Histology Group of Victoria Inc.

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PARAFFINALIA

Volume 17 Number 2

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Contents:

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 2011-2012 are:

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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.

Advertising:
All enquiries for trade advertising in the next edition, please contact: Kristy De George - trade@hgv.org.au

Advertising for the next edition of Paraffinalia closes: **1st June, 2012**

Rates:

A4 Electonically Submitted

Single sided B&W	**$250**
Double sided B&W	**$325**
Per page colour newsletters)	**$250 (Will be colour for e-newsletter and B&W for hard copy**
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50 words – no logos/no pictures	
Positions Vacant	
No Logo up to 75 words	**FREE**
A4 B&W with logo	**$150**

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Author enquiries and readers wishing to contribute articles or reports can contact the Editor - editor@hgv.org.au

Please email articles (preferably Microsoft Word format) for inclusion in the next edition to editor@hgv.org.au
All items submitted for publication will then become the sole property of the Histology Group of Victoria Inc.

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Blurb from the Bush

Preparation has begun for the 2013 National Meeting. The committee has appointed a PCO to assist in the organisation of the event, and are currently in preliminary work on venues and dates. You will be kept up to date on our progress, as we aim to get programs and registration documents prepared as soon as we can to enable our members maximum time to consider and register for the conference.

Work continues also with AIMS on the Cape Schanck conference in August. The program looks to be very exciting and the venue conducive to a relaxed atmosphere. It has been great to see a good working relationship between the two groups for common educational good.

This is the first edition of Paraffinalia that has not been bulked mailed. There was certainly a mad rush to ensure that members made a choice as to how they received the newsletter, but there are still around 200 members who have not yet responded, and as a consequence will not be receiving this edition.

The vast majority of members have agreed to receive Paraffinalia by email. And for the time being we will continue to produce a hard copy for those requesting such. New members will not be given a choice as to how they receive the newsletter. The only option will be email. The committee has also talked about only providing a link via email which will take members directly to the newsletter on the website. Often the size of the newsletter is too large to be accommodated by some members' mailboxes. However for the time being we will continue to send the newsletter with and without advertisements to circumvent this issue.

If any members have suggestions as to any operational component of the HGV, please feel free to contact a committee member or alternately send an email to membership@hgv.org,au

Adrian Warmington
HGV President

Ken Thompson has Retired

Ken was working for Lomb when I first met him. After that he became a part of Grale Scientific and was there for eleven years. If you wanted it Ken could source it despite the sometimes odd and vague request. He served on the histology group committee for three years and his input was always welcome. He took an active interest in histology in Victoria and always knew who was working where and what changes were happening. We talked at length about our profession and where it was going. We knew how to solve all the problems as grumpy old dinosaurs do.

Mr Thompson is a friendly and compassionate bloke. He was concerned for people, he visited them in hospital and attended funerals when they arose. When the bushfires went through my area I received a phone call from China to check upon us.

We had a common interest in fishing. He came on a couple of fishing trips that the histo group organised and he and I went on a couple of charters together. One to Merimbula for a couple of days and a more adventurous one to Cooktown where I out fished him comprehensively. Although I couldn't match him in the art of hooking rocks. He didn't land the rock. His office wall was adorned with photos of fish many of which I suspect were plastic replicas. In recent years he swapped fishing for remote controlled planes.

A health scare led him to reassess his priorities and Ken has decided to lead a less stressful life. I wish him and Lynne well.

Clyde Riley

KRAS and *BRAF* Testing

Presented By: Chelsee Hewitt – Peter MacCallum Cancer Centre

Cancer is a common disease. Approximately 13% of all deaths worldwide are due to cancer. The most common form of cancer is somatic cancer, which means it is NOT inherited but is acquired. This means that the cancer is caused by the accumulation of mutations over time, so the incidence of somatic cancer increases with age. In fact, one in two Australians will be diagnosed with cancer by the age of 85.

In the last decade there has been a shift in therapeutic strategies for some cancers to include targeted therapies. Some of these therapies only work if specific mutations are present, whilst others only work if there are no mutations present. Therefore oncologists need to know this genetic information to allow them to decide which treatment to prescribe each patient. Although this presentation is focused on *KRAS* and *BRAF* testing, our molecular department also tests for *EGFR* mutations in non-small cell lung cancer, and *KIT* and *PDGFRα* mutations in GIST.

KRAS Testing:

We currently perform KRAS mutation analysis for colorectal cancer. The survival rate has doubled in the last decade helped by the introduction of the anti-EGFR monoclonal antibody therapies, such as Erbitux. Following treatment most patients have a 20% tumour reduction which allows for tumour resection which is associated with increased overall survival.

