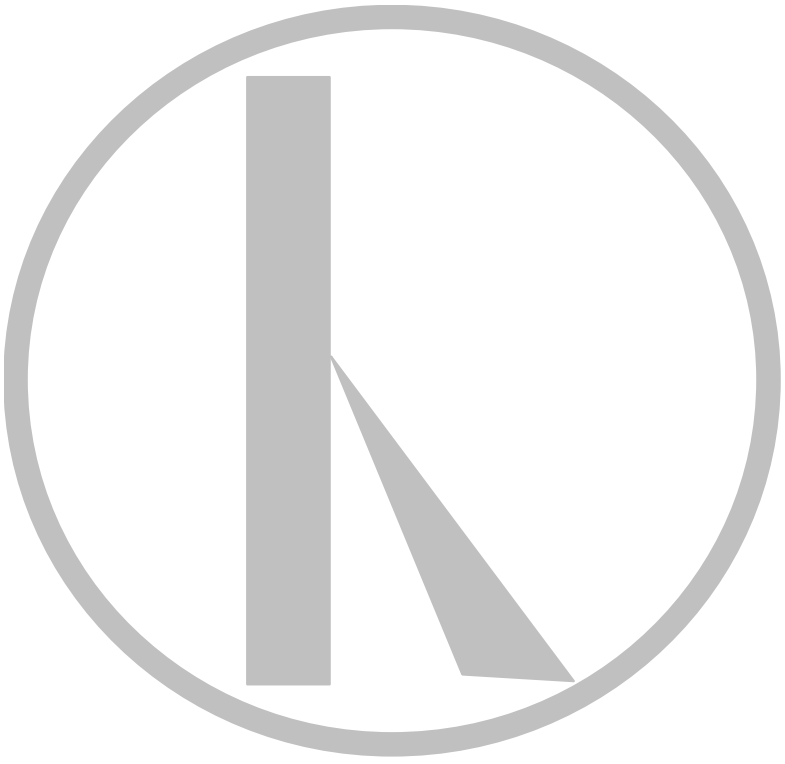
Histograph

www.histonsw.org.au

ISSUE 1
February 2010



Newsletter of the Histotechnology Group of NSW

Contents

Editorial

Another year has passed and we, hopefully, are ready to attack 2010 with verve and exciting expectation or at least with a sarcastic smile. Unfortunately we were only able to get two issues out last year. Why? Well, I have been quite busy and have had a very enjoyable, rewarding year with some great milestones. I started teaching clarinet to a group of primary school students at my wife's school (where she is the Assistant Principal). They gave their first public performance and were brilliant. I attended my first American NSH Meeting in Birmingham Alabama and had a wonderful time.

I was invited to write a review on Microscopic H&E Quality Control for the next Dako issue of Connection. I was invited by the Tropical Division of AIMS to give a presentation on Scientific Histopathology in Townsville and I have been invited onto the editorial advisory board of the Journal of Histotechnology. I have had a very good year.

Having said this, we have co-opted the aid of Linda Gomes from the Children's Hospital at Westmead as Assistant Editor and she will be editing the next issue of the journal. Linda is one of the new breed of Histopathology Scientists with a passion for our discipline. Be excited!

Tony Henwood,
Editor
anthonyh@chw.edu.au

Chairman's Report

While you were enjoying your well earned Christmas break, several important decisions were made. Unfortunately this delayed the publication of this "Histograph" (sorry Tony).

After reviewing a number of potential venues for the 5th National Histology Conference to be held in Sydney in 2011, we have finalised arrangements with our preferred venue.

The Conference will be held at "Rosehill Gardens Event Centre" on the 4th, 5th and 6th of November 2011. Accommodation arrangements are being made through "Rydges-Parramatta" which is located across the road from the venue. We are currently working on a program and costings and will have this information available as soon as possible. We suggest you lock these dates into your calendar.

We have also finalised a meeting with our Newcastle members which will be held on Saturday the 17th and Sunday the 18th of April at "Club Toukley RSL" in Toukley, the Central Coast. This will be a one and a half day meeting, speakers are currently being finalised. A flyer has just been prepared which has been incorporated into this newsletter. The last Newcastle meeting was well supported with excellent speakers and topics. Do not miss this one.

There are also a number of Scientific Meetings planned throughout this year. Our first meeting will be in March at Liverpool Hospital. The topic will relate to immunohistochemistry. More details shortly.

Updated information on these meetings will be posted on our Website, www.histonsw.org.au. Please check this website regularly for updated information.

Cheers,

Trevor Hinwood.
Chairperson.
Histotechnology Group of NSW.

