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Newsletter of the Histotechnology Group of NSW

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Editorial

Well we made it through the National Meeting and Christmas.

I am totally amazed at how the meeting went so well. I unfortunately attended one of the organising committee meetings and half way through wanted to run away screaming. How can so few do so much in such a short period of time.

How's this:

- 24 participants for Penny's workshop
- 6 slots available in hypotheticals, all other workshops are full
- Parking available at Granville TAFE
- Tea, coffee from Granville TAFE
- Morning tea sponsored by Leica at Granville TAFE from TAFE canteen
- Registrations and satchels at Granville by Julie Bilkey& Bharathi Cheerala@ 8am. Contact Sandra Pitchfork
- No T-shirts for delegates registered for only workshops, they buy them at conference on Saturday.
- Kathy Drummond is sent letters to all participants, still need to send parking details
- Aprons and gloves- Julie Bilkey
- Boxed lunches for participants, \$15/box, Julie, Bharathi and Nicole to distribute to delegates attending workshops at TAFE.
- Bus schedule: 8am, 12.45pm and 5 pm from Race course to TAFE. 57people per ride. \$660 per day
- Trevor to go on bus
- Site map to be put on website
- Signage to be taken care of Sandy at TAFE
- Dianne and Trevor to prepare commonly asked questions
- 210 delegates +50trade people +10 speakers
- 7.30am to 8.30am Leica breakfast meeting on Saturday
- Kathy and Lin taking care of registrations on Friday afternoon, Saturday and Sunday
- Natalie to liase with Kathy for lanyards
- Dianne, Margaret and Nicole will be the main information group at Rose hill
- 20 different coloured lanyards from Leica for committee members
- Names to be sorted as alphabetically for registration

And this was from part of one meeting – I am in AWE of our Organising Committee

See you all soon.

Tony Henwood, Editor anthonyh@chw.edu.au



Chairman's Report

We are already into the New Year, does not seem that long ago we were busy with the National Histology Conference. Speaking of which the feedback we have received has been very positive. The committee took on organising the conference, a big job. They need to be thanked for the many hours of work that made the conference what it was, great. Some general comments:

The workshops on the Friday were well received and went beyond our expectations. We had planned on one Surgical Dissection workshop of 12 participants. This filled very quickly so we rearranged our other workshops to add in a second workshop which also filled very quickly and unfortunately we could then not accept further bookings. Two practical Histochemistry workshops were held simultaneously with the Surgical Dissection workshops in the laboratories at Granville TAFE. These workshops were fully subscribed with 30 people in each. The Histo Hypothetical workshop at Rosehill Gardenshad 60 people attend.

Our speakers covered a wide range of topics to ensure there was something of interest for everyone. They were very complimentary of our organisation and members.

We received many complimentary comments about the venue, facilities and staff. The venue has also congratulated us on the running of the conference and the way our delegates conducted themselves, they were very impressed.

There were 23 exhibition booths. A lot of interest was shown in the exhibition; a number of

companies went to a lot of trouble and expense to provide impressive displays. Their support is crucial in making a successful conference. Three companies held user meetings during the conference which were well attended.

We had approximately 260 people attend [delegates, trade and speakers]

It has been confirmed the next National Conference will be hosted by the Histology Group of Victoria in 2013.

Where to from here? Workshops are now an important component of conferences. As a State group we should look at holding workshops during the year as well as our night time meetings. In organising conferences the cost needs to be kept down to maximise the number of attendees. This means the organising committee doing some/ the entire organisation.

This year is already looking busy for the NSW group. We have held our first night time meeting, "Establishing Histological Tissue Parameters of normal Skeletal muscle for a better diagnosis" presented by Dianne Reader.

Future meetings planned are; Sydney University Vet School, Tony Henwood to redo the Hypotheticals workshop, meeting at the "Millennium Foundation" re tissue banking and meeting at the NSW Dept of Agriculture laboratory complex at Menangle. We are also looking at holding several workshops during the year.

Cheers,

Trevor Hinwood. Chairperson. Histotechnology Group of NSW.



Management Corner - Music in the Laboratory

Dorothy writes:

I would like to know what other histology laboratories allow for music players while working. Do you have formal policies about music content or volume?

Do you allow lab space doors to remain closed to muffle the volume of what is being played?

Are headsets allowed?

I personally prefer to work in a quiet environment. I am trying to be open minded, as long as the work gets done. However, one of the techs had a song playing today that I believe was inappropriate for general listening in the lab. Am I just out of touch? Is that dang "F" word just something I'm going to have to learn to accept?

Do you have a written policy? When/how/why was it implemented?

I should mention that it's a small private lab, with minimal patient traffic.

