please log on to www.hgq.org.au for more information & to stay updated.

The highly - anticipated Queensland Histotechnology Conference has now arrived. We have over 100 people registered which include delegates & speakers. There are 15 trade exhibitors showcasing their latest & greatest histology-related products. We hope all attending will enjoy this event and get the most of this continuing education opportunity. Thank you to all our sponsors, trade exhibitors & to all that have contributed to the success of this conference. Your hard work & support is much appreciated.

For any readers wanting to become a HGQ member, 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.

I would like to take this opportunity once again to thank everyone for reading & contributing to the "Tissue Paper". If you'd like to be involved in the "Tissue Paper" please contact us. The more the merrier.

Hope to catch up with you at the conference.

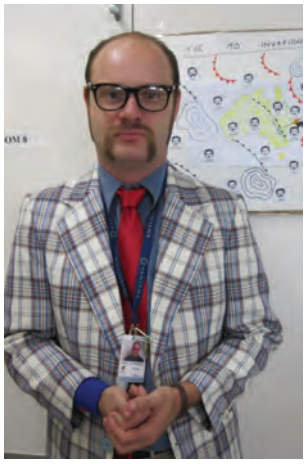
Enjoy!!

Editor's Note

Thanks for taking the time to read this bonus conference edition of the 'Tissue Paper'. I trust that you will find it to be a soothing escape from the hectic and exciting weekend here at Broadbeach.

Thanks to those who have made contributions to this and earlier editions, and also to the trade companies who are willing to advertise with the HGQ. Happy Reading!

Anthony van Zwieten



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02
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Reference: Colley & Stead 2011, Optimized Immunohistochemistry Workflow Facilitated by New Dako Autostainer Link 48 Software
http://www.dako.com/au/index/knowledgecenter/kc_product_info_sites/pathology_instrumentation/dako_autostainerlink_48_prod_info_site/dako_autostainerlink_48_publications.htm

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SOME DAYS YOU JUST NEED A LITTLE LIFT

David Gan -QML

Question from I.M.Dodgy:

"*We sometimes have problems especially with small biopsies that after a few shallow levels the important tissue cuts out and there is no tissue left in the block for further staining. Are there ways of using previously stained H&E slides for other staining?"*

Great Question Mr Dodgy, yes there are many ways that you can reuse slides, which technique that you use depends on a number of factors. Factors can be the number of stains required, the type of stains and the slides that the originals were cut on. You may be able to just destain and restain or you may have to use one of the more complicated techniques. The one technique that I will share is called tissue transfer or tissue lifting and is useful in many different circumstances. This technique involves the lifting of cells from the original slide and transferring them to another slide that is then stained. Using this technique, multiple stains may be performed from one original slide. Most routine special stains, Immunohistochemistry stains and In Situ Hybridization techniques work well using the lifting technique. It should be noted that careful in-house validation must be performed and experienced interpretation is required when using this technique. Apart from this, tissue lifting can be a very useful tool and may be used in many different settings.

For those not familiar with this technique, I have included a summary of the technique that my laboratory uses.

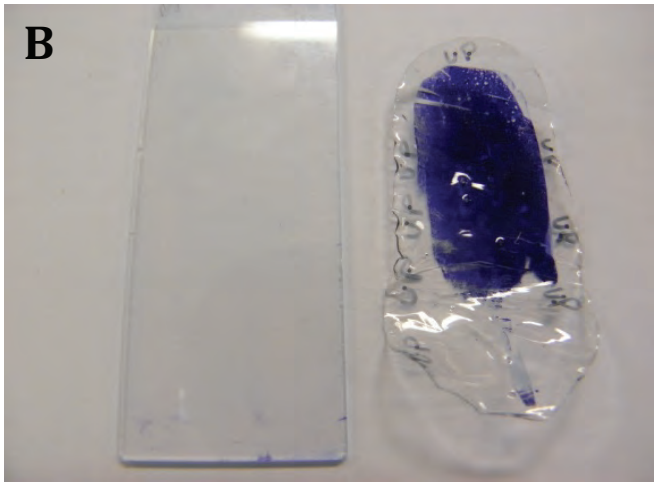
*The technique starts with the removal of the coverslip and all mounting media.

*The slide is air dried and then depex or another commercial product such as Mount-Quick (Newcomer Supply Middleton, WI 53562) is applied to the cells that you would like to lift. I have not used Mount-Quick but their web-site has the technique and others have used it successfully. I have trialed a few different mounting medias and had the best results from depex (probably because it is more viscous).

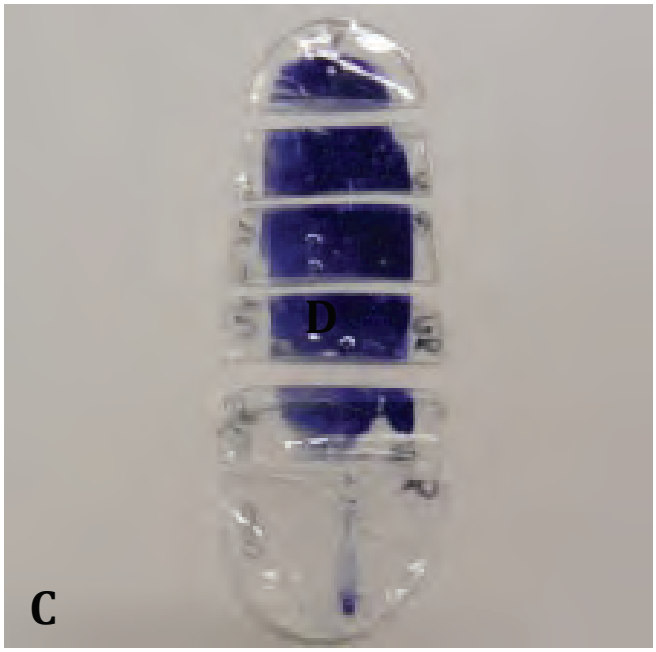
*The slide is then dried in a 55 degree C oven for 4 hours or until just hard (see photo A. all of the cells are covered in this instance). This will vary and if the depex is too hard or soft, the next steps are more difficult.