Committee Members

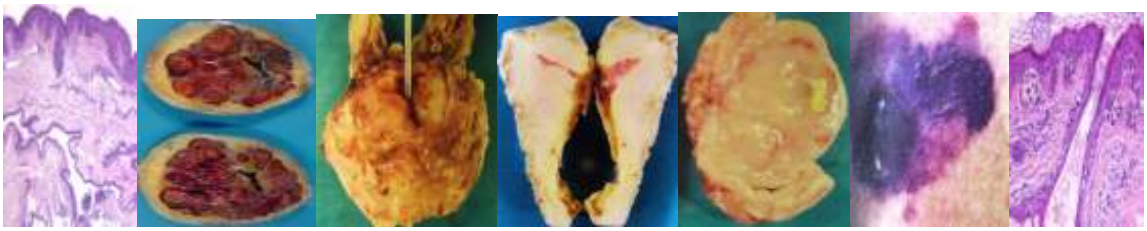
Chairman	Trevor Hinwood	trevor.hinwood@hdscientific.com.au	Product Specialist H D Scientific
Secretary	Kathy Drummond	kdrummond@dhm.com.au	Senior Scientist Histology DHM
Assistant Secretary	Dianne Reader	dreader@nsccahs.health.nsw.gov.au	Senior TO Anatomical Pathology RNSH
Treasurer	Lin Parkes	eyepoint2@bigpond.com	
Membership Secretary	Margaret James	megajam@optusnet.com.au	Dutta Path
Trade Rep			
Country Rep	Margaret McLauchlan	mclauchlans@bigpond.com	Nextpath
Country Rep	George Youssef	george.youssef@gsahs.health.nsw.gov.au	Griffith Base Hospital
Editor	Tony Henwood	anthonyh@chw.edu.au	Histopathology Lab Dept Head CHW
Assistant Editor	Linda Gomes		Scientist, Histopathology CHW
Committee	Bill Sinai	sinai_family2@bigpond.com	Life Member
	Grant Taggart	gtaggart@dhm.com.au	Department Manager Histology DHM
	Julie Bilkey	julie.bilkey@bigpond.com	Sydney Skin Pathology
	Liam O'Donnell	liam@sydneyskinpathology.com.au	Sydney Skin Pathology
	Bharathi Cheerala	bcheerala@dhm.com.au	IHC Histology DHM

HISTOTECHNOLOGY GROUP of NSW

(ABN 63 128 868 343)

nswhistogroup@bigpond.com

Postal address:
P.O. Box 496
Guildford NSW 2161



CENTRAL COAST 2 DAY MEETING
17-18 APRIL 2010

JOIN YOUR PEERS AND COME ALONG TO EXPERIENCE

- **A wide range of professional presenters.**
- **A variety of topics across several areas.**
- **Comprehensive program to keep.**
- **Good networking opportunities.**
- **Opportunity to view what's new through the trade displays.**
- **Central venue and good food.**
- **Chance to catch-up socially at the Saturday night dinner.**

For further details "*watch this space"* or go to our website www.histonsw.org.au

Contact: *Kathy Drummond, kdrummond@dhm.com.au or fax 0298555169*

Marg McLauchlan, mclauchlans@bigpond.com or

Work phone (Mon-Fri 1-7pm) 0249 276580

NSW Histotechnology Group 2 Day Meeting 17-18 April 2010 at Toukley

Application Form

(To be completed and returned with payment RSVP 1 April 2010)

Name:	Address:
Email address:	Phone:
Workplace:	Work phone:

Full 2 day meeting $35	
Saturday only (10.00am – 4.30pm): $30	
Sunday only (10.00am - 12.30pm): $15	

Saturday Night Dinner
Expression of interest
(please circle)

YES **NO**

(The cost of the Saturday night dinner is not included in the meeting subscription).

Application and payment can be made by:

1. *Direct payment to- BSB 802084 Account 94099, mail application form to PO Box 496, Guildford NSW 2161 or email (form is available on website)*

2. ***Cheque to "NSW Histotechnology Group" with application to PO Box 496, Guildford NSW 2161.***

Accommodation

Delegates are required to organise and pay for their own accommodation. Recommended hotels/motels in the vicinity of the meeting venue are:

Toukley Motor Inn	43965666
The Water's Edge Motor Inn Toukley	43964622
Sea and Surf Motel Noraville	43964474
Lake Haven Motor Inn Gorokan	43932224
Bridge View Motel	43923355

End of an Era

With the completion of 11,960 crosswords, it is with great sadness that after 46 years of service to Histology, Michael Konopka is retiring to spend his best years with his family and to pursue his passions for sailing and travelling to exotic places.

Michael began his career as a trainee at the Royal Alexandra Hospital for Children (Camperdown) just before Christmas in 1964. In those days, you studied whilst working in order to qualify as a Medical Technologist (equivalent to BSc) from AIMLS (now AIMS). At the time, the Chief Medical Technologist was a man called Herbert Mitchell, a very talented and knowledgeable man that took Michael under his wing and taught him the art of Histology. Another impressionable person in his early career was Dr Douglas Reye, Director of Pathology, who as Michael recalls was a very tall but quiet man. Michael relays quite fondly his first encounter with Dr Reye, "A day or two after I started he called me into his office, I stood in front of a huge imposing desk, he said "What's your name son" to which I replied "Konopka Sir" there was a moments silence, then "Don't you have a first name"

That set the scene for the next 30 years.

Very few of us in the Histology profession have experienced the evolution of a Histology laboratory Over 46 years Michael has seen many facets, from the manual handling of tissue, to automation, the rapid decline of autopsies performed, introduction of tissue processors ".....we had one piece of automation, a tissue processor, it was a free standing pedestal model that stood about 5 feet six inches tall or 165cm, an original TissueTek…." Michael recalls the times where everything was done manually, slides were washed and dried, solutions were made up including stains and mountants, with no such commodity as calling a supplier to purchase readymade stains and coverslipping was performed manually; to the new era where most of the routine work is automated. For that generation of histotechnologists it was a great learning curve.

As a supervisor and mentor, Michael was tough but fair and passed on his vast knowledge to all who wished to learn. As his mentors had installed in him, he expected all work to be of high quality and nothing less than 100% from his staff and trainees. The teaching received from Michael in Histology produced highly qualified experienced scientific and technical staff.

