



Histology Group of Victoria Inc.

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PARAFFINALIA

Volume 19 Number 5

October, 2014

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Editor: Elizabeth Baranyai

“The HGV aims to provide a dynamic continuing education program in which all persons with an interest in Histology and Histotechnology are freely invited to participate.

Committee Page

The members of the Histology Group of Victoria 2014 are:

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	Tania Marsden	Royal Children's Hospital	9345 5748
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Please feel free to contact any of the committee members listed above with any comments or suggestions. Contributions are always welcome.

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BLURB FROM THE BUSH

This year the one of the key focuses of the committee is to revitalize the scientific meetings to provide relevant interesting topics to attract larger numbers. This has been terrifically successful with numbers of attendees being at levels not seen for several years. Thankyou to all the members that have attended to make these evenings successful. This was capped off recently with an evening centred around immunohistochemistry providing lots of good insight and discussion around specific antibodies. Thankyou to the three presenters and the trade that supported the evening.

The Tasmanian conference is only weeks away, and the committee continues to plan and organise to make sure it is a smoothly run conference that all delegates enjoy. The trade have provided wonderful support with all trade booths selling out and to date over 100 delegates will be in attendance, well in excess of our expectations. The Saturday evening dinner is almost sold out so please look out for emails regarding this as numbers must be capped.

Part of our Tasmanian conference will include our Annual General Meeting. Within this edition of Paraffinalia you will find a nomination form for committee. If you are interested in participating as part of the HGV committee please complete a nomination form. We are always keen to have new members assisting us to deliver ongoing education for the benefit of all.

Adrian Warmington
HGV President

The Good, the Bad and the Clinically Unhelpful

HGV Scientific Meeting review:

The last scientific meeting of the year showed the knowledge and expertise IHC scientists have in their field. There were three excellent speakers, Nick Jene (Peter Mac), Trung Nguyen (Alfred) and Piero Nelva (Monash).

Nick presented two clones of SOX-10. SOX-10 is a nuclear transcription factor important in neural crest development. It is involved in the specification and differentiation of cells of melanocytic lineage including melanocytes and schwann cells. SOX-10 has become useful in the diagnosis of melanocytic and schwannian tumours.

SOX-10 stains the nucleus in both normal and tumour cells. Normal cells: schwann cells, melanocytes, myoepithelial cells of salivary, bronchial and mammary glands. Tumours: melanomas, primary/metastatic (97%), Peripheral nerve sheath tumours, schwannomas and neurosarcomas. Can stain diffuse astrocytomas and breast carcinoma.

SOX-10 is currently being used with S100 to aid in the diagnosis of melanoma's.

Initial Optimisation occurred on a Ventana ULTRA using both Ultraview and Optiview DAB Detection reagents including Amplification Kit. SOX-10 Polyclonal (rabbit) Antibody. Protocol: CC1 High pH retrieval solution at 95°C for 32 minutes and 52 minutes, dilution range 1/100-1/25, 32 minutes at 36 °C plus Amplification.

SOX-10 Monoclonal (mouse) antibody. Biocare (ACI3099C) Clone BC34. Protocol: CC1 High pH retrieval solution at 95°C for 52 minutes, dilution 1/50, 32 minutes at 36 °C plus Amplification.

The polyclonal antibody in Nick's experience has more background staining, needs a higher dilution and is more expensive than the monoclonal. The monoclonal shows a cleaner staining pattern, lower dilution and is cheaper!!