DC:

The "F" word, among several others is not appropriate in a workplace. It's not appropriate anywhere, but we have control over the workplace. I have never, and will never allow inappropriate music at the workplace. The best way to prevent it, without your staff claiming "prejudice" as they so love to do, is ban music in the lab altogether. This is work, not a party.

AG:

I have worked in labs where music was played and loved it. Music keeps you going all morning and even in the mid-afternoon when you hit that "slump" time. If it is not inappropriate music or loud enough to be distracting when someone comes in with a question or when the phone rings I don't think it is a problem.

One of my pathologists always listened to conservative talk radio and turned me on to it so being as how I'm lucky to usually be the only one in my lab I've become a talk radio junkie when I'm not listening to a novel on my iPod.

KD:

Music is a great stress reliever as well. I've always let the group decide if they are all willing to tolerate other kinds of music to be fair. I always ask them to try to keep it clean. Don't want to offend. With that being said, I myself have had to tone it down because I love me some disco music.

Yes, ring my bell and shake your groove thing lol

Without further silliness, music is great in the lab if you can get the people to agree on compromise. If you have someone you know is very strict and you know Katy Perry's 'I kissed a girl' song is going to send them over the edge. Don't do it. Let them use headphones. Because you wouldn't want someone claiming harassment.

GL:

It would be fine, if there could be found a compromise. We have the radio on during cutting time, but it is quiet enough not to "overblow" work. And in the minutes nobody talks it is quite amusing to listen daily infos and music.

And yes, also for me it would be a pain to listen to "hard" music at work all the time.

On the other hand, a pathologist in our department loves classic music at the microscope. - That's also too much for me.

TP:

I have always allowed music to play at work usually left on a radio station since it will keep us updated on the day's information and leave the more offensive language out. I bought the department a system that will play cd and iPods'. The only rule is while playing our iPods', the songs with the strong language must be skipped.

KH:

I could not work without music. Nothing like cutting to the rhythm of good music. I have it on all the time or I would go nuts and probably fall asleep. I set my radio to one radio station. I am the Chief Cook and bottle washer so no one to bother with the music. If someone wants to play hard core obscene music let them put it on their Ipod so not everyone can hear it. I would make a rule that they

have to be able to hear if someone is talking to them.

CG:

Our lab is governed by hospital regulations that no headphones or ear buds be worn anytime. This is considered a safety issue. We do have policies governing codes for behaviour such as dress, hygiene, inappropriate computer use such as streaming music or social media but music is something they allow individual departments to dictate. Our lab has decided to allow desk top radios to be played. I have a few techs that like to listen to talk radio or music so they each have their individual radios set so low that only they can hear it. I think if everyone had their own radio it would be insane but a couple is not too bad. If it were to become distracting or a nuisance, I would ban them completely. We are not in a patient traffic area but we do get a lot of outside visitors walking thru such as Resident's interviewing or clinicians. One must always consider patient care first and foremost and if music is offensive to you because of language then it probably is offensive to others as well. I think you should never have to endure music that is offensive in anyway. Jazz makes me crazy. My question to you would be, do you have a policy stating that music is allowed?

ES:

I am lucky enough to work in a closed lab setting so we can play whatever, until the boss comes in (she can't concentrate with music on). If I couldn't listen to something while sectioning, I think I might die of boredom. But forcing other people to listen to your music is never good, even when you're with friends. If everyone doesn't like it, pick something that everyone does like.

TR:

Regarding the offensive language, many institutions have policies that address the use of inappropriate language. You may find it in something like a "Workplace Harassment/Violence" policy. We too do not permit ear buds or headphones for the same reason.

JS:

Our policy is that if a radio station is to be played in the lab, the choice has to be a unanimous one of everyone working in the lab, it cannot be talk radio (prone to political leanings), it has to play appropriate music for work setting, and it has to be turned down low enough to almost not be audible. Personal iPods with only ONE ear bud used is preferable to a room radio station.

TH:

I suppose naughty words have appeared in songs for years (Chuck Berry's "My Ding-a-ling" must have been a little risky - or is it just my FF (formalin fixed) mind!)

We often have the radio on and one day the staff, especially the young ones, thought I was "quite hip!" as I was bopping along to a song that seemed to contain naughty words. Fortunately I had no idea what the words to the song were (I needed subtitles) - and that seems to be the case with most of them now.

My hearing is probably shot from too much Deep Purple, Black Sabbath and ACDC anyway!!

TG:

I got a pair of Bose speakers for the lab - I like to listen to quality sound while I work and we bring in ipods, phones, whatever. But, everyone has the option of nixing any music selection at any time or opting for no music. Depends on the mood of the lab on any particular day.