Over the years, Michael has had the opportunity to work with many researchers on various projects including the Children's Medical Research Foundation (CMRF) now CMRI at Westmead, the University of Sydney and the University of New South Wales and has presented at numerous national and international conferences.

In 1994 the hospital moved to Westmead (The New Children's Hospital). Moving a Histopathology department after 100 years was quite a task, but Michael took all the mishaps in his stride and as a result a new modern laboratory was founded.

In 1998 after working at Westmead for 3 years, Michael decided it was time for new challenge. He moved to Darlinghurst and took up a position at the Skin & Cancer Foundation Australia (SCFA). The SCFA laboratory is a Dermatopathology laboratory headed up by Prof. Steven Kossard. With the total support from Prof Kossard, the histopathology laboratory was expanded and equipped with state of the art automated equipment. In the last 10 years, his role had drifted from the bench to management, but given his upbringing in the industry the bench was never far away.

It has been my good fortune to work with Michael Konopka as a colleague and friend. I know he will be missed by all who have had the opportunity of working with him.

I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale (Marie Curie).

Maria Sarris

Processing Fatty Breast Tissue & HER2

It seems to be a recurring problem. The high number of poorly processed breast tissue blocks. From Histonet, several recommendations have been communicated and I hope they may be of use:

- Keep the grossed blocks thin
- Ensure they are adequately fixed.
- Increase the processing times.

Maxim Peshkov from Russia used graded alcohols (isopropanol) for dehydration, then mixtures of isopropanol and mineral oil 5:1 and 1:2 at 50°C, then mineral oil instead of xylene. Paraffin infiltration was as usual. These reagents give the "soft" transitions from dehydrant to paraffin. Difficult tissues will cut more easily. Risk of inhalation of toxic chemicals is decreased upto 5 times.

Peshkov's protocol is based on that of Rene Buesa (RJ Buesa, Mineral Oil: The Best Xylene Substiute for Tissue Processing Yet? JOH/Vol.23, No2/June 2000):

Step	Solution	Time	Temperature
1	Isopropanol @70%	1h	RT
2	Isopropanol @80%	1h	RT
3	Isopropanol @90%	1h	RT
4	Isopropanol @92%	1h	RT
5	Isopropanol 99% (fresh)	1h	RT
6	IM 5:1	1.5h	50°C
7	IM 4:2	1.5h	50°C
8	Mineral oil	overnight	50°C
9	Paraffin	1h	60 °C
10	Paraffin	1h	60 °C
11	Paraffin	1h	60 °C
12	Paraffin	1h	60 °C

Rene Buesa has the following recommendations:

The fundament is a GENTLE dehydrant substitution by the infiltrating agent (paraffin) and ELIMINATES the use of an antemedium (xylene).

The general function of an antemedium is the ability to be the "connection" between the dehydrant and the wax (paraffin) because it mixes with both. Xylene (as well as white naphtha or some other aromatic chemicals) can do this, BUT the thing is that mineral oil (MO) is paraffin of a low molecular weight so the antemedium is not needed or a mixture of MO with alcohols constitutes the antemedium.

My procedure uses ethanol to dehydrate and later the antemedium is substituted by the mixture of ethanol + Isopropanol + MO.

Maxim has simplified the procedure because he processes manually and doesn't have the advantage of vacuum, pressure or agitation as I did when developing the method. So it turns out that Maxim's modification is easier and more direct; he just dehydrates with propanol and later goes into the gentle substitution with a mixture of 5 parts of propanol + 1 part of MO heated at 50ºC followed by another mixture of 2 parts of propanol + 1 of MO heated also at 50ºC to obtain the gentle and complete infiltration of ANY type of tissue.

The infiltration with MO gives the tissues softness never achieved with any other antemedium. It also meets the objective of eliminating xylene from the histology lab. The procedure uses two chemicals that are cheaper and when needing to be disposed off, the propanol can be evaporated. The used MO mixed with used paraffin and both disposed of as a solid, cutting costs in disposal.

But let us look at the pre-fixation and fixation issues concerning breast processing.

As a result of the American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 (HER2) Testing in Breast Cancer (1), pathology laboratories must ensure that all breast excision specimens subject to HER2 testing are fixed in 10% neutral buffered formalin for 6-48 hours, and that core biopsies are fixed for at least 1 hour. Alternative fixatives must be validated to ensure that their "performance against the results of testing of the same samples fixed also in buffered formalin and tested with the identical HER2 assay, and concordance in this situation must also be 95%. They also require that time to fixation and duration of fixation if available should be recorded for each sample.

Delay to formalin fixation may invalidate hormone receptors and HER2 analyses. Invalid results of tumor markers could significantly alter the type of adjuvant therapy a patient receives and potentially impact outcome. Khoury et al (2) studied the effects of progressive delay to formalin fixation on breast cancer biomarkers. They found that the mean Q score started to decline at the 2 h mark for estrogen receptor and 1 h mark for progesterone receptor. The lowest score was at the 8 h mark for estrogen receptor and overnight for progesterone receptor. HER2 fluorescence in situ hybridization started to be compromised for interpretation at the 1 h mark and became statistically significant at the 2 h mark ($P<0.03$). To avoid delay to formalin fixation as a factor negatively affecting on breast biomarkers, they recommend not to delay formalin fixation for more than 1 h and not to store specimens overnight.