Figure 1A

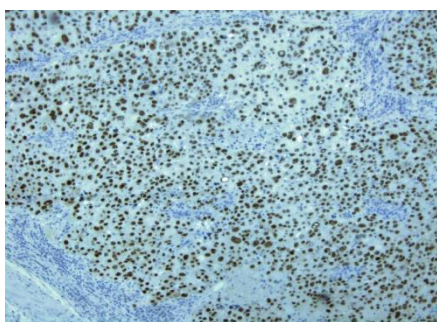


Figure 1B

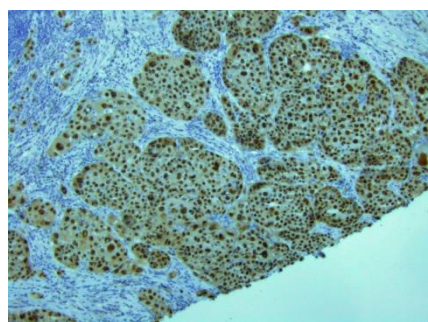


Figure 1A: Section stained with Polyclonal SOX-10. B: Section stained with Monoclonal BC34

Trung presented his good, bad and the ugly IHC. Alfred use the LabVision 360 Autostainer, heat retrieval detection Tris/EDTA pH8 buffer in Dako pressure cooker 120°C, Leica Novolink Max polymer detection and Dako DAB+ chromagen.

The good is Helicobacter Pylori (Rabbit Polyclonal Cell Marque 215A-76, 1:800). It stains great all the time and is reducing the number of Giemsa/Diff Quik stains they are doing. The Helicobacter pylori is not performed on all gastric biopsies, only if clinically indicated.

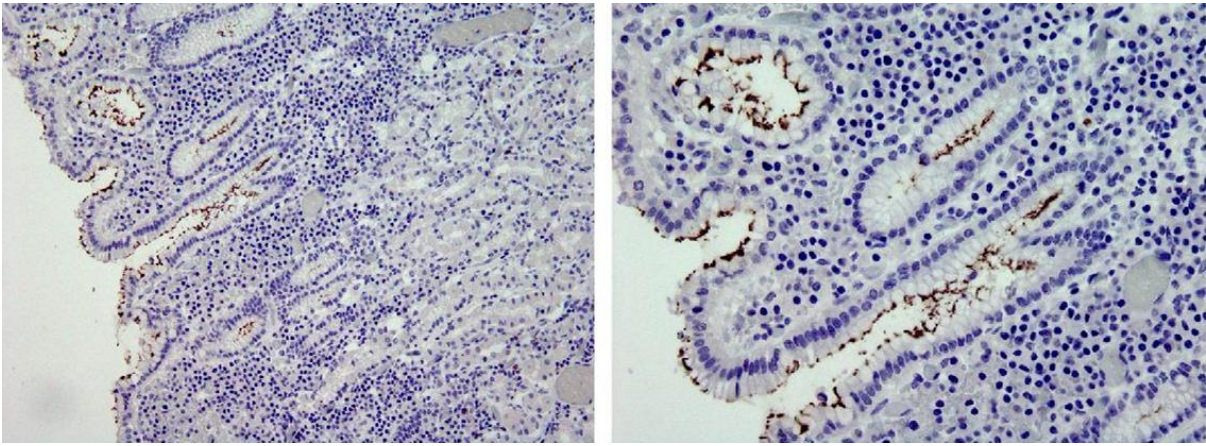


Figure 2 Gastric biopsy stained with Helicobacter pylori.

There were two bads. First, trapped DAB artefact that they see on muscle biopsy frozen IHC sections 8µm (Antibody: Spectrin 1:200 Leica NCL-SPEC1, Clone: Mouse monoclonal RBC2/3D5).

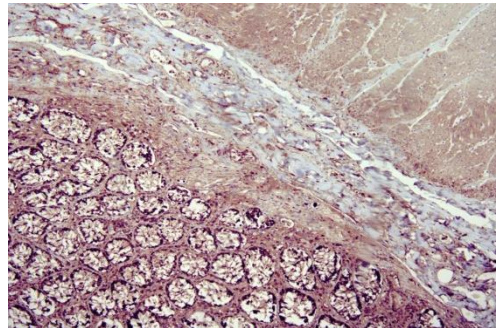
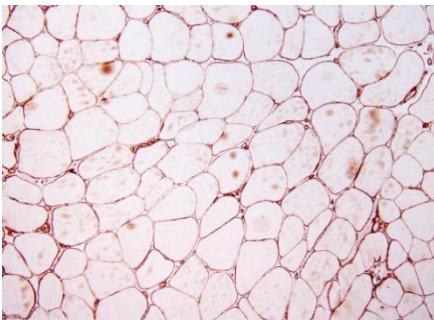


Figure 3: Muscle biopsy stained with Spectrin Figure 4: Colon stained with CK AE1-AE3

The second is fixation/processing artefact seen in large breast tumour sections. The rim of the tissue section is beautifully stained but the staining quality deteriorates as you move into the section. This was seen with ER (Antibody: Estrogen receptor 1:200 Spring M3014 Clone: Rabbit monoclonal SP1).