AB:

I am in the same boat as those that would go nuts without music. I have noticed that I cut a *heck* of a lot faster with music than without. It's a rhythm thing, I can't really explain it. I really can sympathize with those that can't tolerate certain types of music though. Having one tech happy and another with a headache is not helpful or productive. Not everyone will agree on good music though, so you need to try to be versatile and share the air.

For open air music (not personal music with headphones) I like to stick to music that would be played on popular radio stations. That way the FCC can determine what's appropriate. Admittedly that's a bit risky if you have been following the court case about indecency fines, but that leads us to politics and that's likely to draw fire as well. Neutrality is best, NPR works well there. Radiolab was a great suggestion! Thanks for that. If anyone has other good podcasts, please share.

I like headphones, but if it interferes with communication or productivity it's gotta go. I don't

like repeating myself and it is just rude to everyone around you.

Yes, those who manage the lab have the right and responsibility to control the environment and make it conducive to good work practices. Please also consider the other side -- the techs who do work better with a little music (some of us ADD-prone bench folks).

CK:

If you're in a high-volume lab and have a bunch of smart techs, sometimes music keeps the 'drama' down. Put a bunch of smart techs on a highly repetitive bench and we find ways to entertain ourselves! Music can help by keeping a not-fully-occupied brain entertained. By limiting the open area radio stations to those without cuss-words and making volume levels 'personal' or allowing one ear-bud (not two: you have to be able to hear the work around you!!) is one way. Having a rotation of who gets to pick the radio station (taking turns) and having a universal policy is another. Be sure to address those tasks that may be uber-repetitive but require high accuracy for which radios are a conflict such as dictation and specimen entry.

Cutting off radios after they've been in place can be counter-productive. Another way is to ask your techs to come up with a policy that meets their wishes without compromising the needs of the work. Sometimes they'll come up with answers that surprise and really do addresss the work related issues: one group came up with a common radio on during peak cutting times and off during transitional task times so those who like quiet got their time, too!

SR:

I have always had music and encourage it. However, it must be music that ALL people can enjoy and has NO offensive material. If you can't agree....then headsets or nothing. BTW...if you use headsets, the policy should include that the volume must be such that the user MUST be able to hear and respond to spoken communication at all times. It's a safety issue.

GL:

In German-speaking Austria we have the advantage not to understand every word of the songs. So it can happen, that we sing out loud lyrics, that we never took in mouth in our mother-tongue. Sometimes funny, and sometimes much less stress.



Are You Interested in our Surgical Cut Up Wet Workshop 2013?

- Gain practical skills with ethically collected tissue
- Held on Saturdays 2nd March, 4th May, 6th July and 7th Sept 2013.
- Each session runs 10:00am to 3:00pm, morning tea and lunch included
- o Covers the 4 NPAAC guideline divisions:
 - simple transfers (biopsies, punches, cores etc.)
 - simple tissue (skins, gallbladders, appendix etc.)
 - moderately complex skins and organs (uterus etc)
 - complex skins and organs (breast, colon, prostate etc)
- Comprehensive workbook provided with detailed descriptive language
- o Folio generated from work completed NPAAC requirement
- Suitable for all staff, from Associate Diploma equivalency to Degree
- Must have minimum 12 months experience in Cut Up assistance
- Satisfactory completion of all 4 sessions = Certificate of Competency
- Held at Canberra Institute of Technology and run by Anne Prins and Penny Whippy
- Enquire now maximum intake 12 only 6 places already taken!
- Cost: \$110 per session or a total of \$400 if paid before first session.

Contact: Anne Prins 02 6125 4644 <u>anne.prins@anu.edu.au</u> Penny Whippy 02 6174 5667 <u>penelope.whippy@act.gov.au</u>

Safety Corner - Picric Acid - again

I am curious how big an explosion there would be from 1% picric acid in acetone if a little dried around the cap.

Story One:

When I was in college, a bottle of Picric Acid was vibrated off a shelf by an out of balance centrifuge. It was common for students to work with chemicals late at night when taking inorganic chemistry. The student loaded the centrifuge and left the room (went outside for a smoke), bottle dropped to the floor, exploded and left a 6ft x 8ft whole in the counter and wall and pretty much destroyed the 30ft x 30ft lab. Very lucky no one was hurt. At the time, I remember thinking; hey we will get a pass on the next assignment, only got two days off.

The University Safety team had an accurate listing of all chemicals on the shelves and determined it had to be the picric acid. Safety and the Fire marshal did a sweep of the university and found six other bottles in various labs on campus. Never did hear how they disposed and I bet that made a BIG BANG!!