Willmore-Payne et al (3) have found that an increasing number of breast biopsy specimens are being fixed in formalin substitutes. They tested 6 non-formalin-based fixatives to determine their impact on FISH testing for Her-2/neu gene amplification status by comparison with formalin-fixed control specimens from the same neoplasm. Specimens fixed in Pen-Fix, Prefer, Histochoice, UniFix, and GTF were associated with absent or technically compromised staining in at least one of the 3 neoplasms tested for each fixative when compared to the formalin-fixed control. O-Fix did not seem to compromise staining quality in 3 paired specimens tested.

Fritzsche et al (4) were concerned with the safe processing of potentially contaminated tissue material with Creutzfeldt-Jakob disease and other prions. Formic acid pretreatment is considered to be effective in prion inactivation. They evaluated the c-erbB2 and the hormone receptor-status in potentially prion infectious breast cancer tissue after pretreatment with formic acid. Paired breast cancer tissue samples were immunostained with commercially available antibodies against c-erbB2, estrogen receptor, and progesterone receptor with 1 tissue sample of each pair being pretreated with 98% formic acid. Staining was evaluated either according to the HercepTest score or using an immunoreactive score. Additionally, fluorescence in situ hybridization (FISH) analyses were performed for 7 of these cases. Untreated tissues showed strong circumferential staining for c-erbB2 (HercepTest score 3+), whereas the membranous staining of the tissues pretreated with formic acid was significantly weaker. FISH analyses showed no differences in both groups. The hormone receptor expression was not significantly influenced and positivity was maintained in all cases. In breast cancer

patients, the pretreatment of tissue with formic acid for prion-decontamination in the case of suspected Creutzfeldt-Jakob disease or other prion diseases can lead to underestimation of the immunohistologically determined c-erbB2 status. In these cases, a c-erbB2-FISH analysis should be performed. For the immunostaining of hormone receptors in breast cancer, formic acid pretreatment can be applied without negative effects on the sensitivity or specificity of the assay.

References:

1. Wolff et al (2007) "American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer" Arch Pathol Lab Med. 131:18–43.
2. Khoury et al (2009) "Delay to formalin fixation effect on breast biomarkers" Modern Pathology 22: 1457–1467.
3. Willmore-Payne et al (2007) "Effects of Fixative and Fixation Protocols on Assessment of Her-2/neu Oncogene Amplification Status by Fluorescence In Situ Hybridization" Applied Immunohistochemistry & Molecular Morphology. 15(1):84-87.
4. Fritzsche et al (2006) "Tissue Pretreatment With Formic Acid Might Lower HercepTest Scores in Breast Cancer" Diagnostic Molecular Pathology 15(4):237-242.

Histogroup is all at sea – Report on Christmas Meeting.

Some 30 members and guests met for our Annual Christmas Function which was held at North Ryde RSL Club on 27th of November 2009.

Last year we were "Star Gazing" with Dr Richard Jaworski. This year we were "Nautical" with Alan Edenborough a project director and specialist consultant to the "Sydney Heritage Fleet". Alan is "Saving Ships with a little Science".

The "Sydney Heritage Fleet" is a living museum of working vessels. Alan was directly involved in locating, salvaging and the restoration of the 1874 barque "James Craig". Alan took us through the processes involved from beginning to end. There was a lot of scientific and detective work, and a remarkable feat of restoration.

In this living museum there are other unique vessels such as the "Waratah" (1902 coal fired steam tug) and the "Lady Hopetoun" (1902 VIP steam launch). Vessels under restoration include the "John Oxley" (1927 coastal steam ship) and the "Kanangra" (1912 Sydney Harbour ferry).

A very interesting and informative presentation. A good night was had by all.

Trevor Hinwood.

Histograph is expertly printed by:

Guidelines for the Burial of Human Remains

I recently received a request as to what the procedure is for allowing the burial of fetuses (under 20 weeks) by parents in their own backyard or possibly, by inference on Crown land (ie not in a cemetery). I was especially concerned with the infectious risk (see PUBLIC HEALTH (DISPOSAL OF BODIES) REGULATION 2002 - REG 22 paragraph 2). The following was received from Neil Shaw, Manager, General Environmental Health, NSW Department of Health:

"The Public Health (Disposal of Bodies) Regulation 2002 does not apply because the foetus is not registrable as a birth under legislation. Your reference to clause 22 is appropriate but was designed to prevent burial of larger remains, which take considerable time to fully decompose, on suburban and semi-rural properties without forethought.

Technically the foetus is "clinical waste" but does not represent a suitable outcome for some people and how they handle the grieving process. We do not encourage disposal of the foetus at home, nor on public lands without approval. The parents need to think it through particularly as to what happens when they move. Some cemeteries, I understand, are happy to accept human tissue for burial and allow for a memorial if that is their wish.

However, there are many small animals buried in backyards all over metropolitan areas.

It becomes a sanitation issue and the foetus needs to be disposed without causing a public health risk or nuisance. We do not consider that a single foetus poses a public health risk and the procedure you suggest is excellent. We do know that products of conception decompose much more rapidly that child or adult human tissue and given the small amount of tissue (<400g) it should decompose rapidly particularly when in contact with the soil. A period of six months has been suggested for complete decomposition.
I would suggest that burial occur not in a shoe box and as deep as practical to prevent insect and rodent attack. Some topsoil (from the top 100mm of soil as it contains microbes which aid decomposition) be placed immediately around the wrapped tissue and then a barrier such as a piece of fence paling be placed over the tissue. This will minimise rodent attack and also minimise the escape of decomposition odours. I have seen a reference which suggests that rodents can burrow up to 450mm in disturbed soil."

