Histology Group of Victoria Inc.

Org. No. A003523F
ABN 49 725 623 468

PARAFFINALIA

http://www.hgv.org.au

Volume 15 Number 2

April 2010

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 2009-2010 are:

Name	Institution	Phone
Allison Boyd	St. Vincent's Hospital	9288 4288
Judy Brincat	Dorevitch Pathology	9244 0354
Maria Chavez	Monash Medical Centre	9594 3493
Elizabeth Baranyai	Cabrini Health	9508 1263
Erin Little	RCPAQAP	9808 9744
Mark Bromley	Melbourne Pathology	9287 7806
Michelle Zammit	The Alfred Hospital	9076 3088
Nguyen-Hoang, Nguyen	Peter MacCallum Cancer Centre	9656 1844
Cameron Skehan	Monash Medical Centre	9594 3493
Raelene Howlen	Dorevitch Pathology	9244 0354
Adrian Warmington	St. John of God Pathology (Victoria)	5320 1171

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 advertising in the next edition, please contact: TBA
trade@hgv.org.au
Advertising for the next editions of Paraffinalia closes: 1st June, 2010

- Single sided A4 Black and White $200
- Double sided A4 Black and White $325
- Colour insert (Supplied by company) $325

PLEASE DO NOT POST ADVERTISEMENTS TO THE COLLINGWOOD MAIL BOX

Advertising rates:

Submissions:
Author enquiries and readers wishing to contribute articles or reports can contact the Editor—email: editor@hgv.org.au or post directly to:

The Histology Group of Victoria Inc.
P.O. Box 1461
Collingwood Victoria 3066
Australia

Please send articles on floppy disc (preferably Microsoft Word format) for inclusion in the next edition. All articles submitted for publication will then become the sole property of the Histology Group of Victoria.

Disclaimer:
Any opinions expressed in this publication are solely those of the contributing author and are not necessarily reflective of the Histology Group of Victoria or the editor.

NOTE: No responsibility is assumed by the Histology Group of Victoria for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herin. It is the users responsibility to ensure that all procedures are carried out according to appropriate Health and Safety requirements.

WE ARE GOING GREEN!

With a membership of over 550 and in an era where we have a heightened awareness of our environment and the impact we have on it, we are proposing to commence sending our newsletter electronically.

Initially the result of receiving your newsletter electronically is that all the advertising material may not be able to be included, as it will create a document that is too large to send. However we will be placing the complete newsletter on the website.

If you would be happy to receive the newsletter electronically, please send us an email with the subject "GOING GREEN" from the email address that you wish to receive the newsletter to

membership@hgv.org.au

Additionally if there are newsletters getting delivered to your workplace of former employees please let us know at address above.

We would also value your input into the content of our newsletter. If you have 5 minutes, and have not done so yet, please visit

http://www.surveymonkey.com/s/3CK3ZD8

and complete a short survey.

Blurb from the Bush

To start I would like to congratulate the committee on once again putting on a One Day Seminar that not only attracted great numbers but delivered a great array of speakers and topics in a well oiled manner. Over 140 registrants attended two workshops and seminar over March 19th and 20th. Visitors from overseas and interstate were in attendance which is again testament to the reputation the HGV holds in delivering scientific meetings.

The membership should be very proud of what is currently a relatively inexperienced committee. An event such as this takes an enormous amount of planning which commenced over 12 months ago. Your committee volunteer valuable time and considerable effort to make our One Day Seminar appear professional and dynamic. We ensure we focus on excellence of program, participation of trade and provision of quality social events at a cost other discipline professional groups who always have profit on the agenda can only dream of achieving for their members.

The committee is considering our options in relation to provision of our newsletter electronically. We are conscious that we are now in an era where electronic media is dominating both for social and environmental reasons. Our issue surrounds the size of the document when it includes all the advertising material that in essence currently pays for the newsletter production. As such we will be asking all members how they would like to receive their newsletter and what components of the newsletter are important to them. Please take the time to respond.