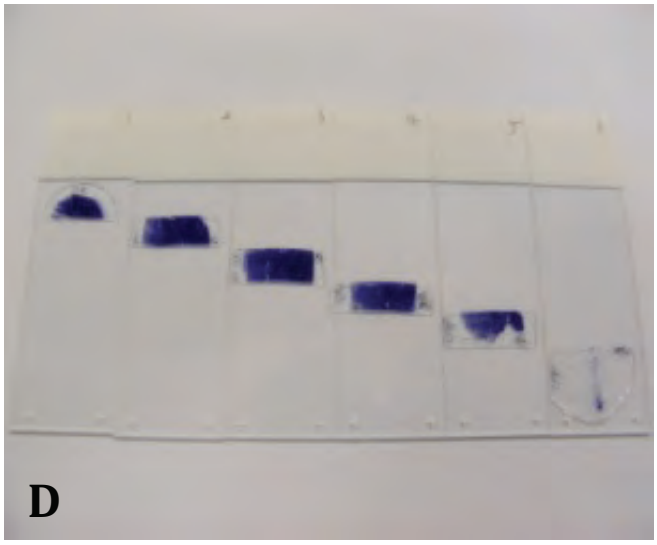
A

*Next is to soak the slide in water for 5 minutes, blot dry and then, using a sharp blade carefully cut off the depex with the underlying cells attached (see photo B). It is important to keep the depex orientated and not to turn it over. If cells on the original slide are to be retained, just recoverslip it at this stage.



*The depex can then be cut into multiple pieces depending on the number of stains required and the amount of diagnostic material and then placed on charged slides (see photos C and D).



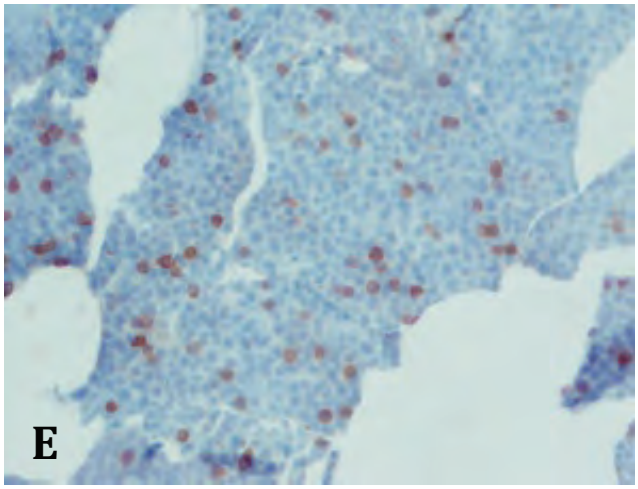


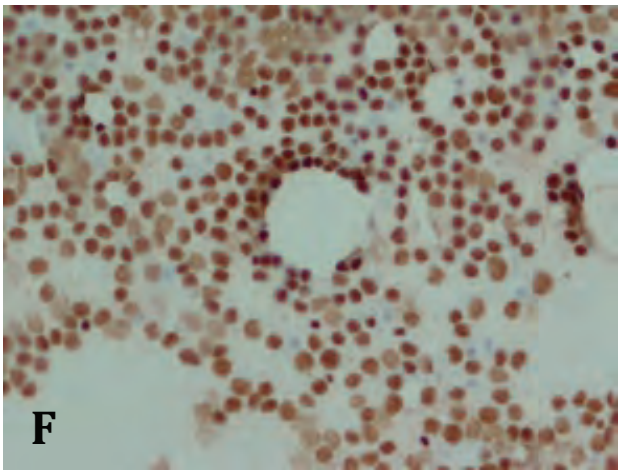
*The depex should be pushed down quite firmly to help it adhere to the slide properly. *The mounting media can then be dissolved in xylene, dried and then stained in the usual manner from water. In my experience, the cells may fall off more easily if the slide is washed in ethanol after xylene.

I have found that some stains work much better than others after being lifted, probably due to the cells drying during the technique. Some stains that are problematic are IHC stains that have either no pretreatment or an enzyme pretreatment. When prepared properly, there is minimal damage to the tissue other than fraying, lifting or cracking in some areas. I must stress again the importance of validation and interpretation of this method. Internal positive staining is an excellent control measure while totally negative staining should be viewed with caution.