The current Children's Hospital at Westmead policy is as follows:

1. Rinse the fetus in gentle running water for 10 minutes or so
2. To 500ml of tap water, add 5ml of ammonia and leave fetus for 5 minutes or so (helps neutralise the formalin smell)
3. Remove the fetus and pat dry with absorbent paper
4. Place in a sealed biodegradable bag
5. Place bag in a shoe box appropriately covered with paper
6. Seal the box with tape
7. Hand the box over to the patient's representative with a copy of this policy and the NBF MSDS.
8. Ensure that the box is buried at least 2 feet below the surface.
9. It is a good idea to plant a small tree or bush above the site (the formalin breaks down to methenamine - a fertiliser, and with the phosphate salts present in the NBF tends to cause the plant to thrive).There is no risk of animals digging up the formalin fixed fetus, since they find the formalin smell too obnoxious and will leave the area alone for several years - an unfixed specimen would be a different matter.

Is Lymphocytic (Hashimoto) Thyroiditis Associated With Suicide?

Stephen J. Cina, MD; Joshua A. Perper, MD, LLB, MSc
The American Journal of Forensic Medicine and Pathology:
September 2009 - Volume 30 - Issue 3 - pp 235-237

The histologic diagnosis of lymphocytic (Hashimoto) thyroiditis requires lymphocytic inflammation of the thyroid gland in combination with Hurthle cell metaplasia of follicular epithelial cells. Clinically, this autoimmune process has been associated with hypothyroidism and psychiatric conditions including depression. This retrospective study was designed to quantify the incidence and severity of lymphocytic thyroiditis in a series of non-consecutive suicides compared with a cohort of motor vehicle accident victim controls. Eighty-one suicide victims (61 male, 20 female; age range 13-79 years, average 43) were compared with 88 age and gender matched controls (64 males, 24 females; age range 19-85 years, average 36). The degree of lymphocytic inflammation of the thyroid gland was graded on a scale of 0 to 3 (0 = no inflammation, 1 = mild inflammation, 2-3 moderate-to-marked inflammation with Hőurthle cell metaplasia). Slides from each case were reviewed while blinded to the cause and manner of death in each case. Of these 169 total cases, 8 (4.7%) received a score of 3, whereas additional 7 (4.1%) received a grade of 2. Eighty-six percent of all of the cases showed no significant inflammation and recorded a score of 0. Of the 81 suicides, 3 had a score of 3, and 3 had a score of 2 (combined incidence of 7.4%). Within the control group, 5 of 88 cases scored 3 and another 4 scored 2 (combined incidence = 10.2%). Three males and 5 females scored 3 with an age range of 23 to 63 years, average 42. Incidental data tabulated showed that 19% of suicide victims were on psychoactive medications compared with 6% in the motor vehicle accident control group. No one on this study was on thyroid hormone replacement therapy. Depression is strongly linked to suicide and lymphocytic thyroiditis may be a cause of depression. Based on this study, however, the presence of lymphocytic thyroiditis cannot be used as a histologic adjunct to discriminate between suicide and accident in problematic cases (ie, overdose deaths). This diagnosis is rendered with essentially equal frequency in suicide victims versus controls.

S.O.S: save our slides. A technique to salvage sections adhering to detached plastic coverslips

The automated cellophane coverslipper, when it first appeared, appeared to be a godsend. Reducing manual coverslipping and reduced xylene exposure hazards being its biggest advantage. Unfortunately with time plastic coverslips often detach, taking tissue sections with them and leaving behind drawers of blank slides and loose coverslips. Each lost slide may create a medico-legal risk or further deplete a teaching slide collection. Lountzisa & Elstona (Journal of Cutaneous Pathology 2009: 36: 1227–1228) have described a technique to salvage detached or detaching slides. Because of its uniqueness and importance, I have summarized the article as follows.

Step 1: Identify the tissue on the detached plastic coverslip and cut away as much of the surrounding plastic as possible. Leave enough space so that the plastic coverslip can be picked-up with forceps. Maintain orientation as to the top and undersurface of the coverslip as this is essential for proper re-adhesion of the section.

Step 2: Place the newly cut plastic coverslip with tissue section on the desired location on a fresh glass slide. For partially detached slides, isolate the tissue on the slide using a scalpel blade and remove the surrounding plastic coverslip.

Step 3: Take a dropper full of acetone and place five to eight drops over the top of the plastic-covered tissue section. Continue to add acetone to keep the plastic-covered tissue section immersed, as the acetone will quickly dry before proper separation occurs. It may be best to perform this on a shallow glass plate or proper work area in case spillage should occur.

Step 4: Wait 3–5 min for the acetone to degrade the plastic coverslip. This will be evident as the plastic coverslip begins to wrinkle. This will separate the coverslip from the tissue, and the tissue section will adhere to the new glass slide.

Step 5: Carefully remove the softened plastic coverslip from the glass slide with tweezers. Caution should be taken as the tissue may be loose and folding may occur. Adhesion may be improved by using a adhesive pre-treated glass slide.

Step 6: Remove any excess plastic debris by placing additional acetone on the slide or gently washing in an acetone bath.

Step 7: At this point, the slide can be de-stained and re-stained if needed as many detached sections also lose colour intensity. Re-staining should be done by hand because automated staining may remove the loose tissue sections.

Step 8: The slide can finally be re-coverslipped with DPX and glass.

The article has accompanying colour photographs that clearly show the procedure and I highly recommend that you obtain a copy.