BRAF Testing:

Metastatic Melanoma is an aggressive disease with few effective therapies and has a poor prognosis. Between 40-60% carry a mutation in the *BRAF* gene. 90% of these mutations result in the substitution of glutamic acid at codon 600 for valine (V600E). There is a small molecule inhibitor called VEMURAFINEB which has strong anti-cancer activity against cells producing the V600E protein. VEMURAFFINEB improves progression free and overall survival of melanoma patients.

Issues Encountered with the Somatic Molecular Testing Process:

Since somatic cancers are caused by the acquisition of mutations rather than inheritance, we need to perform our testing on the actual tumour cells. Clinical samples are generally only available as formalin fixed paraffin embedded blocks, which cause us problems. Some issues are: the fixation process results in highly degraded and damaged DNA; tumours can contain both normal and cancer cells and natural pigments such as haem and melanin can inhibit molecular techniques.

Requirements for Testing:

1. A fully completed request form
2. Tissue in a paraffin block/s containing tumour prior to treatment where possible **OR** 10 x 5 micron unstained sections on uncharged slides
3. Payment details if not covered by a pharmaceutical company

We will make an attempt to test all samples as long as they contain tumour cells. If you have been requested to send a block that either contains no tumour or the sample has been cut out or you think there may be a problem with it – prior to sending the sample, please call the Peter Mac molecular laboratory for further discussion on how to proceed.

The Testing Processes:

The techniques we use to test for *KRAS* and *BRAF* mutations are similar as the most common mutations that occur in these genes are located in small hot spot regions. The majority of *KRAS* mutations are single base changes in 2 codons whilst the majority of *BRAF* mutations occur at one particular base. In both cases, we know that 50% of patients will be positive.

We therefore use an Initial mutation screening technique called High Resolution Melting (HRM) analysis to identify those samples carrying a DNA change at these hot spot areas. We then sequence samples that are positive to characterise the sequence change identified.

Testing at the Peter Mac:
The number of molecular requests have increased exponentially over the past 5 years, mainly due to an increase in somatic testing. We see typical increases in the number of requests following announcements regarding the sensitivity of cancers harbouring particular genotypes to new drugs or when drugs become available on the Pharmaceutical Benefits Scheme (PBS).

The turnaround time (TAT) for *KRAS* and *BRAF* testing is nine days, but we are often able to report results in a quicker time frame. Our TATs are calculated from the day we receive the sample to the day the case is reported. As the mutation analysis results are often required for oncologists to make treatment decisions for end stage cancer patients we have a large number of urgent cases that require a shorter TAT. It is therefore important that histology laboratories aim to send through the tumour sample as soon as possible.

KRAS and *BRAF* molecular testing are important for targeted cancer treatment and decision making for end stage colorectal and melanoma patients. We provide the services through HRM screening analysis and sequencing of positives. Rapidly increasing number of requests and calls for faster TAT are pushing us to new technologies e.g. next generation sequencing.

The molecular lab would like to thank all histology labs who have kindly sent us samples in such a timely manner in the past.

For any queries or further discussion in regards to any molecular testing, please contact Chelsee Hewitt.
Phone: (03) 9656-3695
Email: chelsee.hewitt@petermac.org

Reported By: Nguyen Nguyen
Peter MacCallum Cancer Centre

The 2nd International Workshop in Diagnostic Immunohistochemistry

Crowne Plaza Gold Coast QLD

February 3rd- 5th 2012

The meeting was opened by the director, Dr Eugene Petcu, who universally introduced the invited speakers as well-known experts in their field with extensive experience in immunohistochemistry.

The welcome address delivered by Murray Mitchell was followed by **Glenn Francis**, who delivered an update on the RCPA-QAP with particular emphasis on immunohistochemistry and in-situ hybridisation and their associated accreditation standards. The results of KRAS,BRAF and EGFR testing influence the application of targeted therapies for patients, so the importance of test standardisation and accuracy of results cannot be underestimated. All breast cancers diagnosed in Australia are subjected to ISH testing for Her-2, again, the patient's treatment is affected by the outcome of the test. All laboratories performing hormone receptor IHC are required to audit the results of 100 individual patients per year. The Newfoundland breast cancer "scandal" was described, where during the years of 1997 – 2005, of 1013 ER negative breast cancer diagnoses, 383 patients received incorrect treatment and 108 patients died. Worth remembering each time we are asked to perform the dreaded audit!