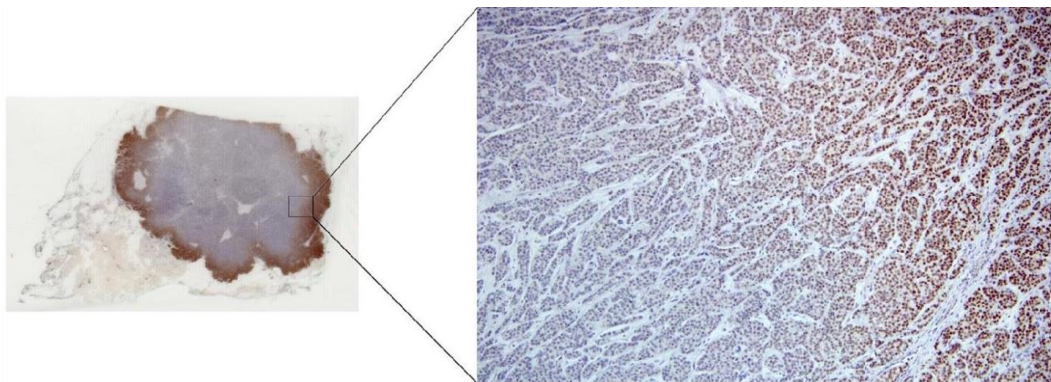


Figure 5: Breast section stained with ER showing fixation/processing artefact

The ugly was another fixation/processing artefact seen in CK AE1-AE3 (antibody: Cytokeratin 1:500 (Dako M3515) Clone: Mouse monoclonal AE1 & AE3). A segment of bowel was received in the lab on Easter Thursday and kept in the fridge until Easter Tuesday before formalin was added. The results was staining everywhere, showing the importance of good fixation.

Trung concluded with a beautiful section of CK5/6.

Piero spoke about GATA3. GATA3 is a transcription factor involved in development and differentiation in many tissues and cell type including breast epithelium, urothelium, hair follicles, adipocytes, T lymphocytes, thymocytes and sympathetic nervous system.

GATA3 is seen in breast carcinoma. Ductal, lobular, primary and metastatic, hormone positive and negative. There is a slight decrease in sensitivity in high grade / poorly differentiated lesions but still >80%. It is more sensitive than GCDFP. For Piero, this is a good antibody. So, what makes a Good antibody? It works! Reliable, easy to implement, comes with a picture and it is relevant.