I have found the Journal of Cutaneous Pathology to be an excellent journal and it especially has some interesting technical articles.

Chairman's Report AGM August 2009

It has been a busy and successful year.

We have held seven group functions:

- "The Pathology of Skins ", Dr Vicky Howard, AGM, North Ryde RSL Club.
- "What is PCR and how does it correlate with Histology ", Neisha Jeoffreys, ICPMR, Westmead.
- "Pancreatic Research, where this research is currently and future directions ", Dr David Chang, The Garvin Institute of Medical Research.
- Newcastle Scientific Meeting, hosted by the Histology members in Newcastle, The Adamstown Club.
- Christmas Function, "Join the Stars "[Astronomy orientated], Dr Richard Jaworski [Pathologist, DHM], North Ryde RSL Club.
- "Prostate Update ", Dr Warwick Delprado [Pathologist, DHM], Douglass Hanly Moir .
- "Is breast cancer sexually transmitted [by HPV]? ", Dr Jim Lawson and Dr Noel Whittaker [University of NSW], Douglass Hanly Moir.

The presentations and functions this year continued to be well supported. Although we are regularly having around 40 people attending these meetings, we would like to see this number increase even further.

Special mention needs to be made of the Newcastle meeting which was held in November last year. The meeting was well organised with Tony Chow [Nextpath] being the master of ceremonies. It was a busy day with six medical speakers presenting a range of topics including; reconstruction surgery, ophthalmic surgery, forensic pathology, anatomical pathology and retirement after pathology.

Thankyou to the Newcastle organisers for a great day, we certainly want to do this again in the future. It would have been good to see more people from Sydney make the journey to Newcastle, maybe next time.

At the last " National Histology Conference " in Adelaide, the decision was made to have the next " National Conference " in Sydney in the latter part of 2011. Our committee has already commenced investigating possible venues and is also looking at possible guest speakers.

With some experienced conference organisers on our committee we are confident that we can produce a conference that will equal or surpass the high standards set by previous conferences.

Tony Henwood, our editor, continues to quietly put in the hard work producing the "Histograph "which continues to be an important part of communication with our members. We would also like to thank the companies who continue to support the "Histograph "with advertising.

Our redesigned website [www.histonsw.org.au] was completed earlier this year following a lot of work by Margaret James. The Website will continue to be developed and we see this as another important communication tool with our members.

A concern at the moment is the renewal of membership. We realise it is easy to forget to complete the paperwork and send it in. The fact is we really do need you to renew for this coming year so we can fund and support the meetings that you attend. Remember, membership funds are our main form of revenue raising.

While mention has been made of several committee members, I could mention others who have given a great deal of their time to ensure the group continues to grow and flourish. So thank you to all the committee members for their support over the past year.

We again need to thank Douglass Hanly Moir Pathology for their support in making rooms available for committee meetings and some of our functions and presentations.

We look forward to your continued support in the coming year.

Trevor Hinwood.
Chairperson.
Histotechnology Group of NSW.

Russell Thomas Allison RIP

Many of you would have had the privilege of meeting Russ Allison a few years ago at our State Scientific Meeting. You would therefore be saddened to hear of his passing. The following obituary appeared in the British Institute of Biomedical Scientist's Journal (http://www.ibms.org/go/media-centre:monthly-comment).

The 100-year history of this Institute is decorated with the names of those who repeatedly gave more back to the profession than they received. Given the length of time involved, this list is now quite long, but, given the number of members who have come and gone, in reality it is quite short.

One of those names is Russell Thomas Allison, known universally as 'Russ'. There are few people in life who are recognisable by only one name, but Russ came close to achieving that distinction within biomedical science. It was with great sadness that Council noted recently the untimely death of Russ Allison. Russ was President of the Institute from 2002 to 2004 and during this period oversaw many of the changes that resulted in the reinstatement of our professional examinations. However, his main contribution now affects the professional life of all our members (including the President and Chief Executive!). It was due to Russ' foresight more than 20 years ago that the Institute became one of the first in the UK to introduce a Continuing Professional Development scheme.

Men of vision and commitment are not particularly common, but Russ ticked both boxes. His work at the University of Wales College of Medicine in Cardiff included developing practical facilities, supervising the training of undergraduates, and publishing research into histological methodology – a previously neglected area. In fact, his publications are legion. This led to invitations to lecture at various levels, from degree courses to international specialist meetings around the world. He was a senior research fellow on the academic staff of the University of Wales College of Medicine Dental School.

Russ joined the Institute in 1959 and quickly became a stalwart of his local IBMS branch committee (Cardiff), which he joined in 1963. He was at various times chairman, secretary and vice chairman of the branch, and was a member of the South West region committee for 36 years. It was as a regional member that he was elected to Council in 1984 and he served continuously in this capacity until 2005. His main interest and passion was for science and he will remain the Institute's longest-serving chair of the Science Committee. He served on the editorial board of the British Journal of Biomedical Science, *a publication that he supported passionately and to which he contributed significantly during its development.*

Russ had an easy-going manner, which made him many friends, and it was his ability to network that made him a key figure in the development of the IBMS Congress programme. Those who knew him personally will be aware of his passion for Welsh rugby, and the local amateur team that he championed was a major part of his life. Those who knew him really well will also have seen a softer side. He loved animals of all kind, but in particular dogs, and his daily 'walkies' were part of a routine that he loved.

Like others, I counted Russ as one of my friends, but within this profession there are many more who owe him thanks. The life of Russell Thomas Allison, within his chosen profession, really did make a difference.