Story Two:

Way back in 1976, I had just been disgorged from graduate school with a MS degree in microbiology, and I landed a job in a hospital lab in a Chicago suburb as the micro supervisor. Since I was the new guy, and no one else wanted the (unpaid) job, I was also appointed as Laboratory Safety Officer. One morning I sallied forth into the histology lab with my clipboard and flashlight to look for safety hazards. Everything was in good shape until I looked under a sink. There was a brown glass gallon bottle at the back of the

cabinet, which I dragged out and plunked down on the bench. The label was yellow with age (and the pigment of picric acid which had leaked from a small crack in the bottle.) The label identified the contents as liquid picric acid, which was now a single solid crystal, since all of the liquid had evaporated. It would have been about a half-gallon if it had still been liquid. I recalled my clinical chemistry class, in which we learned that the picric acid we used for serum creatinine was explosive in the crystalline state. I called the local fire department, and they were first concerned that we had suffered an acid spill, but I explained that this acid was a solid, but potentially explosive. Since there was no chemical spill, not they were concerned, and said they would get back to me.

About an hour later, the bomb squad showed up in full regalia. The fire department had looked up picric acid and found it was 2,4,6 trinitrophenol, a close relative of 2,4,6 trinitrotoluene (TNT). They evacuated that wing of the hospital (the entire lab and about 50 patients on the two floors above the lab), and carried the bottle of picric acid out in their bomb disposal device.

They detonated it in a field far away from the hospital by firing a rifle shot into it. It left a crater about 20 feet in diameter and ten feet deep. It was featured on the evening news by at least two of the Chicago TV stations. They had nice video of patients on gurneys being rolled down the halls, and a great view of the exploding bottle. Mythbusters could have learned from that video. Unfortunately, the hospital administration was not amused by the publicity, and we had to explain to multiple committees why we had such a hazardous substance in the lab.

The final comment on this incident is that the bottle had been under the sink for years. No one working in the lab at that time could remember when it was last used. This cabinet was where the histotechs stored their purses (back in the days when nearly all histotechs were female). They would come in at the beginning of their shift and toss (literally) their purses into the cabinet. Virchow be praised, they had never hit the bottle with enough force to detonate it.

Story 3:

The largest disaster I know of related to picric acid (among other things) is one that everyone working with it should keep in mind. The Halifax explosion basically leveled the city of Halifax, Nova Scotia, Canada in 1917. While they were carrying much more than the 500 ml to 1 gallon that we might use it is worth noting the magnitude of what this caused. There is a really good Wikipedia article on it here: http://en.wikipedia.org/wiki/Halifax_Explosion

This was a terrible disaster and it underscores why we need to be really conscious of the chemicals we work with, and even the ones we haven't used in years.

I would also like to play Devil's advocate here though. Yes there are inherent hazards with many chemicals we work with. But, we also need to be able use these chemicals in a safe manner. If used safely, these chemicals can be used for stains that cannot really be replicated with substitutes. Picro-sirius red is a good example of this.

The solution to hazardous chemicals is not getting rid of them and burying your head in the sand. It is education and understanding of the hazards and using them properly.

Story 4:

I whole-heartedly agree with and applaud with the above. I certainly would never discount the degree of danger with what is a high explosive and would take all due caution using disposal people who know what they are doing. But there is a use for the substance and one needs to separate mystery and uncertainty and incomplete facts and anecdote from actual fact. It is understanding while that crystalline picric acid might be unstable and shock sensitive (and a danger to a degree); it is the metal or salt picrates that are way, way more dangerous. Thus warnings in chemistry for picric acid; don't use metal spatula's. No metal cans or metal caps. Don't drop on concrete (silica and other things). Don't dispose down drain (lead or other metals). When viewing the chemistry guy in college who "blows up" a minute amount of picric acid on an asbestos pad over a Bunsen burner it is NOT pure picric acid but PA plus lead salt. While artillery shells might have been filled with picric acid they were relatively stable but they became way more unstable if the picric acid reacts with the metal casing or fuse casing (the infamous Halifax Explosion???). Thus to my utter dismay, a bomb disposal unit blew up a glass jar of dangerous picric acid in the field right next to a soccer goal post. As a former soccer goalie who used to dive all over the ground my question is; is anyone picking up the

thousands of shards of glass from the soccer field? And what about the dispersed picric acid since no chemical reaction is efficient and there must 100% dangerous picric acid (residue) all over the place. It is not well known but prior to the Trinity blast there was a lot of study of encasing the bomb in an enormous containment vessel called Jumbo. If the multi-firing shaped, pin, explosive nest surrounding the sub-critcal fissionable mass had misfired by even a millisecond, the core would have blown to one side instead of attaining criticality by being compressed. As there was very little fissionable grade material on the face of the earth at that point, they wanted to retrieve it "clean it from the environments and re-use it" by containing it. What if they had used Jumbo (it still sits, unused hundreds of yards away from detonation point), while most of it would have vaporized in the successful test, might there not be shards of highly radioactive bits of metal scattered and raining down for hundreds of square miles including on sports fields near towns. Where soccer goalies play?