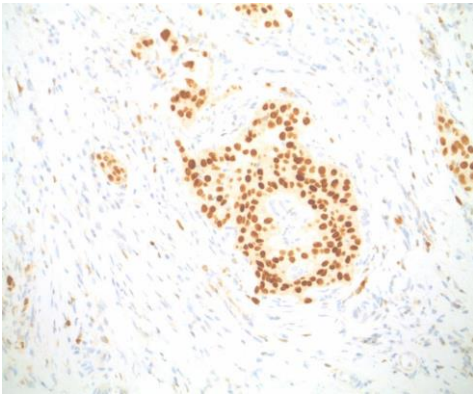


Figure 6A: Renal mass stained with GATA3

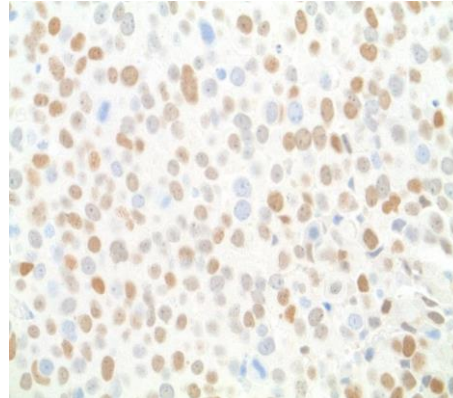


Figure 6B: Temporal mass stained with GATA3

A difficult antibody is CD10. It is widely used for diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. Monash Health diagnoses approximately 110 new lymphomas per year and is involved in GOYA and Gallium studies. It is a critical antibody for Monash. Piero's favourite clone (56C6) was no longer available through his current supplier so he had to try a new clone SP67. For Piero, this clone is not a bad clone but he was unable to demonstrate crisp membranous staining. The staining pattern seen was weak, inconsistent and had background staining. When compared with the clone 56C6, there was beautiful crisp membranous staining with a clean background.

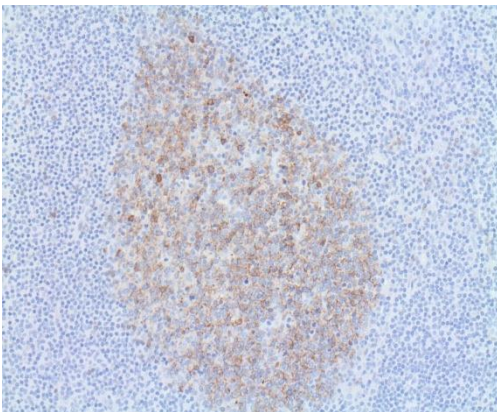


Figure 7A: Tonsil stained with SP67 clone.

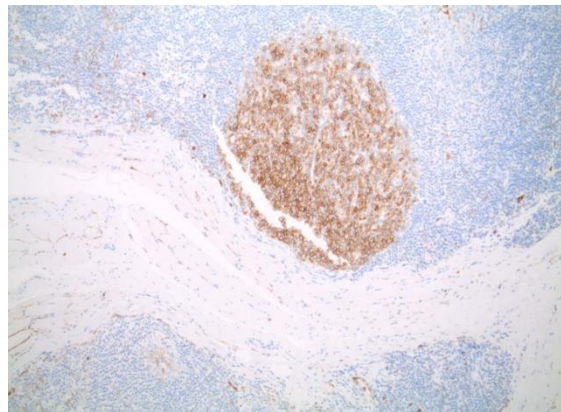


Figure 7B: Tonsil stained with 56C6 clone

Tania Marsden
RCH

Under the Microscope with Rebecca Anderson

Q1: What was your first job?

A: My first job was at Coles working in the bakery.

Q2: How long have you worked for in histology?

A: 14 years.

Q3: When people ask "So, what do you do?" How do you explain histology?

A: It depends on how much time I have to explain but my short version is 'I slice & dice human body parts for a living?'

Q4: Who would you most like to have dinner with & why?

A: My husband because he works evening shifts & we don't get to have dinner together often.

Q5: What is your all-time favourite movie?

A: The Shawshank Redemption.

Q6: What is your favourite stain?

A: Either a MSB or a LFB.

Q7: What is your favourite food/restaurant?

A: Anyone that knows me well knows that I could never pick a favourite because I love all types of food!

Q8: What is the best conference you have ever attended?

A: Histology conference at the Gold Coast, Surfers Paradise 2006 where the topics presented were fantastic & then there was the awesome beaches & shopping to enjoy later.

Q9: Favourite beverage?

A: Lemon, lime & bitters.

Q10: What is your dream holiday destination & why?

A: Canada because there's so much to see & do there across all seasons.

Reported by: Kellie Vukovic
Peter Mac

Waxing Lyrical

Spring has Sprung a Leak

As the football season fades into memory and after more exposure to brown and yellow than a biochemist on faecal fat duty, the lab plant and I look forward to warm shafts of spring sun shimmering through our solitary pane.