Alan R Potter
Chief Executive

Safety Corner - Neutral Buffered Formalin Storage

Rebecca Riesen, from the NCH Healthcare Systems posted the following concern on Histonet:

> *We have been directed by our Safety Officer to store all formalin (37% and 10% NBF) in a flammable storage room, cabinet or container. Yes, 37% Formalin we do store in this manner, but I have never heard of this requirement for 10%NBF. I looked on-line at many MSDS sheets from different vendors and found only one that stated such storage requirements for 10% NBF. During this search I found all but one company state that formalin is not flammable. I brought this to the Safety Officer. He agrees that it is not "flammable" but that it IS "combustible". Combustible=Flash point of 100°F to 200°F. Of the dozen sites I visited I found the following data concerning the Flash Point of 10% NBF: from "NA" to ">200°F" and "122°F to 185°F". The NFPA (National Fire Protection Agency) guideline is no more than 1 gallon in a flammable storage container and 1 gallon outside of a safety cabinet/container per 100 square feet is already quite limiting. Using this guideline, we have calculated acceptable volumes of the known flammables (Alcohols and Xylenes) we can store. Adding 10% NBF to the equation will have us travelling to our "bulk" storage area constantly. Does anyone out there store 10%NBF in flammable cans/cabinets?*

Boyd states that they only store 37% formalin in a flammable cabinet. Ten percent formalin is not considered a flammable substance according to the MSDS for Richard Allen 10% formalin. Your storage should be according to the manufacturer's MSDS. Grant it, they should be all on the same page since it is a concrete formula.

Rebecca received many responses and only one laboratory reported that they store all chemicals, including formalin, in safety cabinets. She noted that all MSDS's she studied stated that 10% NBF indeed is NOT flammable, but the Flash Point is under 200°F on all but one manufacturer's product. The NFPA99 11.7.2.3.1 and 11.7.2.3.2 rules on flammables storage include all Class I, II and IIIA liquids. Class IIIA liquids include those with a Flashpoint of less than 200°F. That would include 10% NBF. It appears to be that the formaldehyde fume (which we all know very well) is the combustible portion no matter if it is in 96.7% water. Most manufacturers' recommendations are for storage in a tightly sealed container, probably to keep those nasty fumes inside. One would think that would be sufficient.

Allen Smith reports that formaldehyde is flammable; formalin is not. Above 122°F enough formaldehyde evaporates from formalin to create a modest fire hazard in the fumes just above the liquid. Try this: pour 3 ml of formalin (37% formaldehyde) into a watch glass under a fume hood (fan off). Touch a match to it. The match will flare briefly in the fumes and that is all (unless the temperature is above 122°F).

Staining Paraffin Sections without Prior Removal of the Wax

John Kiernan in 1996 published an article in Biotechnic and Histochemistry (Volume 71, Number 6 pages 304 — 310) that attempted to answer a question that many of us have wondered about. I don't know how many times I have reminded trainees to dewax paraffin sections before staining. One asked me what stains would work on non-dewaxed sections. Ah, well, I googled and found this article.

John found it possible to apply aqueous dye solutions without first removing the wax.

The factor having the greatest effect on stainability was melting of the wax on the slides. No staining could be obtained at room temperature or at 45°C after a slide had been heated enough to liquefy the wax. Leaving slides on a hot plate overnight or longer at 50°C led to softening of the paraffin without liquefaction. The ribbons became wider and the wax separated from the edges of the tissue. If this happened, subsequent staining was slow and inconsistent. To avoid this problem, sections were flattened on water no hotter than 45°C and dried overnight at 40°C. Unsatisfactory staining was also observed if ribbons were mounted upside-down on the slides, with the shiny side up and the dull side on the glass, even though mounting and drying were carried out at low temperatures.

It is not surprising to find that melting or spreading of paraffin over a mounted section completely blocked the penetration of any dye solution in a solvent that could not dissolve wax. It was also necessary for the sections to be mounted with the dull side of the ribbon facing up. The surface of a paraffin section that has passed over the bevel of a microtome knife probably owes its glossy appearance to smearing of momentarily softened wax across the cut surface of the tissue. Consequently, the shiny surface obstructs the penetration of aqueous reagents. The angle between the face of the block and the bevel of the knife edge prevents polishing of the upper surface of the ribbon resulting in a dull surface. It is on this surface that aqueous reagents can come into contact with the embedded tissue.

Minor technical modifications to the staining procedures were needed. Mercury deposits were removed with iodine, and a 3% solution of sodium thiosulphate in 60% ethanol was needed to remove the iodine from paraffin sections. The alcoholic solution is made by adding 30ml of ethanol to 20ml of 5% aqueous sodium thiosulphate. At room temperature, progressive staining takes 10-20 times longer for sections in paraffin than for hydrated sections. At 45°C, this can be shortened to about three times the regular staining time. After staining, the slides are rinsed in water, air dried, dewaxed with xylene, and coverslipped in the usual way. Nuclear staining in the presence of wax was achieved with toluidine blue, alum-hematoxylin and Weigert's iron-hematoxylin. Eosin and van Gieson's picric acid-acid fuchsine were effective anionic counterstains. A one-step trichrome mixture (Gabe's one-step trichrome) containing 3 anionic dyes and phosphomolybdic acid was unsuitable for sections in wax because it imparted colours that were uninformative and quite different from those obtained with hydrated sections.