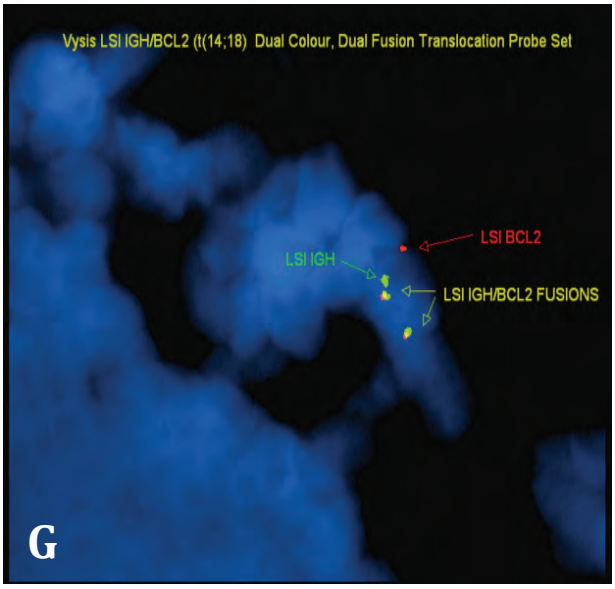
This technique can be used for staining:

Cytological smears (photo E is a CD3 stained from a Diff Quik FNA from a dog lymph node and photo F is a PAX5 from the same slide).

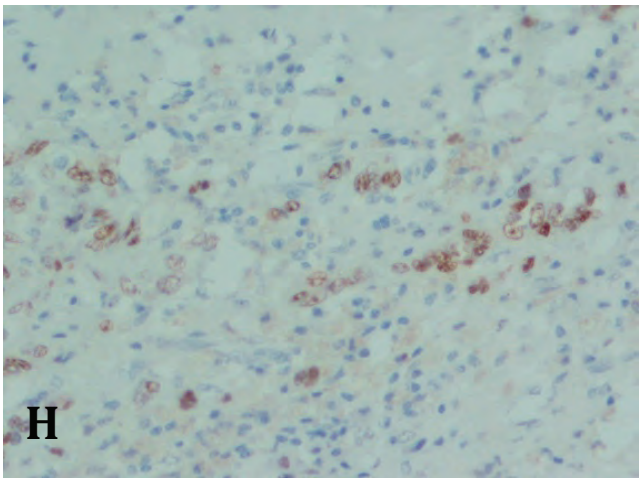




Cases where there is minimal diagnostic tissue (photo G is a FISH t(14:18) preformed from a lifted H&E level of a small bowel biopsy)



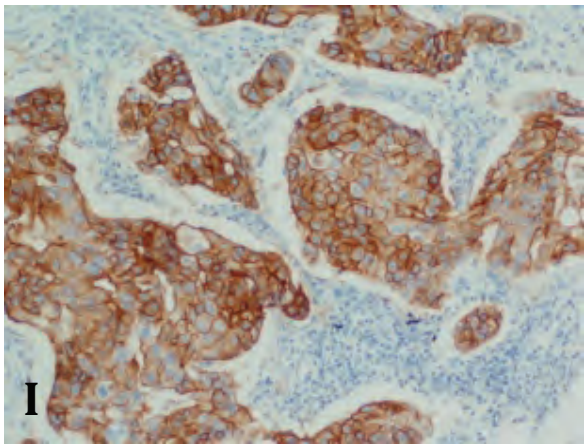
Consultations Cases where no block has been sent (photo H is an ER lifted from a slide sent from a liver specimen querying breast metastases.)

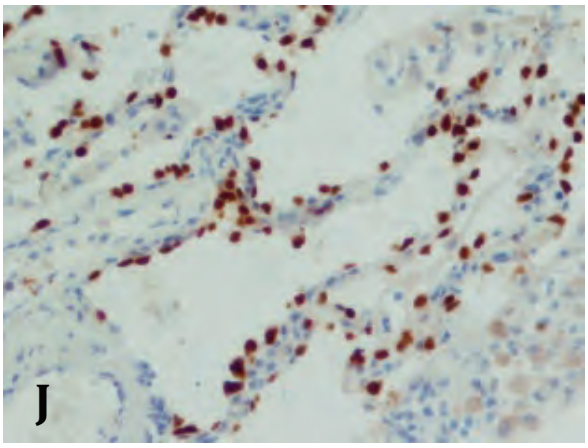


Lifting could be quite useful in research situations where blocks may be very difficult to get a hold of, while limited slides are available and multiple stains required.

Other examples of staining from lifted sections are:

Photo I- Her2 IHC, breast Ca Photo J- TTF1 IHC, lung tissue





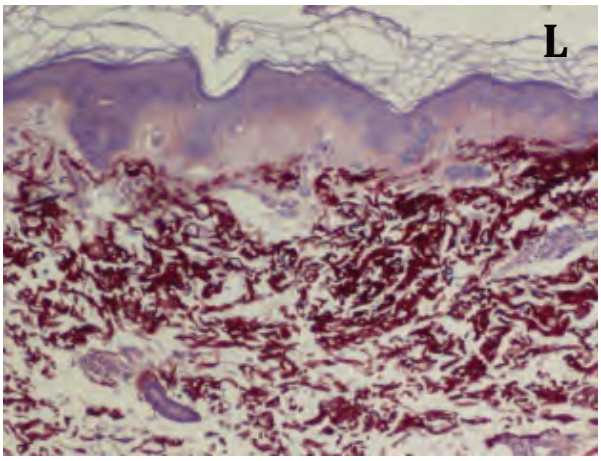
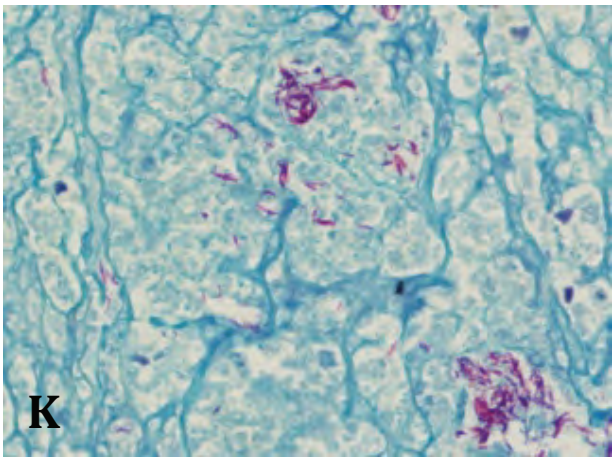