It's a great advantage having ten years of street grime and the results of last month's staining machine waste hose incident diffusing the UV in a lovely lacework effect. I sometimes wonder how a single leaf in a dank polystyrene mix has thrived to become a multi-fronded abundantly prolific bush in the histolene-laden atmosphere of the histology lab. Then, I observe my colleagues and realise they too demonstrate the benefits of orange juice with a little alcohol thrown in from time to time.

Speaking of alcohol, there was no requirement for the SJOG/V/Line cleanup team following the Trivia night this year, but I suspect there may be a need to post an all-systems alert on the Spirit next month. I'm going on the boat. My 'Mad Manager' has insisted I use a couple of my 23 RDO's and pop down to Hobart for the conference. I have agreed reluctantly so I can appease the continuing professional development pleadings of the Quality Manager and savour the local seafood. I figure it's only fair as I will be feeding them on the way down. It is also a wonderful opportunity to observe the HGV committee members with their 'hair down'. Look out for me at the Trade display – I am reliably told that is where all lab news and pens come from. Does the water go down the plughole the same way in Tasmania? I will report back.

Whilst delivering cell blocks to Cytology, whispers floating between microscopes wafted news of Trish O'Neill departing Peter Mac for the green pastures and rejuvenating sea air at Limosa Rise, Wilson's Promontory. Trish has left the bronchial brushings for the Bed and Breakfast business. I'm sure there will be special rates for HGV members if you mention this column and she may even re-screen your 'hard ones' if you bring them down.

Congratulations to Rebecca Anderson from the Alfred on the birth of her daughter Rachael Isabella. Mother and baby are doing well, despite Rebecca blowing a blood vessel in her eye pushing out the baby. Looks like she did 15 rounds with Mike Tyson.

Tanja Dimitrijevska of Cabrini has recently returned from a six week sojourn tracking fashion trends in DFO's around Europe. I can recommend long sleeve gowns to protect this season's Dior at special stains, Tanja. In contrast, a reputable source has revealed the secret hideaway of Julian Richardson. The philosophising Histology historian shows his wild side, regularly returning to nature for 'Man Camps' amongst blueberries, olives and animals. Rumours of Doomsday Prepping and guns cannot be verified. I'm having a visual...

I find myself contemplating Spring colour as I re-sharpen the dissecting scissors our Professional Practice student used to trim his swim fins. The rapidly developing rosaceous glow of at least two fingers indicates the chemical properties of that innocent clear puddle near the Schiff's bottle. My colour palette is settled then for racing season. Pink goes with everything at the TAB. Purple also has its place at this time of year - the colour of a pathologist's face on Monday after you forget to set the processors for Daylight Saving time. I suppose it's more like puce really. Art and Science – what a combination.

Article Review by Samantha Arandelovic, SJOG

Immunohistochemistry in formalin- gel fixed tissues

Amadeu Borges-Ferro, Ana Bastos Santos, Joana Louro Filipe

Journal of Histopathology 2014, Vol 37, No2

Professionals who work in histopathology laboratories are exposed to several hazards such as chemical, biological and mechanical, on a daily basis. Formaldehyde is one of the chemical hazards to which such professionals are routinely exposed, therefore laboratory staff members must ensure that their professional activity is set to the highest standard while complying with the best safety procedure.

Formaldehyde, being a gas, is colourless and has pungent odour, typically used as a 37-40% aqueous solution called formalin that is diluted to the final concentration of a 10% buffered solution. It is a toxic substance with carcinogenic, mutagenic and teratogenic potential in a case of chronic exposure, however it is widely used due to the excellent results obtained in tissue fixation. It provides preservation of cell integrity, ceasing autolysis and putrefaction and it enables the tissue sample to withstand other histological processing.

Formaldehyde is a great fixation reagent, however it directly affects the immunohistochemistry (IHC) by masking antigens and compromising their antibody recognition. It has the ability to create methylene bridges between amino groups on different proteins therefore changing their three-dimensional structure and causing functional inactivation.

Recently, a new form of formalin fixative has been released, in the same concentration and pH but in a form of gel instead of aqueous solution. Being a gel is a much safer option than liquid, however IHC is greatly affected by fixation so the most important question is "Is the IHC quality affected by the use of formalin gel as a fixative"?