Adrian Warmington
President

ARTICLE REVIEW:

Citraconic anhydride: a new antigen retrieval solution

Anthony S-Y Leong and Zenibia Haffajee- Pathology- Journal of the Royal College of Pathologists of Australasia (Jan 2010), **42(1)**, 77-81

This study examined the possibility of a suitable retrieval reagent for antibodies which fail to work or stain weakly in fixed paraffin embedded tissue section when using citrate buffer – currently the widely embraced "universal" retrieval solution for immunohistchemisty (IHC). Although citrate buffer at pH 6.0 is effective over a wide range of diagnostic antibodies, there are some antibodies that remain capricious and yield poor staining results.

Two sets of 4µm thick sections of control tissue blocks were cut, deparaffinised, rehydrated and subjected to antigen retrieval. One set of sections was treated by microwave irradiation for 10 minutes at 98°C on 0.1M citrate buffer at pH 6.0 and the other set was subjected to the same protocol but with 0.05% citraconic anhydride at pH 7.4 as the retrieval solution. Antigen retrieval was performed by completely immersing the slides in the retrieval solutions and irradiating in a computerised oven that allowed control of time and temperature. A standard streptavidin-biotin peroxidise method was used with diaminobenzidine as the chromagen, performed on an automated stainer.

Thirty-five of the sixty-five (53.8%) antibodies tested showed more intense staining following retrieval in the citraconic anhydride. The only instance where the citraconic anhydride failed to improve staining was with CD21 and in fact resulted in no staining at all. In all sections, no difference in cytomorphological preservation was detected following retrieval in the two solutions.

Antibodies directed against melanoma antigens benefited from retrieval in citraconic anhydride. These included Melan A, HMB45 and MiTF. Other, more unpredictable antibodies such as TdT, MyoD1, myogenin perforin, TIA-1, RET protein and surfactant protein A produced distinctly better staining following irradiation in citraconic anhydride. Even difficult to detect antigens such as CD4, cyclin D1, granzyme B, bcl-6, CD25 and lambda chain revealed significantly more enhanced staining than after conventional antigen retrieval methods.

The study confirms that 0.05% citraconic anhydride is a useful antigen retrieval solution, especially when used with microwave irradiation especially for antibodies which stain weakly or fail to work with conventional methods.

Sarah Morabito – Pathology Diagnostics

Under the Microscope

Reported by Maria Chavez

Natalie Kvalheim
Histology Scientist
Department of Primary Industries
Veterinary Diagnostic Services

1. **What was your first job?**
   I worked in the kitchen at a local catering business in Echuca. I'll never forget serving someone tea and accidentally pouring coffee in it instead of hot water on one of the few times I was allowed out of the kitchen to waitress.

2. **What attracted you to Histology?**
   I love the practical aspects of histology it's a fun job and getting a result that's interesting to look at and sometimes get a nice photo is rewarding. Cutting sections can be quite relaxing.

3. **What is the worst decision you have ever made?**
   Nothing comes to mind, must be very optimistic or I've buried the experience somewhere down deep in my memory.

4. **What is the best decision you have ever made?**
   Getting into SCUBA diving where I met my husband and lots of other interesting characters. There's lots of colourful coral in our temperate waters, a ship's graveyard and a recent addition the Canberra that I can't wait to explore when I get the chance.

5. **Who would you most like to have dinner with and why?**
   Elizabeth Blackburn. Great to see an Ausie woman win the Nobel Prize. The link between telomeres and cancer is interesting, her views on stem cells and cloning were controversial and she's got some interesting things to say about working in medical research.

6. **What music do you enjoy listening to?**
   I like old style rock or R&B like Hendrix, Rolling Stones and the Doors and new bands like Red Hot Chilli Peppers and Kings of Leon.

7. **What is your favourite stain?**
   I like doing ISH because there is so much involved and understanding molecular techniques, much more interesting as a picture than a band on a gel or a wiggly graph.

8. **What is your favourite food/Restaurant?**
   Indian or Sri Lankan

9. **What are you reading at the moment?**
   Patricia Cornwall, I 'm gradually working my way through all her Scarpetta series books. I like books about murder mysteries. One of her latest books mentions histology and it made me laugh.