Photo K- Ziehl-Neelsen, mycobacterium lung ; Photo L- Orcein Elastic, skin



As a whole, this technique is not the easiest to perform but in some instances has been essential for a specific diagnosis with the tissue available.

If there is anyone interested in this, or similar techniques the two references below are excellent, or feel free to contact me.

Acknowledgements

Dr Bryan Knight

"Technical Immunohistochemistry" Dr Rodney Miller 2001 ProPath Mount-Quick- Newcomersupply Middleton, WI

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A Report from the Wide Bay Scientific Group Conference

Saturday 3rd March, Tranquil Park, Maleny

Andre Heiser, QML Buderim

The day started with Dr Peter Ellis giving us a fascinating look at identifying the remains of fallen Australian soldiers at Fromelles. The challenges were daunting as there were roughly 9,000 dead or wounded on both sides during "the worst 24 hours in Australia's entire history." Identification of the remains used every conceivable method as well as a few inconceivable ones such as the number of eyelets on the boots. It was like a cross between *Cold Case* and *CSI: Fromelles*.

Dr David Sowden was the next speaker and discussed the difficulties in diagnosing *Clostridium difficile* infections. *C. difficile* can arise in the GIT after antibiotic treatments and Dr Sowden explained the various ways it can be diagnosed.

After that was a light-hearted discussion of lymphomas by Dr Jerome Lai. He focused on the history of classification of lymphomas from the old days of big, medium and small to the more clinically-useful categories used today. He also shared some good advice for all pathologists which can be summarised blandly as: "Don't think you know it all."

QML's Lynda Dial spoke siderolastic anaemia and the dangers of certain complementary medicines such as some Ayurvedic medicines which can contain dangerous levels of lead. Whilst locally-produced alternative medicines are controlled by the TGA, ones purchased from overseas may contain lead. Another lead poisoning/SDA case study demonstrated that gunfire can be dangerous in more ways than the obvious.

At this point we all went for a nice buffet lunch overlooking the Glass House Mountains National Park where we could all look at the rolling green pastures and argue over which particular rock was Mount [Insert Name Here].

Upon our return, the group split in two. I attended the Scientific Program rather than the Phlebotomy Program. This was good for me as I learned from Mike Caffery the ins and outs of the Gram Stain. I have not done one of these since university (the Brown and Hopps doesn't count) so his refresher course in everything Gram (from its chemistry to the correct way to use the microscope) was welcome.

After that, Kim Adkins presented a good talk on cytology of the thyroid—made all the more interesting by the fact that she was the patient used as the examples. Kim found her tumour by pure chance and became her own case study, bravely discussing the specimen collection methods all the way through to her own diagnosis.

Next was the amusingly-titled HONK, HONK! by Alison Hall who discussed Hyperosmotic Non-Ketotic coma (also known as Hyperosmolar Non-Ketotic State). Using a case study, she explained that HONK was usually a consequence of undiagnosed Type-2 Diabetes. In addition, she discussed the difference between HONK and Diabetic ketoacidosis (which is similar in many ways).

Before the afternoon tea (cheese and crackers), Robyn Wells spoke on paroxysmal cold hemoglobinuria—an auto-immune haemolytic anaemia caused by antibodies binding preferentially at cold temperatures followed by complement activation once the body has warmed up (presumably to the point where the complement proteins are re-natured) causing haemloysis.

After the cheese and crackers (and the free alcohol), Dr Jenny Grew spoke on HPV vaccines (*Gardasil* and *Cervarix*), their safety and effectiveness as well as the expansion of its use across the world and for males to further enhance the herd immunity. She also discussed the reasons why it is necessary, focussing on the consequences of HPV infection.

The final technical speaker was Shane Byrne who explained the relatively new field of molecular microbiology. The biggest take-home message of his talk was that molecular microbiology was still a new field and we have yet to see the limits of the technology. The second subtext of his talk was that people still don't know where molecular microbiology fits in pathology laboratory taxonomy.

I did not stay for the AGM (the food was disagreeing with me and my bowels) but I am sure it was interesting too.

Histotechnology Group of Queensland

If you have been to an interesting conference or come across an interesting case then why not tell the HGQ's "Tissue Paper"

There's more to me than Histology!

Dr David Godbolt is an anatomical pathologist working at Pathology Queensland – The Prince Charles Hospital. On weekends and when he gets the chance, he won't be found without the use of a GPS and/or a compass. He is a daredevil aerobatic pilot, mountain climber and all-round adventurer looking for thrills and most worryingly, spills. All of this and he is a committed husband and father of three.

He has kindly **but** reluctantly agreed to an interview with me for the second installment of "There's more to me than Histology"

First and most important question, how many unassisted chin-ups can you perform in one minute?