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National Meeting Abstracts

Scientific Cut-Up for Technicians

Penelope Whippy and Anne Prins

For many years cut-up has been the province of registrars in the hospital setting and pathologists in the private sector — with scientists/technicians performing simple specimens and transfers. This has changed over the last few years as cost efficiency and increased workload for registrars have inclined both public and private pathology to look at the alternative of scientists/technicians performing the bulk of cut-up.

In line with the four tiered NPAAC Surgical Cut Up for Technicians policy Anne Prins and Penny Whippy have put together a **practical workshop** approach to surgical cutup that can be taken back to the laboratory for refining to suit the individual laboratory's requirements.

In this wet workshop, the policies, requirements, and techniques will be discussed and then a number of simple to complex specimens will be available to practice those techniques — under the guidance of Anne and Penny.

Histochemistry Workshop

Linda Prasad and Tony Henwood

Histopathology Department, The Children's Hospital at Westmead

Scientific Consultants: Karen Walker (TAFE Granville), Nicole Mackie (University of Sydney)

With the support and technical resources of HD Scientific and Thermo Fischer

The aims of this workshop are to:

 Introduce some novel histochemical stains that hopefully you have not previously used and might be useful.

- Experience microwave technology in order to decrease turn-around times.
- Do these stains xylene-free!

We do not claim these techniques to be better than what you currently use but the intention is to offer alternatives that, following modification, might be beneficial.

Histo Hypotheticals

Tony Henwood

Histopathology Department, The Children's Hospital at Westmead

Scientific Histopathology has developed greatly in the last ten years. We have seen an exponential growth in proteins demonstrable using immunohistochemistry. We have developed more rapid processing methods, and expected a higher quality in our microscopic product. As well as this we have been made aware of more stringent occupational, health and safety requirements. AND Accreditation is always just around the corner.

We are confronted with problems caused by preanalytical, analytical and post-analytical variations. This will tax our scientific knowledge (and dare I say prowess?).

How are we to meet these challenges?

This interactive workshop presents problems that affect the quality of our results and participants will be encouraged to advice as to a suitable course of action. With each case, the current research and developments will be presented.

The Xylene free Histology Laboratory

René J. Buesa

The mass production of tissue processors in the 1940s resulted in a standard protocol with xylene as ante-medium which eliminated the use of many very toxic "clearing" agents but by late 1970s there was evidence that its acute neurotoxicity was greater than that of benzene. This determined that many chemical companies started developing xylene substitutes for tissue processing even when the staff is more exposed while staining.

The participants will receive information about 22 D-Limonene and 35 alkane-based substitutes along with the possibility of totally eliminating "clearing" and "ante-medium" agents. The experimental validations of alcohols and mineral oil mixtures as clearing-ante-medium, now standard procedure of several histology laboratories, will be presented and discussed.

The use of dishwasher soap solutions to dewax sections before staining and oven drying the stained sections before coverslipping are two additional techniques now standard procedure in some histology laboratories also.

Cleaning tissue processors and metal embedding molds with a strong glassware laboratory detergent will eliminate xylene from this task.

These four methods will transform the histology laboratory into a xylene free environment, with significant health benefits and reduction of operations costs.

The causes of the resistance to the implementation of these techniques will be discussed with the participants.

Molecular Pathology for patients with Chronic Myeloid Leukaemia

Dr Susan Branford

Department of Molecular Pathology, Centre for Cancer Biology, SA Pathology and School of Medicine, Adelaide University, Adelaide, Australia

Treatment outcomes for patients with chronic myeloid leukaemia have improved substantially over the last decade with the introduction of targeted therapy. The disease is caused by the constitutively activated BCR-ABL1 tyrosine kinase. The natural course of the disease is presentation in a relatively benign chronic phase, which progresses to an invariably fatal acute leukaemia after approximately 3 to 6 years. The first kinase inhibitor of BCR-ABL1 was imatinib, and most patients respond very well to the inhibitor. The survival for patients who achieve a major molecular response, which is approximately a 3 log depletion of the leukaemic cells, is predicted to be near normal. The success of imatinib was the culmination of 40 years of research, which involved careful elucidation of the mechanism of disease activation. Once the cause was known, development of targeted therapy followed. Although most patients respond very well to imatinib, approximately 20% develop drug resistance. The most common mechanism of resistance is mutation within the BCR-ABL1 kinase domain, which interfere with imatinib binding. More potent inhibitors are now in use for patients with resistance. Imatinib was one of the first targeted therapies to be used. Research is ongoing to identify other molecular targets, and many targeted therapies are now in use for solid tumours and other malignancies. Molecular monitoring for patients with CML provides an example for the development of similar methods to monitor molecular targets of other inhibitors.