10. **What is the best conference you have ever attended?**
    The HGQ meeting in 2006, it was a good program but mainly it was great to get out of the lab to somewhere warm and by the beach.

11. **Are there any current projects you are working on at the moment?**
    Expanding our range of IHCs for animal infectious diseases and looking for antibodies.

Contaminated pathology specimen resulting in an unnecessary procedure

A patient (Patient A) was referred to a specialist Oncologist following pathology results indicating features indicative of a poorly differentiated carcinoma.

The patient was referred for additional radiological investigations. Stage 1 cancer could not be excluded. Due to the young age of the patient they underwent radical surgery.

The pathology results from the specimens taken during the surgery indicated that there was no tumour. Following these results, clinical doubt was raised about the original pathology results that indicated a poorly differentiated carcinoma.

Pathology undertook an investigation which highlighted the possibility of another patient's specimen being present in the slides. DNA testing confirmed that the tumour cells in the slides had a different DNA profile to Patient A. The contaminant was subsequently found to be from another patient (patient

B) whose specimen was processed in pathology immediately prior to patient A and reported as showing poorly differentiated adenocarcinoma. Patient B received the correct treatment.

A full open discussion was commenced with Patient A as soon as the clinical suspicion for contamination was raised.

Conclusions

It was not possible to ascertain the exact point of contamination therefore traditional Root Cause Analysis methodology was not used. Process mapping of the entire specimen handling process was carried out – from specimen collection in theatre to final reporting of slides in pathology

Using a modified hazard barrier target analysis, each of these points was then analysed in terms of probability, sources of contamination, barriers, failures and solutions.

How did the health service address these issues?

An external review of anatomical pathology is to be undertaken and will include:

- Review of cleaning processes to ensure adherence to best practice standards
- Review of specimen handling processes to ensure potential sources of cross contamination is minimised, including when formalin is added
- Review of quality control processes related to cross-contamination and floaters
- Review of all relevant protocols

In the interim, the following must be implemented in anatomical pathology:

- Where forceps and other cutting instruments are used, they shall be single use or single patient use.
- Where equipment has to be re-used (such as embedding centre, microtome)single use tissues/towels are to be used during the cleaning process.

Pathology to develop an equipment replacement / purchase plan (intended to decrease risks associated with specimen cross-contamination) which must include:

- Purchase of additional water baths, to allow cleaning between patients
- Purchase of a flat-bed single slice stainer

Pathology to implement a broad based, formalised education program for all pathology staff (inclusive of consultants, registrars and scientists).

The operating theatre management are to review specimen handling processes within theatre to ensure that all potential sources for contamination are minimised.

Histology Group of Victoria Inc.
Org. No. A0035235F

A Series of Short Presentations

Date: Thursday 6th May, 2010

Time: 6:00 – 6:45 Refreshments

6:45 – 7:45 Presentation

Venue: **Brockhoff Lecture Theatre**
Level 3, Smorgan Family Building

Peter MacCallum Cancer Institute
7 St. Andrews Place
East Melbourne

Attendance at this meeting contributes to APACE points

Histology Group of Victoria Inc.
Org. No. A0035235F

Future Scientific Meetings: 2010

~~**19-20th March**~~
~~One Day Seminar~~
~~Venue – St. Vincent's Hospital, Melbourne~~

6th May
HGV/ASC Scientific Meeting – Student Presentations
Venue – PeterMac

24th June
Scientific Meeting – Forensics
Venue – Victorian Institute of Forensic Medicine (VIFM)

30th July
Social Event – Trivia Night
Venue – TBA

9th September
Scientific Meeting – Renal Biopsies
Speaker - Professor John Dowling
Venue - PeterMac

11th November
Scientific Meeting - AGM
Venue – PeterMac

ASCT *2010 Introduction to the Cytopreparation Laboratory* Now Available

The ASCT has recently revised its “Introduction to the Cytopreparation Laboratory” Course. The web course, hosted by blackboard.com, continues to be updated to fulfill the need expressed by the ASCT membership to obtain assistance in training cytopreparatory personnel.