More than you ever can, Sunshine (laughs)

When on holidays, what is your favourite destination?

A toss-up between Lord Howe Island for its array of activities – Mountain climbing and hiking, deep sea scuba diving and surfing; and Namibia for its vastness and low density population which makes it relatively safe to explore. Unfortunately my goal to paraglide through the desert did not come to fruition as I wouldn't have survived the flight.

Speaking of mountain climbing, what is the highest peak you have climbed?

Mt Kenya (17,000 feet) was the highest but not the scariest (laughs). I save the fun for freestyle waterfall climbs in the Border Ranges (see below)

As a pathologist, when did you get the time to become a qualified pilot?

When studying a B. Medical Science I had some time to get it. It's something I always wanted to do.

Does your pilot licence cover a range of different aircraft?

The smallest would be ultra light drifters and the largest I can fly is a 10 seater.

Apart from yours truly, how many passengers have been physically sick in the air when you perform aerobatics?

At least 90%, so not just you (laughs). It seems to happen when I don't give any notice to my next manoeuvrer.

At least it was my morning coffee only that landed on the windows

(Laughs) It was a fun morning.

What is your favourite flight path from Redcliffe aerodrome?

East to Moreton Island is always good especially during whale season. Down to the Gold Coast is very scenic. If I had a choice I'd rather be performing aerobatics.

What makes Pathology interesting to you?

Especially with interesting cases, going back to first principles to make a diagnosis.

Is it true that you are intrigued by a hunting magazine called “Bacon Busters”?

I’ve been trying to source its annual calendar to pass onto a friend researching Boars in Scotland. I’ve had trouble locating it at the newsagent but someone has been kind enough to give me a subscription for 2012. My Scottish friend will be very excited!

Are you inspired by Bear Grylls and his television show “Man Vs Wild”?

I find him more amusing than inspired, especially following his improvised guano enema in the ocean.

Is there another adventurer who you are inspired by?

Harry Butler’s “In the Wild” was fascinating growing up on a property. I attempted to follow his witchetty grub diet but didn’t have the stomach for it.

With your escapades outdoors, how do you find working inside a small pathologists office?

To be honest it is an absolute struggle

Why did you choose Pathology as a profession?

It was an interesting subject at medical school and the working hours suit my lifestyle

What would you be doing if not Pathology?

Either a tour guide somewhere exotic or a part-time socialite / part-time adventurer when my celebrity wife allowed me to.

How old do you think Jerres is?

35

If you are or know of someone worth reading about, then it needs to be in the HGQ’s “Tissue Paper”

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Some Connective Tissue Stains

Amanda Russell
RBWH – Anatomical Pathology

Thanks to Amanda for sending in this edited powerpoint presentation on these important histological techniques

- **Haematoxylin Van Gieson – Collagen**
- **Verhoff's Van Gieson – Elastic Fibres**
- **Orcein –Elastic Fibres, Hepatitis B Surface Antigen and Copper Associated Protein**

Collagen

- Is the most common protein in the body
- Provides strength to tissue
- An increase indicates fibrotic change
  - eg. Cirrhosis in the liver

Haematoxylin Van Gieson

- Routine stain for liver biopsies
- In-built control
- Nuclear staining can be achieved two ways:
  - Sequential staining with Celestine blue and an aluminium Hx (eg. Mayers)
  - Use of an iron Hx (eg. Weigerts)
- Sequential staining with Celestine blue and an alum Hx acts like a mordant to prevent the loss of nuclear staining when the sections are treated with Van Gieson (VG) soln
- VG counterstain - collagen red, muscle and cytoplasm yellow

STAINING PROCEDURE at RBWH

1. Deparaffinise and hydrate slides from xylene through alcohols and then into running water.
2. Stain in Celestine Blue Solution for 3-5 min.
3. Rinse in tap water.
4. Stain in Mayer's Hx for 1 min.
5. Wash in running tap water.
6. Blue in Scott's Blueing Solution.
7. Wash in running tap water.
8. Stain in Van Gieson's Solution mixture for 30 seconds.
9. Blot sections and dehydrate quickly, clear and mount in synthetic mountant.

Results: Collagen – Red; Nuclei – Blue/ black;