Chris Andry then spoke about the US perspective of "Advances in automation, quality and patient safety in diagnostic immunohistochemistry".....or 'The Quest for Quality". In 1988 the Federal Government of the USA mandated regulatory oversight of diagnostic laboratories with the Clinical Laboratory Improvements Act. The College of American Pathologists(CAP) is the accrediting body deemed authorised to validate the quality and safety of diagnostic laboratories, by way of making unannounced inspections!! There is a detailed list of accreditation standards, and laboratories are inspected by peers and professional accreditation inspectors. For example: the IHC check-list includes: Patient Safety; Fixation; Antibody integrity; Control Tissue; pH and temperature; Slides; Stains; Staff Competency; Quality Control and reporting. There should be evidence of compliance. Positive tissue controls should be used for each antibody.

The **pre-analytical** variables which affect IHC staining include: time to fixation of the specimen, its handling and dissection, subsequent fixation, processing, embedding and incubation post microtomy. **Analytical** variables include: slide management, a validated and reproducible procedure, quality control and external validation. **Post-analytical** variables include image analysis which may be quantitative, and comprehensive, accurate, standardised and clear reporting of the staining results.

The Cancer Human Biobank in the US was created in response to the critical and growing need for high quality, well-documented biospecimens for cancer research. The 2011 Best Practices for Biospecimen Resources can be found at www.cahub.cancer.gov.

Fixation is identified as the most critical variable in the pre-analytical phase. The following guidelines have been developed: tissue < 3mm thick requires 12-72 hrs fixation time. For example breast biopsies and lumpectomies require at least 6 hours and up to 48 hours fixation X10 volume of the specimen in NBF to adequately and reliably demonstrate HER-2

and ER/PR staining. Ideally, the time the specimen is placed in fixative should be recorded on the request form. A qualified and experienced pathologist, high-quality sections, validated dilutions/titrations of antibodies, incubation times, detection systems and testing protocols, all of which should be documented. What type of controls are used? Internal positive controls as well as tissue arrays and multi-tissue "sausage" blocks. The use of expired antibodies is *strongly discouraged!*

Useful reference: "Quality Management in Immunohistochemistry" Richard N.Eisen, Diagnostic Histopathology Vol 14 No 7, July 2008.

Sonya Prasad and Sean Phefley from RCPAQAP spoke about "Performance testing in IHC". The QAP provides external proficiency testing in accordance with ISO/IEC 17043. The program runs clinically relevant exercises. The antigenicity of each block used is tested on the first and last slide taken from the block. Every 20th section is stained with H & E to ensure the relevant morphologic features are still intact.

It should be mentioned here that the RCPAQAP has developed a new website, one of the new features being the provision for multiple logins per laboratory.

Some of the factors contributing to a low score at assessment include: over-retrieval resulting in compromised nuclear morphology; edge artefact (due to pre-analytical factors); over-staining with the counterstain; strong back-ground staining as a result of either insufficient blocking, or the antibody is too strong.

ER staining is generally performed poorly as a result of variation of susceptibility to fixation between the different clones.

Afternoon tea time! The beach beckoned, but back I went, to hear Claude Cuvelier speak about the IHC QA system at Ghent University, Belgium. It has been identified that the explosive growth in world trade induced the need for uniformity in quality systems, this has been addressed by ISO, which stands for International Organisation for Standardisation, or which makes more sense for we English speakers, International Standardisation Organisation. The ISO 9000 "family" of standards addresses what an organisation does to fulfil: the customer's quality requirements and applicable regulatory requirements while aiming to enhance customer satisfaction, and achieve continual improvement of its performance in pursuit of these objectives. ISO 15189 covers the particular requirements for quality and competence of medical laboratories. In Belgium the King is the head of state, so all laws are passed as royal decrees!

The next speaker was **Jane Dahlstrom** who discussed common issues in IHC. IHC is a powerful tool in the context of "personalised" medicine and targeted cancer treatment, which generally proceeds along the following pathway: Diagnosis →sub-typing → staging → gene mutations or chromosomal translocations → guiding treatment → prognosis and patterns of spread. The cost to the community also needs to be taken into account. A common theme throughout the meeting was the factors affecting IHC testing, many of the speakers touched on the subject. Intrinsic factors relate to the tissue itself, extrinsic factors have already been discussed, and will be again!! Pre-analytical factors include collection, tissue type, properties of the antigen and its level of expression, fixative type and length of time, and subsequent storage of the specimen. Collection problems resulting in artefacts can include necrosis, crush artefact, diathermy and autolysis. Decalcification techniques can lead to either false

positive or false negative results, depending on the antigen. Formic acid, acetic acid and EDTA are better but slower-acting than the stronger acids such as nitric acid.