This course is intended to provide fundamental information about the cytopreparation laboratory, thus supplementing the orientation materials that are typically given to new cytoprep employees when they are initially hired. The course is basic and assumes that the participant has no knowledge of laboratory procedures. The content is also suitable for new cytotechnology students, histotechnologists or others who may benefit from learning about the activities in the cytopreparation laboratory.

The course chapters are:

- Introduction
- Specimen Receipt
- Specimen Preparation
- Cytology Equipment Orientation
- Staining Theory and Purpose
- Troubleshooting Common Problems
- Quality Assurance
- Lab Safety
- Fine Needle Aspiration

In addition to the chapter text, the 2010 edition includes a lab terminology dictionary, self-assessment exercises and corresponding PowerPoint files to reinforce the information presented in some of the chapters.

Participants can print their own self-assessment test results. There is no minimum score required, nor passing grades defined. Laboratories, who wish to, may monitor the tests and set Pass/Fail requirements. Individuals who complete the course may request a Certificate of Completion from the ASCT.

An Individual subscription is $95/person, with discounts for multiple registrations per single laboratory. A fixed price of $100 per training program is available for Cytotechnology and Histotechnology schools and/or other educational programs. All subscriptions last for 12 months from the date of registration and access to the course and its resources are available for the entire 12 month period.

Go to the website www.asct.com for registration information or email info@asct.com.

For those of you who were unable to attend the workshops or seminars, we are providing a copy of the abstracts which were printed in the ODS Handbook.

HGV ODS Workshops

Friday 19th March

Workshop 1 - Basic Presentation Photography for the Medical Scientist

The presentation will consist of a 30-40 minute explanation of the techniques involved and approx 2-2 1/2 hours of exercises designed to allow participants to become familiar with the basics of photo manipulation in Photoshop Elements

The explanation of the basics will include: background to digital photography, getting the photo, basic macro and micro photography, equipment alternatives and where to get them, current simple photo manipulation and the use of collages as a tool for presentation.
Photo manipulation will include simple use of layers, use of brightness and contrast, Cloning, Cleaning up images from various sources, optimization of size of files and file type and filing and storage. Photos will be supplied for each exercise.

Presenter: Julian Richardson

Workshop 2 – Assessing the Quality of Tissue Processing: It's Not Easy!

Have you ever tried to comparatively evaluate the quality of processed tissue blocks? Is protocol A really producing better results than protocol B – or am I just imagining it? Is this new xylene substitute better than xylene? Can we reduce our processing time without reducing quality? While gross differences in processing quality may be fairly easy to identify small differences are another matter. Without a systematic approach which controls all the variables that can influence the quality of the final tissue section, together with an objective scoring system, you will find it difficult or impossible to achieve - and to thus make informed processing decisions.

This workshop will outline the difficulties in assessing the quality of tissue processing, discuss the variables that must be controlled in any worthwhile test, describe how specimen panels should be structured and explain a scoring system which has been successfully used for this purpose. An opportunity will be provided for participants to try their hand at scoring.

At the conclusion of the workshop participants will:

1. be aware of the major problems in evaluating the quality of tissue processing,
2. be able to devise a processing test in which important variables are controlled,
3. be familiar with a scoring system that can produce an objective evaluation of tissue processing.

Presenters: Geoffrey Rolls, Neville Farmer

Abstracts

Saturday 20th March

If You Have the Muscle; I Have the Nerve: Part 1 - Muscle Biopsy Basics

Jean Mitchell
Neuromuscular Laboratory, University of Wisconsin Hospital and Clinics

Preparation of muscle biopsy tissue for clinical diagnosis presents a unique challenge to the histologist. A brief overview of anatomy and physiology of the normal human skeletal muscular system will be presented along with a review of abnormal clinical findings and symptoms that warrant a patient to undergo a muscle biopsy procedure. Transporting, handling and the special procedures that muscle biopsies require for optimal results will be discussed. The panel of non-enzyme and enzyme stains routinely employed for muscle biopsies with pathologic changes demonstrated by each stain and their relevance to disease states will be mentioned along with troubleshooting suggestions to ensure optimal staining results. The significance of immunohistochemical procedures and the use of electron microscopy to enhance/confirm muscle disease diagnosis will be presented.