A study was conducted to compare the 10% neutral buffered formalin and formalin gel fixation after 12, 24 and 48 hours by analysing the IHC quality of CKAE1/AE3, CD3, CD20, Ki-67 and Vimentin antibodies in human placenta.

Two groups of 30 human placenta samples, subdivided into three groups of 12, 24 and 48 hours fixation processing, embedded and cut at 3µ with a total of 360 sections mounted on SuperFrost Plus slides. IHC was performed using Ventana BenchMark ULTRA staining machine and Ventana antibodies with a high pH antigen retrieval, horseradish peroxidase and Ventana ultraView Universal DAB Detection Kit.

All samples were analysed using a qualitative criteria defined by Carson and Hladik and screened by three independent assessors.

All specimens from formalin liquid and formalin gel revealed nuclei with a variety of chromatin patterns and crisp blue nuclear membrane, no nuclear bubbling, smudginess or fading. The cell cytoplasm was well preserved, no artifactual spaces between individual cells or cell shrinkage.

It was determined that there were no statistical differences in the quality of IHC due to fixation. Formalin gel can match the quality of fixation given by formalin liquid, providing advantageous alternative in terms of safety however further studies should be conducted that contain larger diversity of tissue and antibodies in order to standardise the reliability of formalin gel.



Histology Group of Victoria Inc.

Org. No. A0035235F

Nomination Form for Election to the Committee of Management of the Histology Group of Victoria Inc.

Saturday 15th November, 2014 RACV/RACT Hobart Apartment Hotel

Nominated Person.....

Institution.....

Email Address.....

Position Nominated For	President	<input type="checkbox"/>
(Please tick box)	Treasurer	<input type="checkbox"/>
	Secretary	<input type="checkbox"/>
	Committee Member	<input type="checkbox"/>

All nominations must be signed by two HGV members
(If you receive Paraffinalia you are a member)

Name of Member.....Signature.....

Name of Member.....Signature.....

Nominations must have the consent of the nominee

Signature of Nominee.....

Nominations must be returned no later than Saturday 8th November, 2014.

Please send nomination form to:
The Secretary
Histology Group of Victoria
PO Box 1461
Collingwood, VIC 3166

Scanned and emailed to
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HISTOLOGY GROUP OF VICTORIA EXPERIENCE TASMANIA HOBART CONFERENCE NOVEMBER 15-16TH 2014



THE HGV COMMITTEE PRESENT AN EXCITING CONFERENCE WITH OUR SOUTHERN MOST MEMBERS IN TASMANIA. THIS PROMISES TO BE AN EXCITING PROGRAM SET WITHIN THE MAGNIFICENT SURROUNDS OF HOBART. THIS IS A WONDERFUL OPPORTUNITY FOR MAINLAND HGV MEMBERS TO TRAVEL TO ONE OF AUSTRALIA'S PREMIER TOURIST LOCATIONS AND ENJOY QUALITY HISTOLOGY EDUCATION AND SOME LOCAL PRODUCE.

REGISTRATION

FULL REGISTRATION

\$210

INCLUDES CONFERENCE REGISTRATION (SATURDAY MORNING TEA, LUNCH AND AFTERNOON TEA; SUNDAY MORNING TEA) AND CONFERENCE DINNER (3 COURSE MEAL WITH LIMITED DRINKS)

CONFERENCE REGISTRATION ONLY

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CANCELLATION 7 DAYS OR LESS PRIOR TO THE EVENT WILL FORFEIT ALL PAYMENTS

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ACCOMMODATION

ACCOMMODATION MUST BE ORGANISED BY THE DELEGATE

THE CONFERENCE VENUE ACCOMMODATION HAS SOLD OUT
THERE ARE SEVERAL OTHER WALKING DISTANCE OPTIONS AVAILABLE

TRAVEL

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DEVONPORT – HOBART 300KM (3HR 15MIN DRIVE TIME)

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VENUES

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154 - 156 COLLINS STREET HOBART