Muscle – Yellow; Cytoplasm - Yellow

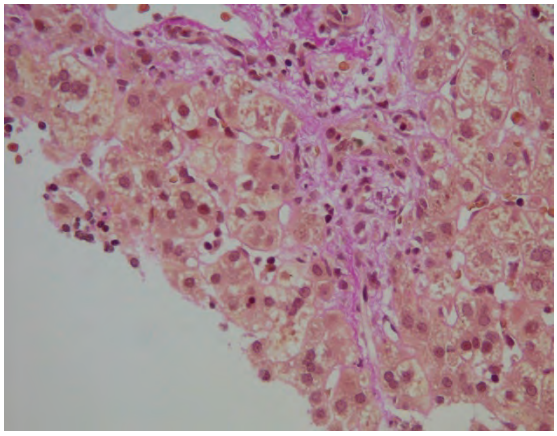


Image from RBWH staining manual

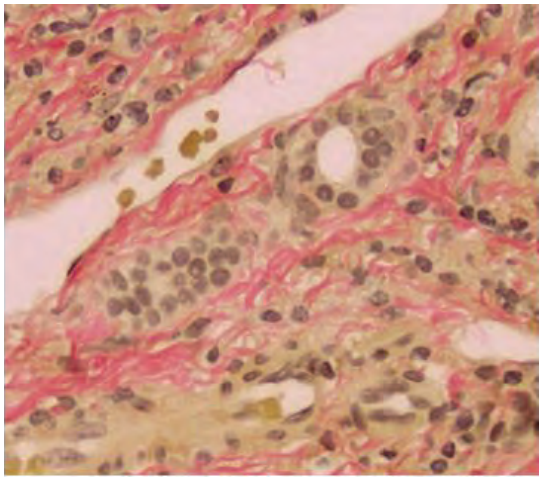


Image from RCPA website

Elastic Fibres – Elastin

- Is a type of connective tissue fibre
- Allows tissue to stretch
- Prominent in arteries, lung, intestines, urinary bladder and the skin
- The size and arrangement of the fibres varies between organs
- Stain with acidic dyes

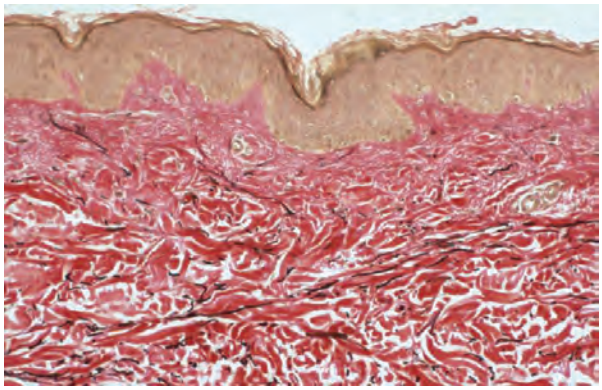


Image from Cell and Tissue Biology Website

- Demonstration of the fibres using special stains may show
  - Fibre atrophy
  - Thinning
  - Loss of fibres
  - Brakes or splitting
  - Reduplication
  - Can be used to show if tumour has invaded blood vessels (metastasis/ progression)

Verhoff's Van Gieson

- Regressive stain
- In-built control
- Routine for temporal artery bx
- Tissue is initially over stained with the Verhoff's solution
- The FeCL3 and iodine act as mordants for the Hx
- Dilute FeCL3 is then used to differentiate – this breaks the tissue-mordant-dye complex
- The elastic fibres have the strongest affinity for the complex, and therefore retain the stain for longer
- The proposed dye binding mechanism is Hydrogen bonds
- Over differentiation - results in the loss of staining in finer elastic fibres
- Sections are to be differentiated independently
- 95% ethanol - to remove excess iodine
- VG is used at the counterstain – staining collagen red and other tissue elements yellow
- Excess time in the VG will continue to differentiate the section

STAINING PROCEDURE at RBWH

1. Deparaffinise and hydrate slides from xylene through the alcohols and then into running water.
2. Stain in Coplin jar of filtered Verhoeff's stain for 15 minutes.
3. Wash slides in running tap water.
4. Differentiate sections in 2% Ferric Chloride in a Coplin jar until only elastic fibers and nuclei are stained. This differentiation is monitored by washing in water, then examining sections under the microscope. If differentiation is not adequate, return the sections to the differentiator.
5. Place slides in 95% Alcohol to remove excess iodine for 5 minutes.
6. Rinse sections in distilled water.
7. Counterstain sections with filtered Van Gieson Solution for 30 seconds.
8. Blot sections.
9. Dehydrate, clear and mount in synthetic mountant.

Results: Elastic Fibres – black; Nuclei – Blue/ black; Collagen – Red; Other tissue elements – Yellow

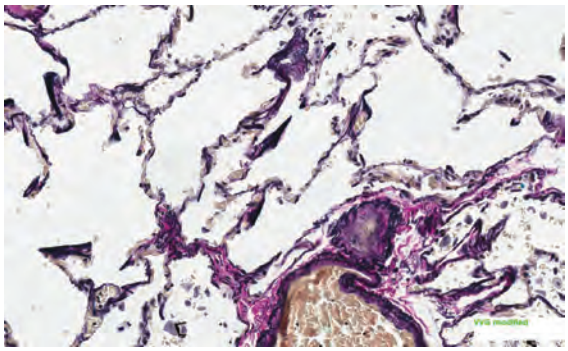
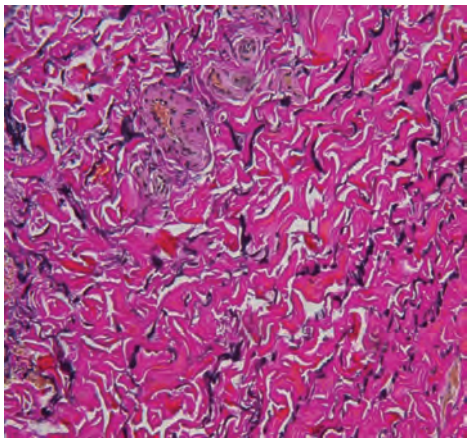
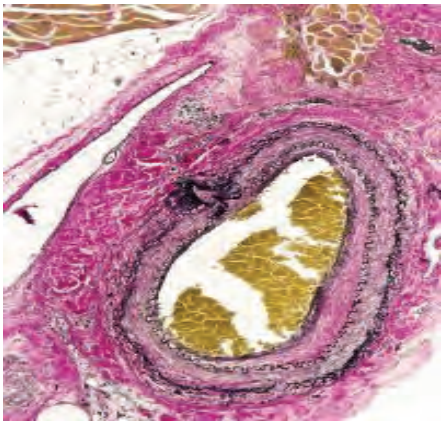