Basal Cell Carcinomas in Australia

Dr Geoffrey O'Brien

Basal cell carcinoma in Australia may well be the condition presenting the biggest workload to histopathology laboratories. In its various guises, basal cell carcinoma is at times a diagnostic challenge demanding the utilization of the complete skills of the laboratory team in providing certainty in identification, orderly blocking of specimens, processing, cutting, staining, immune-profiling and in microscopy.

I present some of the experiences of my 30 years of reporting these tumours.

Acid-Fast Staining: Separating the Sprinters from the Stayers

Colin Gordon

The first description of acid-fast (AF) staining was promulgated by Ehrlich in 1882. A number of improvements to this technique were elucidated by various individuals before a method attributed Ziehl and Neelsen gained widespread acceptance. Over time many variations of the Ziehl-Neelsen (ZN) have been espoused to enhance selectivity, sensitivity and robustness of the stain or to provide a simpler, safer, rapid, more cost effective result. The nature of the clinical material can also influence staining procedures. Regardless of approach, the ability to achieve standardised staining in ZN based methods is often complicated by the diverse nature of the "basic fuchsine" dye powders. Basic fuchsine is the collective name for any combination of pararosaniline, rosaniline, magenta II or new fuchsine. Manufacture of such dyes involves complex organic chemistry which yields powders in which the coloured and noncoloured components can be variable and often poorly defined. The impact of this variability is often underestimated and still not well understood.

In developed countries, basic fuchsine powders or their derived staining solutions have undergone external or "in house" quality assessment. In contrast, basic fuchsine powders used in "resource challenged" settings are often derived from unreliable sources and rarely or never undergo quality assurance testing. The failure of such powders to perform can have significant impact in national screening programs for detection of Mycobacterium tuberculosis, whose detection is a requirement for initiating treatment. It is critical therefore to utilise simple, cost-effective staining methodology to achieve robust, selective and sensitive acid-fast staining. In these settings, methods based on the original Ziehl-Neelsen are still favoured. Thus, the selection of appropriate dye powders is critical to successful outcomes.

The Marriage of Orthopaedic Pathology with Clinical and Radiological features

Drs Fiona Maclean, Julie Schatz and Richard Boyle

To report orthopaedic pathology well, careful clinical and radiological correlation is often required. This concept will be illustrated along the lines of that old marriage adage - something old, something new, something borrowed, something blue. A selection of interesting orthopaedic cases will be presented with a panel discussion including an orthopaedic surgeon, a musculoskeletal radiologist and an orthopaedic pathologist. The session will be largely visual in nature, illustrated with macroscopic and microscopic images and radiology, and will encompass a wide variety of concepts including even the causes of the fall of the Roman Empire!

Familial Hypercholesterolaemia

Dr Daniel Talmont M.D.

Familial Hypercholesterolaemia (FH) is a genetic condition highly prevalent in Australian Lebanese, Afrikaans and Canadians from Quebec (approx 1 in 60) whilst in the general Australian community FH affects 1 in 500 people. If diagnosed early FH can nowadays be managed and treated with lipid regulating medications. It is crucial to make the diagnosis early as life- threatening situations such as heart attacks may occur if the condition is not discovered early enough or left untreated. Clinical examination and biological testing in Lipid Clinics together with "Family cascade screening" are crucial and can save lives.

The Glioma Challenge

Manuel B. Graeber

Brain Tumour Research Laboratories, The Brain and Mind Research Institute, University of Sydney, Sydney, Australia

Gliomas are common brain neoplasms, and glioblastoma (GBM), the most malignant variant, constitutes more than 20% of all adult primary malignant brain tumors. Due to their highly diffuse infiltration of brain tissue, GBMs cannot be completely resected, and less than half of patients survive more than one year. It follows that patients cannot be helped most effectively by research that is limited to an analysis of the glioma itself. We therefore decided to focus on the guestion what the brain and its defence cells, the microglia, do or do not do to these tumours. Microglia are cells that sense pathological tissue alterations. They can develop into brain macrophages and perform immunological as well as CNS-specific functions. Genetically enhanced, i.e. reprogrammed microglia might revolutionize the treatment of CNS diseases, one step short of synthetic biology (1). It is our goal to develop the techniques that will enable the reprogramming of microglia. A number of years ago we discovered that microglia support glioma growth, and we

have subsequently obtained evidence that many if not most of the microglia/brain macrophages in malignant glioma derive from bone marrow-precursors (2). Thus, experimental glioma should provide an ideal setting to test genetically modified microglia and to develop an entirely novel and hopefully more effective treatment for glioblastoma as a result. We will combine experimental neuropathological with some of the latest molecular genetic techniques to bring genetically enhanced microglia into the tumour and its microenvironment.