We are familiar with immersing trimmed blocks in water to improve the sectioning of objects containing tough materials such as dense collagen and over-dehydrated (brittle) tissues (eg spleen and thyroid). The water enters and softens the dehydrated tissue despite the presence of wax. The entry of water indicates that the hydrophobic embedding medium does not displace all of the water originally present in the tissue.

Advantages of staining in the presence of wax include economy of solvents, reduced risk of overstaining and strong adhesion of sections to slides.

Plants in the Histology Lab

In my visits to Histopathology laboratories, it is interesting the number of labs that have a healthy range of indoor plants flourishing on benches, shelves and in windows. A list of air filtering plants was compiled by NASA as part of the NASA Clean Air Study, which researched ways to clean air in space stations. As well as absorbing carbon dioxide and releasing oxygen, as all plants do, these plants also eliminate significant amounts of benzene, formaldehyde and/or trichloroethylene.

Wolverton et al (2) found a 50% reduction in formaldehyde concentration with Scindapsus aureus and Syngonium podophyllum. They also found rapid decrease in formaldehyde concentration with Chlorophytum elatum (Spider Plant) from 14 to 2ppm in six hours and below the detection limit of 2ppm from 6 to 12 hours.

Researchers are studying the ability of plants to reduce formaldehyde levels in the air. A study led by Kwang Jin Kim of Korea's National Horticultural Research Institute compared the absorption rate of two types of houseplants. The results of the experiment on Weeping Fig (Ficus benjamina) and Fatsia japonica, an evergreen shrub, were published in the Journal of American Society for Horticultural Science.
During the study, equal amounts of formaldehyde were pumped into containers holding each type of plant in three configurations: whole, roots-only with the leafy portion cut off, and aerial-only, with the below-ground portion sealed off, leaving the stem and leaves exposed (1).

The results showed the combined total of aerial-only and roots-only portions was similar to the amount removed by whole plants. Complete plants removed approximately 80% of the formaldehyde within 4 hours. Control chambers pumped with the same amount of formaldehyde, but not containing any plant parts, decreased by 7.3% during the day and 6.9% overnight within 5 hours. As the length of exposure increased, the amount of absorption decreased, which appeared to be due to the reduced concentration of the gas (1).

Aerial parts of reduced more formaldehyde during the day than at night. This suggests the role played by stomata, tiny slits on the surface of the leaves that are only open during the day. The portion of formaldehyde that was reduced during the night was most likely absorbed through a thin film on the plant's surface known as the cuticle. Root zones of ficus removed similar amounts between night and day. However, japonica root zones removed more formaldehyde at night (1).

Researchers consider micro-organisms living among the soil and root system to be a major contributor to the reduction. Japonica were planted in larger pots than the ficus, which may account for the lower night reduction rate of the latter. More knowledge of the contributions of microorganisms is cited by the study to be important in further understanding the air purifying potential of plants (1).

Other beneficial plants that may be grown in the lab include, blue lungwort, dead man's fingers, mistletoe, cancer weed, devil's gut, bonewort, kidney vetch, liverwort and Hawaii birdnest spleenwort but please not marijuana plants.

References:
1 American Society for Horticultural Science (2009, February 20). Indoor Plants Can Reduce Formaldehyde Levels. *ScienceDaily*. Retrieved November 2, 2009, from http://www.sciencedaily.com /releases/2009/02/090217141419.htm
2 Wolverton et al (1984) Economic Botany 38(2):224-228

HISTOTECHNOLOGY GROUP of NSW

ABN: 63 128 868 343
nswhistogroup@bigpond.com

I wish to become a member of the Histotechnology Group of N.S.W. and enclose

PLEASE TICK: ☐ $38.50 for annual subscription of $35.00 and $3.50 GST.

☐ $16.50 for student subscription of $15.00 and $1.50 GST
(Full-time or working toward first qualification)

☐ $82.50 for company subscription of $75.00 and $7.50 GST
(2 representatives, one of whom must be a NSW representative)

Please make cheques payable to the Histotechnology Group of NSW
Or: I**nternet Banking**: BSB:**802 084**; Account number: **94099;**
Account name: **Histotechnology Group of NSW**.
******Reference: **[Name – for member identification]**

☐ RENEWALS ☐ ANY CHANGES TO PREVIOUS DETAILS.

PLEASE **PRINT** ALL INFORMATION.

SURNAME_____ GIVEN NAME_____

TITLE: MR, MRS, MS, DR, MISS. (Circle one)

OCCUPATION_____ POSITION_____

INSTITUTION_____ DEPARTMENT_____

ADDRESS for CORRESPONDENCE:

STREET/P.O.BOX._____

CITY,TOWN,SUBURB,_____ POSTCODE._____

IS THIS ADDRESS HOME OR BUSINESS ? (Circle One).

PHONE No. WORK_____ EXT_____ HOME_____

E-MAIL ADDRESS: _____

2ND COMPANY CONTACT

SURNAME_____ GIVEN NAME_____

TITLE: MR, MRS, MS, DR, MISS. (Circle one)

POSITION_____ INSTITUTION_____

ADDRESS STREET/P.O.BOX._____

CITY,TOWN,SUBURB,_____POSTCODE._____

PHONE No. WORK_____ HOME_____

E-MAIL ADDRESS: _____

SIGNATURE_____**DATE**_____

RETURN TO:

SECRETARY
HISTOTECHNOLOGY GROUP of N.S.W.
P.O. BOX 496
GUILDFORD NSW 2161

Office use only

Receipt	
Recorded	