Both endogenous biotin (in liver and kidney particularly) and peroxidase can interfere with the staining result. Often a variable staining pattern can be observed throughout a tumour, using a panel of antibodies can sometimes help to interpret such occurrences.

Fixation is critical (you'll hear that again!) and results vary with the type of fixation used eg coagulative vs heat (microwave) or cross-linking. Cross-linking is very slow, taking up to 48 hours to saturate all reactive sites, and formalin is a Class 1 carcinogen. However, under-fixed tissue cannot withstand harsh retrieval methods. It is desirable to optimise the fixation time, breast tissue requires up to 8 hours fixation post cut-up before the commencement of processing. We all know why! If alcohol fixation occurs (coagulative) rather than cross-linkage (formalin) subsequent antigen retrieval (which undoes the cross-lining) is unsuccessful and the demonstration of the antigen is sub-optimal. Given that some treatments eg Herceptin depend on "quantitative" IHC results it is imperative that fixation is optimised! Other analytical factors impacting on results include section thickness (staining intensity increases with section thickness); antibody type: monoclonal antibodies have a higher purity, but polyclonal antibodies have a higher affinity; the target epitope; the method of retrieval; the "processing' system and the visualisation technique. A sound knowledge of staining patterns and localisation of antigens is essential for accurate interpretation of results. The data sheet accompanying commercially available antibodies provides all kinds of useful information including suggested dilution, appropriate positive control material and resultant staining pattern. It's worth a read! Rabbit vs mouse antibodies. Antibodies raised in rabbits have a high affinity for human epitopes with no loss of specificity.

Different antigens respond to different retrieval methods, such as heat vs enzyme. HIER depends on the heating device used, the temperature achieved and the length of time required as well as the pH of the solution eg the Ventana system uses either CC1 (tris buffer pH 8) or CC2 (citrate buffer pH6). It is important to "know your system", particularly for results requiring some measure of quantitation. There are significant differences in detection systems currently in use. Standardisation is more critical now that techniques are being applied in a quantitative manner. *Take home point: always correlate the IHC findings with the H & E staining results.*

Two more to go before the end of day 1. **David Gan** delivered an extraordinary presentation entitled "Making the most of your specimen in IHC"

Re-staining can be attempted on FFPE sections on charged slides, or even smears. It may be worth trying even if the section or smear is not on a coated slide, fix the smear in formalin first before proceeding. If the section to be re-stained has already had IHC, as long as the pre-treatment is the same there is no need to repeat it for a different antibody. As long as a different chromogen can be used there is no need to de-stain.

Double and triple staining also helps to make the most out of available tissue, as only 1 slide is needed for 2 or 3 antibodies. Pre-treatment must be the same, and the antibodies should complement each other eg p63 (brown nuclear) 34βe12 (brown cytoplasmic) p504s (red cytoplasmic).

Hand staining, although labour intensive could be considered for delicate or fragile tissue sections.

Cell blocking can be applied to FFPE tissue sections or smears. Remove the coverslip and bring the slide to water. Scrape off an area of tissue from the slide, make a paraffin block, cut and stain!! Sounds easy, and probably is, but.....not surprisingly, the architecture is destroyed.

Lifting . The tissue is lifted from the original slide, divided up and placed onto coated slides by pressing firmly and then adhered by DPX, which is then removed with xylene. Apparently the slides can then be dried and stained.

Tissue protection. This can also be applied to FFPE sections or smears. Some of the tissue on the slide is left in its original state, while the rest is stained (by hand). This is achieved by covering the part to be conserved with DPX, performing the staining technique, dissolving the DPX and then coverslipping. See "Tissue Protection Immunohistochemistry" Patty Kubier and Rodnet T Miller Am J Clin Pathol 2002;117: 194 -198.

Do these techniques work? I guess it has to be worth a try if there is enough pressure from the requesting pathologist, and you have unlimited time!

The final session before the close of day 1 (which then allowed us to indulge in a beverage or two at the welcome reception) was from **Camile Farah** who spoke about IHC markers in oral cancer. Oral cancer involves the mouth, lips, tongue, salivary glands, gums or oropharynx. It is the sixth most common cancer world-wide, and is three times more prevalent than cervical cancer. The survival rates are poor. In Australia, oral cancers make up 3-5% of all cancers. 90% are SCC, 60% involve the tongue and or floor of the mouth. Treatment is usually deformative.