The presentation will start off with a review of the most important glioma subtypes in humans followed by an update on molecular diagnostics. The third part will deal with our research. Furthermore, we will illustrate why and in what areas histology is likely to remain an important laboratory technique.

References

- 1. Graeber MB (2010) Changing face of microglia. Science 330: 783-788
- 2. Graeber MB, Flügel A (2010) Bone marrow-derived microglia in experimental glioma. 40th Annual Meeting, Society for Neuroscience, San Diego, USA, 13-17 November 2010

Haematoxylin Blues

Mike Rentsch

At some time during a Technologist or Scientist's career, one experiences failures in staining performance with Haematoxylin; these frequently go unexplained or are just replaced with fresh solution/formulation. Some common and not so common causes are examined, with an emphasis on Basic Chemistry, recognition and preventative measures.

Do You Know What I Did Last Summer? – SPHERE Programme

Bill Sinai and Penny Whippy

We were invited in November 2010 to participate in a multi country programme in the Asian region in 2011 aimed at improving detection and interpretation of HER2 overexpression in Breast and Gastric cancers.

This involved the preparation of training material and the presentation of that material to delegates from all 9 participating counties that formed the SPHERE Steering Committee in Korea in January 2011. After consultation and several rewrites; we presented the material in the form of three 2 day meetings with dry and wet workshops in Korea and Thailand as "Train the Trainers" events. The materials were translated into local languages and rolled out in the member countries.

This has been a rewarding experience for all and we are still involved in SPHERE network via a newsletter and individual contact as well as upcoming data collection.

Productivity in the Histology Laboratory

René J. Buesa

Productivity has to be measured for the laboratory work flow as a whole to later evaluate any shortcomings that may exist. The histotechnologist's work output is just a limited aspect of the overall productivity and should be the manager's goal to create the conditions for an efficient outcome of the operation.

How many blocks a histotechnologist should embed or cut daily is just one of many aspects to consider. The peculiarities of the histology laboratory tasks within the general medical laboratory, especially those with a high level of decision making, will be presented.

A perspective of the evolution of mechanical technology since the late XVIII century to present will be used to explain its effects on the productivity of individual tasks and the overall work flow.

The participants will receive an evaluation of their laboratories by answering a questionnaire handed before the conference and will learn how that evaluation came about. They will be able also to compare with that of other histology laboratories worldwide.

The participants will learn how to extrapolate the "average modular productivity" to the characteristics of their particular histolabs and find out how much time it should take to complete their daily tasks while improving their turnaround time.

The ratio investment costs to results obtained when trying to reduce the turnaround time of the laboratory by implementing "Lean" approaches and other management techniques will be discussed.



National Meeting Poster Abstracts

Hemokinin-immunoreactivity in Immune Cells in Inflamed Colon

French M^1 , Peck C^1 , Liu L^2 , Burcher E^2 , Southwell BR^1 .

¹Murdoch Childrens Research Institute, Royal Childrens Hospital, Melbourne. ²Dept. Pharmacology, School of Medical Sciences, University of NSW, Sydney.

Background: Hemokinin-1 (HK-1) is a new tachykinin, encoded by the TAC4 gene. HK-1 was recently found in inflammatory cells and may be involved in the pathophysiology of inflammatory bowel disease. Substance P (SP) is a neuropeptide encoded by the TAC1 gene, present in sensory neurons and motor neurons in the myenteric ganglia and in nerve fibres in the ganglia, muscle and mucosa. TAC4 mRNA is expressed at low levels in muscle layers and mucosa in normal colon and is 10 fold more abundant in mucosa from patients with ulcerative colitis (UC) than in control mucosa (Liu et al 2011, Neurogastro & Motility 23, 475-485, e180). HK-1 binds selectively to the NK₁ receptor leading to the suggestion that HK-1 is an endogenous SP-like endocrine agonist and may mediate actions previously ascribed to SP. Aim: To compare the localisation of HK-1 and SP immunoreactivity (IR) and the location of cells containing HK-1 in human colon.

Methods: Full thickness biopsies were collected from control adults and adults with ulcerative colitis or Crohn's disease. Biopsies were fixed in Zamboni's fixative, frozen and 10μm sections were incubated with anti-HK-1 or anti-SP antibody followed by fluorescent secondary antibodies or DAB. The HK-1 antibody was raised against the unique N-terminus of HK-1. TAC1 and TAC4 mRNA levels were determined by real-time PCR.