TASMANIA AUSTRALIA 7000

TEL: 03 6270 8600

EMAIL: HOBART@RACV.COM.AU

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PARKING AT THE VENUE IS AVAILABLE BUT IS LIMITED AND COSTS \$15 PER DAY



SATURDAY DINNER

MURES UPPER DECK

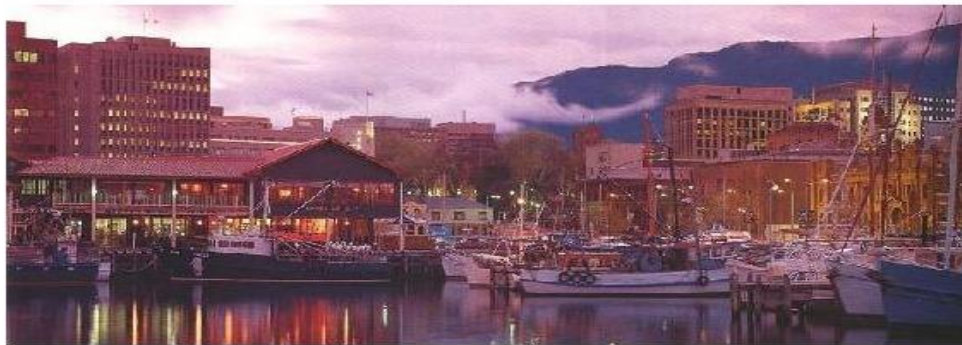
DAVEY STREET

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TASMANIA AUSTRALIA 7000

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NON SEAFOOD AND VEGETARIAN DISHES ARE CATERED



SATURDAY		PRESENTER	TITLE	TIME (min)
8:00 AM	Registration			
9:00 AM	Session 1	Dane Hayes	Determining the Immunophenotype of Devil Facial Tumour Disease (DFTD)	25
		Anne Prins	Heparan Sulphate staining in Type 1 Diabetes Research	15
		Elizabeth Baranyai	Case Study: Rosai-Dorfman disease co-existing with Hodgkin's Lymphoma	15
		Hazel Chambers-Smith	Case Study: An unusual paediatric small round cell tumour	15
		Paheerathan Suthanathan	Implementation and use of new antibodies including caviolin-1	15
10:30 AM	Morning Tea			
11:00 AM	Session 2	HGV AGM	HGV AGM	5
		Margaret Dimech	Online RCPA Macroscopic Cut-up Manual	20
		Janine Danks	Proposed RMIT Cut-up program for complex specimens	15
		Courtney Savill	Role and Training of the Pathologists' Assistants	25
		Panel	Pathologists' Assistant/Cut-up Training Panel Discussion (Janine Danks/Courtney Savill)	20
12:30 PM	Lunch			
1:30 PM	Session 3	Melanie Archer	Forensic Entomology including Blow Fly Embryology techniques	45
		Dr Sukhwinder Sohal	Epithelial Mesenchymal Transition (EMT) In Small And Large Airways of Smokers: Potential Role in Both Airway Fibrosis And Cancer	45
3:00 PM	Afternoon Tea			
3:30 PM	Session 4	Bronwyn Christiansen	Optimization of C5b-9 antibody to aid in detection of Gestational Alloimmune Liver Disease (GALD)	25
		Randal Hodgson	The presumed last case of Hydatids (<i>Echinococcus granulosus</i>) acquired by a human in Tasmania	20
		Kerrie Scott-Dowell	Bring the Fab back into the Lab 1	45
		Fiona Tarbet		
5:00 PM	Close Day 1			
7:00 PM	Dinner Mures Restaurant			
SUNDAY		PRESENTER	TITLE	TIME (min)
9:00 AM	Session 5	Kerrie Scott-Dowell	Bring the Fab back into the Lab 2	45
		Fiona Tarbet		
		Dr Rajesh Bhatia	Pancreatic EUS	25
		Dr Eileen Long		
		Deanne Lamb		
		Gillian Phillips	Cervical screening program update	25
10:30 AM	Morning Tea			
11:00 AM	Session 6	Aysha Du	ZN control tissue and survey (TBA)	15
		Sue Sturrock/Fiona Tarbet	Issues with Tissues - What are we doing to DNA?	25
		Mark Bromley	Histology in the Congo	45
12:30 PM	Close Day 2 Depart			