Electron microscopy- Is it needed or just an expensive toy ?

Paul Crammer
Monash Medical Centre

Microscopes have existed since approximately the year 1590 when two Dutch eye glass makers placed multiple lenses into a tube. Improvements to this forerunner of the compound microscope continued for the next four hundred years and to this day.
It wasn't until 1931 the first electron microscope was invented by Ernst Ruska. An electron microscope depends on electrons rather than light to view an object. Electrons are speeded up in a vacuum until their wavelength is extremely short, only one hundred-thousandth that of white light. This way, the resolution is improved and it is possible to view objects as small as the diameter of an atom.
Preparation of biological material for electron microscopy is similar to that for light microscopy. Main differences include the choice of fixative and the embedding media. Sections are then cut using a diamond knife on an ultramicrotome, stained and viewed under the electron microscope. Digital images are obtained of relevant diagnostic areas and stored on a hard drive or disk.
Due to the advent of immunohistochemistry, the use of electron microscopy for tumour diagnosis has decreased significantly. Occasionally, there is still a need to process tissue to aid in the diagnosis. Unfortunately this means using formalin fixed or paraffin embedded material. As you would expect, this does not provide an ideal outcome as far as preservation of the material. Most electron microscopy is performed on renal biopsies, where the tissue can be added to fixative immediately. This will provide valuable information to aid in the diagnosis of glomerulonephritides.

Abstracts

Saturday 20th March

Veterinary Histology

Natalie Kvalheim
Veterinary Diagnostic Services – Attwood

Veterinary Diagnostic Services - Attwood provides a veterinary diagnostic pathology service on behalf of the Biosciences Research Division of the Department of Primary Industries, Victoria. This service supports the Animal Health work done by Biosecurity Victoria and the Victorian Chief Veterinary Officer. Generally laboratory submissions are made to compliment investigations into unusual disease events involving production animals or wildlife and to carry out routine surveillance for exotic disease agents. Laboratory testing is carried out of a large range of terrestrial and aquatic animal species including mammals, birds, reptiles, fish and molluscs. Such a variety means that the anatomy and histology can be both challenging and interesting especially compared to human medical anatomy and histology. A range of the unique features of veterinary anatomy and histology will be described and illustrated during this presentation using case material generated by ruminant, bird, pig, fish and abalone submissions to Veterinary Diagnostic Services – Attwood. Commonly used histological tests will also be covered in this presentation.

The Forensic Aspects of the Victorian Bushfire Disaster

Associate Professor Chris Briggs

On Saturday 7th February 2009 Victoria suffered the most devastating bushfires in its history, resulting in catastrophic loss of life and public and private property. Within hours of the disaster members of the forensic community were mobilized to initiate the examination and identification of remains (at the Victorian Institute of Forensic Medicine); while some attended scenes. The ultimate aim of all DVI operations is to establish the identity of every victim by comparing and matching accurate antemortem (AM) and postmortem (PM) data. The identification and coronial reconciliation process continued over the next three months and resulted in the positive identification of all victims.

Contaminants in Histology

Alex Laslowski
Monash Medical Centre

In Anatomical Pathology the final diagnosis is always questioned when there is a possible misdiagnosis, however the entire histological process starting from how we handle the patient specimens through to the interpretation of the pathologists needs to be critically assessed. The potential for patient cross contamination, tissue floaters or carry over artifacts is a major cause for concern even in the best laboratory settings. The presence of contaminants still remains a major cause of potentially misleading or incorrect diagnoses. Identifying these contaminants as being foreign to the patient is also a challenging task. When a tumour is unexpectedly found the proof of origin of a tissue sample may be questioned; for this there are numerous techniques to help facilitate this namely PCR. In this presentation we will look at the extent of laboratory contaminations with regards to potential sources, how we can best identify them proactively and the potential for eliminating contaminations.