Image from RCPA website



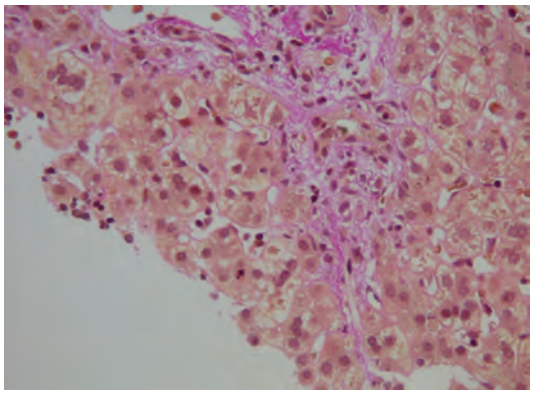


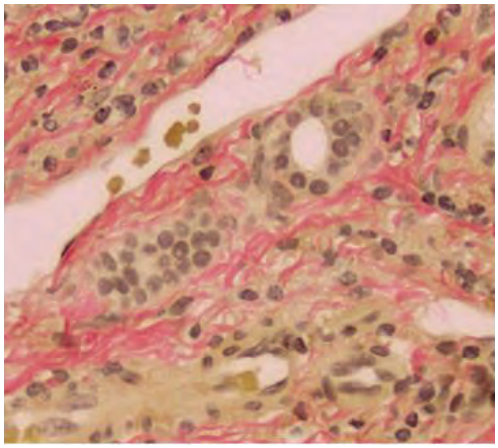
Images from University of Washington Department of Pathology Website

Van Gieson Solution

- Used in VVG and HVG as a counterstain
- Strongly acidic
- Consists of acid fuchsin, saturated aqueous picric acid, nitric acid and glycerol
- Picric acid provides acidic pH and also stains muscle and cytoplasm
- The acid fuchsin selectively stains collagen red
- **Must use saturated picric acid – otherwise the collagen will stain pale pink – orange and cause poor differentiation between collagen, cytoplasm and muscle**

Note the difference between the two images on the right





- Staining mechanism - based on the size of the molecules in the stain
- Picric Acid is a small molecule anionic dye
- Acid Fuchsin is a large molecule anionic dye
- When the VG is applied to the tissue, the picric acid permeates the tissue and binds to susceptible sites
- The acid fuchsin is able to permeate the collagen only - due to its size
- When the section is dehydrated, the picric acid is removed from the collagen – leaving the red coloration of the acid fuchsin
- The addition of HCl or HNO3 in the VG sharpens the contrast between collagen and muscle
- Sections should not be returned to water after treatment with VG as it removes the picric acid
- Any nuclear stain used must be able to resist the acidic VG solution

Orcein

- Routine liver bx stain
- Has great affinity for elastic fibres – thought to bind via van der Waals forces
- Shikata's modified method includes pre-oxidisation with acidified potassium permanganate and then oxalic acid to remove colouration
- Where Shikata's modified method is used Hep B surface antigen (Australian ag), copper-associated protein (CAP) and intestinally sulfated mucins also stain
- The permanganate oxidises sulfur containing proteins to sulphonate residues that react with the orcein
- Presence of CAP – indicates Wilsons disease
- Control tissue: liver with Hep B antigen, CAP and elastic fibres
- Stain in orcein solution
- Use of 1% HCL in 70% Ethanol to decolourise background
- Decolourisation must be controlled – generally little is required

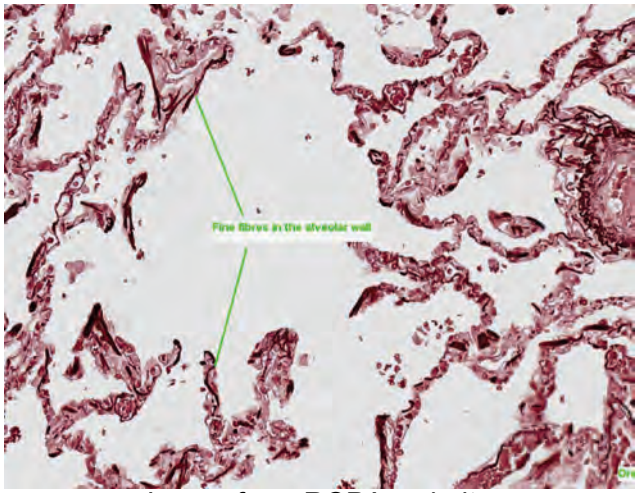
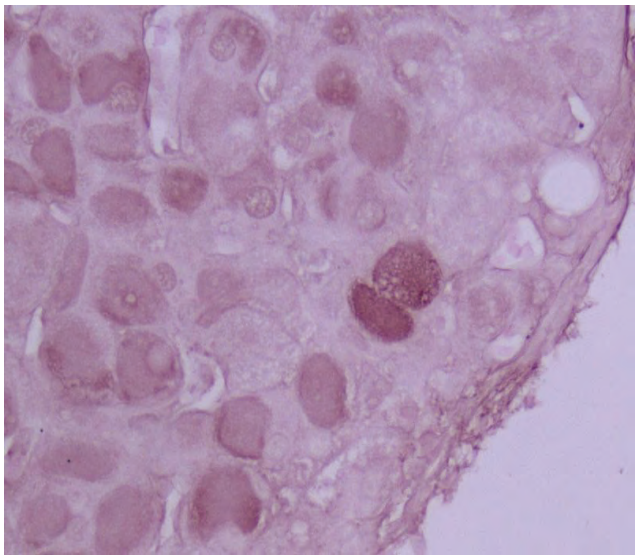