THE HGV ORGANISING COMMITTEE RESERVES THE RIGHT TO CHANGE THE PROGRAM AT ANY TIME AS REQUIRED

Know Pathology Know Healthcare update



It has been a busy few months for *Know Pathology Know Healthcare* (www.knowpathology.com.au), the national initiative to increase recognition of the value of pathology. With 100% of cancers diagnosed through pathology and 70% of medical treatment decisions guided by results, Australians are highly dependent upon good pathology services. The campaign is engaging with the public and showcasing the 25,000 highly skilled Australians working behind the scenes in the engine room of healthcare.

June saw the campaign's public launch at Parliament House, Canberra, where former Australian of the Year and Olympic gold medallist Cathy Freeman OAM told parliamentarians how much she relies on pathology to manage her Type 2 diabetes. A personal tour of a laboratory in preparation for the launch left her amazed by how many samples are analysed each day. She shared her thoughts on ABC News Breakfast. (<http://vimeo.com/londonagency/review/98604504/b37dba7870>)

Since the launch Federal politicians around the country have been queuing up to tour their local pathology laboratories and importantly, share their impressions on social media and in local media. From Perth to Hobart, these lab tours have been a resounding success in revealing to our national leaders and local residents just how integral pathology is to good healthcare. You can see some TV footage of a Hobart lab tour here. <http://vimeo.com/londonagency/review/106346528/2175fbb9f6>

The campaign is also reaching out directly to patients. Collection staff across Australia are being coached to talk briefly with patients on the value of pathology and are encouraging them to complete supporter postcards. This part of the campaign began in June and is being rolled out nationally throughout 2014 – 2015.

On the 27th October, *Know Pathology Know Healthcare* is hosting a morning tea in Canberra to highlight the importance of pathology to cancer prevention, diagnosis and management. As this coincides with Cancer Council's *Pink Ribbon Day*, the developer of the Gardasil® vaccine, Professor Ian Frazer and the CEO of the Cancer Council, Professor Ian Olver, will be addressing attendees about how Australia's high quality pathology services underpin successful cervical cancer screening programmes.

Anatomical pathology is crucial to cancer diagnosis, yet many Australians will not have heard the term before. The high level of skill required means that these departments contain minimal automation. It's time the hard work and expertise of people working in pathology was recognised.

If you haven't registered to show your support already, please visit www.knowpathology.com.au - we cannot expect the public to value pathology if we are unwilling to explain why **we** value pathology.





Histology Group of Victoria Inc.

Org. No. A0035235F

Future Events:

2014

Thursday 5th February

Scientific Meeting- RIMT Student Project Presentations

Venue: Peter Mac

March 8-9

Joint Meeting (Histo SA & HGV)

Venue: The Quality Inn Presidential, Mt. Gambier, South Australia

March 21-23

4th International Workshop in Diagnostic Immunohistochemistry

Venue: Outrigger Twin Towns Resort

Coolangatta-Tweed Heads, NSW

April 9-11

Cut-up Workshop

Venue: CIT, Bruce, ACT

Thursday 15th May

Scientific Meeting

Venue: Peter Mac

Friday 25th July

Trivia night

Venue: The Metropolitan Hotel

263 William St.

Melbourne VIC 3000

August 21-27

NSH Symposium Convention

Venue: Austin, Texas, USA

September 4-7

AIMS National Scientific Meeting 2014

Venue: Rydges World Square, Sydney, NSW

Thursday September 18th

Scientific Meeting

Venue: Peter Mac

September 24-26

Cut-up Workshop

Venue: CIT, Bruce, ACT

November 15-16

Scientific Meeting/AGM Venue:

RACV/RACT HOBART APARTMENT HOTEL

154 - 156 COLLINS STREET HOBART

TASMANIA AUSTRALIA 7000