Early diagnostic features

Genetic defects	↓		↑	
Molecular changes	↓	Tumour	↑	Diagnostic
Cellular changes	↓	development	↑	process
Clinical changes	↓		↑	

Oral cancer stem cell markers include: Aldh1;p75;CD44;Lgr5;CD24. Are they useful in detecting and quantitating dysplasia? Conclusion: Aldh1,Lgr5 and CdD24 have a possible role in malignant transformation of oral cancer.

CLSP;ELF3;IFI44;VSP18;CXCL13 are expressors of genes or proteins.

Epithelial to mesenchymal transition: Malignant transformation is associated with the loss of epithelial features and the acquisition of mesenchymal features. Do β-catenein, E-Cadherin,APC and Vimentin predict malignant transformation? I didn't write down an answer to that question, so I guess I'll have to go to the next workshop to find out.

More next edition of Paraffinalia. *Judy Brincat*

Under the Microscope : Giao Tran

Histology Department
Scientist Grade 2
Melbourne Pathology

Reported by: Kellie Vukovic and Rebecca Forrester

1**. What was your first job?**

Research Tech at the Diabetic Complications and Cytokine Laboratory (Dept. of Medicine, Melbourne University)

2. How long have you worked in histology?

12 years.

3. When people ask, "So, what do you do?" How do you explain Histology?

I say: "Histology is the study of cellular structure and we process tissue samples." Then I would go on to give scenarios and examples of what kind of specimens would be sent to us.

4. Who would you most like to have dinner with and why?

My grandparents. They've passed away many years now but I still miss them a lot.

5. What is your all-time favourite movie?
I have a lot of favourite movies ranging from Classics, Drama/Romance to Art House. Movies like the 1950s *"Rashomon"* by Akira Kurosawa, 1962 *"Jules et Jim"* by **François Truffaut**, Terry Gilliam's 1985 *"Brazil"*, and Zhang Yi-Bai's 2002 *"Spring Subway"* to the 2004 *"Le Pont des Arts"* by Eugène Green. I also love Zhang Yimou's movies. The list is so long and we are so lucky to have such riches!

6. What is your favourite stain?

Perhaps the PAS stain.

7. What is your favourite food/Restaurant?

I like fine dining with cuisines from all corners of the globe. However, I always find that the best meals are the ones prepared by those that care enough to cook for me.

8. What is the best conference you have ever attended?

I haven't been to many conferences in that span of 12 years but I had the most fun at the 2006 Gold Coast Histology Conference.

9. Favourite beverage?

I don't have a favourite. It's whatever suits the meal, the occasion or my mood at the time.

10. What is your dream holiday destination and why?

Base camp at the foot of Mt Everest in the Himalayas. I love the tranquillity (when there's no avalanche) and the opportunity to mingle with people who have or will embark on the challenge of reaching its' dizzy heights. Of course, in between all that I hope to learn something new!

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PRELIMINARY ANNOUNCEMENT

NATIONAL HISTOLOGY CONFERENCE

APRIL 26-28, 2013

CROWN CASINO

MELBOURNE

Start planning your attendance at the National Conference in the Autumn of 2013 in Melbourne.

We are calling for proffered papers and posters.

Use your skills to design the Conference logo and win a free conference dinner.

Send your entries to:

secretary@hgv.org.au

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Renal Failure to Transplant

Speaker: **Julian Richardson (Cabrini Hospital)**

Date: Thursday 3rd May, 2012

Time: 6:00 – 6:45 Refreshments
6:45 – 7:45 Presentation

Venue: Brockhoff Lecture Theatre
Level 3, Smorgan Family Building

Peter MacCallum Cancer Centre
St. Andrew's Place, East Melbourne

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Attendance at this meeting contributes to APACE points

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Future Scientific Meetings:

2012

~~**22nd March**~~
~~HGV/ASC Scientific Meeting – Student Presentations~~
~~Venue – Peter Mac~~

3rd May
HGV Scientific Meeting – Julian Richardson (Cabrini) – Renal Failure to Transplant
Venue – PeterMac

28th June
HGV Scientific Meeting – TBA
Venue – Peter Mac

27nd July
Social Event – Trivia Night
Venue – TBA

18th – 19th August
HGV/HDV Joint Meeting - Mornington Penninsula

13th September
Scientific Meeting -TBA
Venue: Peter Mac

24th – 27th September
AIMS Conference – Darwin

28th – 3rd October
NSH Conference

15th November
HGV Scientific Meeting/AGM – Paul Kennedy/Veronika Gazdik (VNLS)
Venue – PeterMac