Image from RCPA website



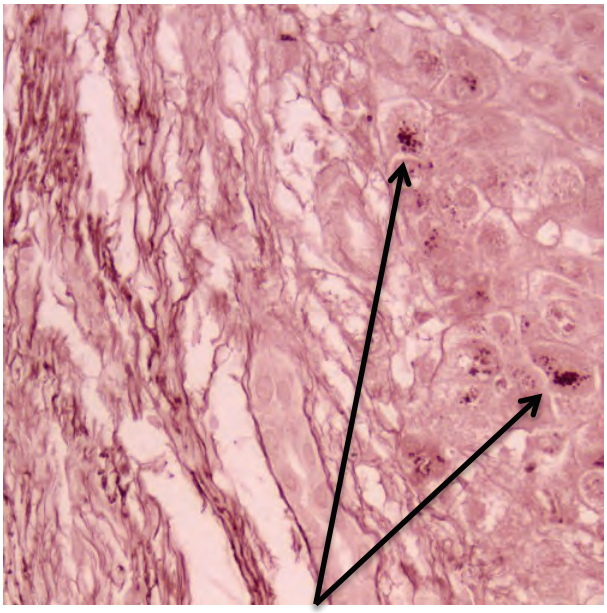
'Ground glass' cytoplasm of hepatocytes infected with Hepatitis B

STAINING PROCEDURE at RBWH

1. Deparaffinise and hydrate slides from xylene through the alcohols and then into running water.
2. Oxidise sections in the Acidified Potassium Permanganate for 5 minutes.
3. Wash the sections in distilled water.
4. Decolourise sections in 1% Oxalic Acid for 1-3 minute.
5. Wash sections in distilled water.
6. Stain sections in the Orcein Staining Solution for 1 hour at room temperature.
7. Differentiate the sections in 1% Acid Alcohol until the background is a pale brown buff colour.
8. Dehydrate, clear and mount in synthetic mountant.

Results: Elastic fibres - dark red brown (maroon);

Hepatitis B Antigen positive hepatocytes - light brown/ maroon with ground glass cytoplasm; Copper Associated Protein - dark red brown (maroon); Background - pale brown buff colour



Copper Associated Protein

Orcein/ H&E

- Orcein with a H&E counterstain
- No need for Shikata's pre-treatment
- Same orcein solution is used
- Is routine in post lung transplant biopsies (TPCH) – airway vs vascular rejection
- Used in lung tumour cases to determine whether or not the tumour has breached the two elastic fibre layers near the pleura
- H&E stain allows identification of tumour and proximity to elastic fibres
- Do not want H&E stain to overpower the orcein stained elastic fibres

Results: Elastic Fibres – maroon (or purple/ brown); Nuclei – Blue; Other tissue elements – Pink

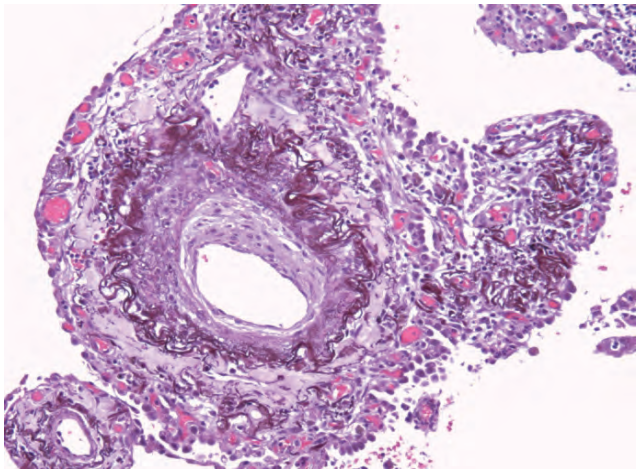


Image courtesy of Lydia Kalpakos - TPCH

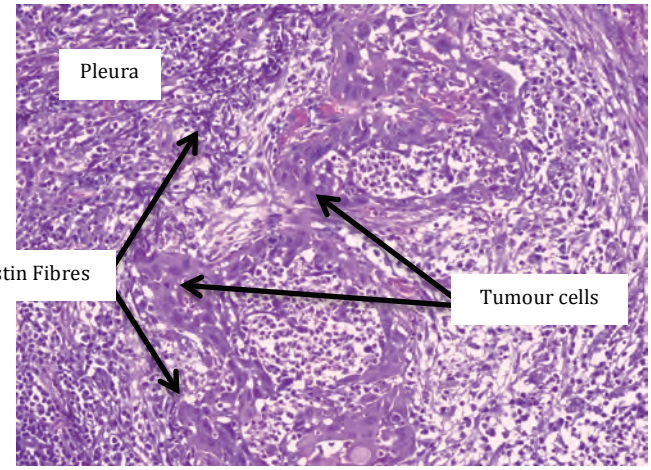


Image Left: Elastin fibres demonstrating location of pleura and invasion of tumour cells

Thanks and Resources

RCPA website – Images

Lydia Kalpakos (TPCH) – Images and information about Orcein/H&E staining

For other references please contact the editor of this newsletter

HGQ members at past conferences

Can you see them at the Sofitel?