Abstracts

Saturday 20th March

The blame game in histology (Difficult specimens)

Ms. Kerrie Scott Dowell

In Histology, we accept that there are some individual tissue samples that will be difficult to section despite being well processed. However, when there are multiple “problem” blocks in a processing batch, is it time to launch an investigation? Is it time to play the Blame Game? We will look at a systematic approach to assessing the situation, the pitfalls and the possible courses of action to deal with the immediate problem and to prevent it happening again.

The Influenza A(H1N1) 2009 pandemic in Australia

Anne Kelso
WHO Collaborating Centre for Reference and Research on Influenza, Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria 3051

Following its emergence in North America in April 2009, the new influenza A(H1N1) virus was first detected in Australia on 20 May in a Melbourne family recently returned from the US. In fact, by this time community transmission was already underway in Victoria. The virus spread rapidly to other states and territories, with laboratory-confirmed cases peaking in July then declining to low levels by September, shortly before the pandemic influenza vaccine was ready for distribution. Despite the low impact of the disease on most of the population, the outbreak presented many challenges for diagnostic laboratories, clinical services (particularly intensive care units) and public health departments and the Australian experience provided valuable insights for many northern hemisphere countries as they prepared for, and then responded, to a heightened second wave of infections in their autumn and early winter. At the time of writing, it is unclear whether new outbreaks will occur in Australia over the summer, for example when travellers return from overseas and children return to school, or whether our inevitable second wave will be delayed until the usual winter influenza season. In the meantime, our Centre and its equivalents in the US, UK, Japan and China continue to monitor pandemic influenza viruses for antigenic and genetic changes that might affect their transmission or virulence or the effectiveness of antiviral drugs and vaccines.

Medicine in remote Papua New Guinea

Jacqueline Boyd
Royal Darwin Hospital

Kompiam District Hospital is a small health service providing medical care to a large population within Enga Province, in the highlands of Papua New Guinea. I have worked there as one of two doctors for about 16 months intermittently over the last few years.
Working as a medical officer in remote areas of a developing country brings with it many challenges in both adequately diagnosing and treating medical conditions. Our limited diagnostics included Sahli haemoglobins, malaria films, rapid HIV tests and basic crossmatches. No biochemistry or microbiology and histopathology takes 3 months at best for a result, needing to be sent to Port Moresby. If and When a diagnosis is reached, there still remains the challenge of administering adequate treatment to the patient.
This talk includes some of my experiences in managing patients within this setting, a little about the tropical diseases prevalent up in Enga and some interesting cases I've come across.

Abstracts

Saturday 20th March

If You Have the Muscle; I Have the Nerve: Part 2 - Nerve Biopsy Basics and Case Histories

Jean Mitchell
Neuromuscular Laboratory, University of Wisconsin Hospital and Clinics

There are many facets to artifact free preparation of nerve biopsy tissue for clinical diagnosis that can present challenges to the histologist. A brief overview of anatomy and physiology of the normal human nervous system will be presented along with a review of abnormal clinical findings and symptoms that warrant a patient to undergo a nerve biopsy. Nerve electron microscopy and the unique method of single nerve fiber teasing will be discussed along with the relevance of these techniques in the diagnosis of nerve abnormalities. Case histories will be used to show the importance and impact nerve and muscle biopsies have in patient care.

Back to the Future –New Applications for Histopathology in Cancer Diagnosis

Sue Sturrock
Peter MacCallum Cancer Centre

Surgery remains the first choice of therapy for most solid tumours. Subsequent histology provides valuable diagnostic information including type, stage, grade and margins of tumour growth, but remains virtually unchanged in its technical performance. Advances in immunohistochemistry and in situ hybridisation have revealed protein expression and DNA sequences associated with particular tumour types. Diagnostic information obtained using these techniques has been coupled directly to new successful companion therapeutics as part of the first wave of personalised medicine. The foundation for new treatments of solid tumours is the paraffin block. The future is in wax.

****