Results: SP-IR was prominent in nerve fibres in the muscle, submucosa, ganglia and mucosa. HK-1-IR was not present in nerve fibres in the mucosa or muscle layers, showing that the antibody did not recognise SP. HK-1-IR was present in a few immune cells in control mucosa. In UC biopsies, there was a substantial increase in mucosal immune cells, and many contained HK-1-IR. The HK-1 antibody was successfully preabsorbed with peptide (1:250). TAC1 and TAC4 mRNA were increased in UC mucosa.

Conclusion: Both HK-1 and SP mRNA are upregulated in UC. HK-1 is present on immune cells in the mucosa in UC while SP is present in nerve fibres. Since HK-1 binds to the NK-1 receptor, it may mediate some pro-inflammatory actions previously ascribed to SP.

Enkephalin Nerve Fibres Are Reduced In Some Children with Slow-Transit Constipation

Ijap NA, Yik IY, Farmer P, French M, Bodemer D, Hutson JM, Southwell BR.

Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne.

Background: Peripherally-active opiates reduce colonic motility and produce constipation. The endogenous opiate enkephalin (ENK) is present in enteric nerve fibres including some containing the excitatory neurotransmitter, substance P (SP). Slow-transit constipation (STC) is a form of chronic constipation where motility of the proximal colon is reduced. In STC adults, the number of nerve fibres containing SP and ENK are reduced. In STC children, 23% have decreased SP nerve fibres in colonic circular muscle. ENK may be present in Vasoactive Intestinal Peptide (VIP) & Nitric Oxide (NO) inhibitory nerve fibres. Aim: To determine if ENK nerve fibres are reduced in paediatric STC.

Methods: Seromuscular biopsies from the right transverse colon were obtained from 51 children (aged 4-14 years) with STC and two control patients with familial adenomatous polyposis processed for fluorescence (FAP), immunohistochemistry to detect SP/ENK and ENK/VIP/NO immunoreactivity in nerves and imaged using confocal microscopy. Biopsies were qualitatively categorised as having 'low' or 'normal' levels of SP nerve fibres. Then 12 patients (6 normal and 6 low SP) were randomly selected from the cohort and densities of SP⁺/ENK⁻ (SP only), ENK⁺/SP⁻ (ENK only) and SP⁺/ENK⁺ (co-localised) nerve populations were quantified and analysed for co-localisation.

Results: In control (FAP) colon, ENK+ nerve fibres were abundant in the circular and longitudinal muscle but absent from the mucosa. The density of SP and ENK nerve fibres was the same in FAP and 'normal' SP patients. In circular muscle, the density of ENK nerve fibres was double the density of SP nerve fibres. There were nerve fibres containing ENK only, SP only and both ENK+/SP+ with 34% of ENK nerve fibres containing SP and 41% of SP nerve fibres containing ENK. In the 'low' SP patients, there was a 50% reduction in the density of SP nerve fibres (p=0.01) and ENK nerve fibres (p=0.05) compared to patients with normal level SP. There was a reduction in ENK only (p=0.03) and SP only nerve fibres (p=0.007) but not in SP+/ENK+ nerve fibres. ENK was not in VIP or NO inhibitory nerve fibres. Statistical analysis: unpaired, two-tailed Ttests.

Summary: The opiate ENK is abundant in nerve fibres in the muscle layers. One third of the ENK nerve fibres contain SP. One quarter of STC children had low levels of SP nerves and there was a reduction in ENK only and SP only nerve fibres in these patients. There was no reduction in SP+/ENK+ nerve fibres between 'normal' and 'low' level SP patients. Exogenous opiates cause

constipation. Reduced ENK in nerve fibres in the muscle in STC children may contribute to delayed colonic transit. Loss of SP and ENK is more common in adults with STC than in STC children.

Novel use of anti-human cytokeratin (CAM5.2) to diagnose a case of canine atypical apocrine tumor

Jarrod Phillips¹, Sarah Morabito¹ & Geoff Orbell²

¹Histopathology Department, Healthscope Pathology, Clayton VICTORIA, AUSTRALIA²Gribbles Veterinary Pathology, Clayton VICTORIA, AUSTRALIA

Introduction:

An eight year old female labrador presented to her vet with a mass on the 3rd digit on her front left paw. Previous fine needle aspiration of the lesion had been suggestive of a sarcoma. Complete digit amputation was performed requesting identification and classification of the tumour. The gross specimen sent to the laboratory was a toe with intact nail 60mm x 30mm x 20mm bearing a 20mm x 20mm nodule beneath the skin surface adjacent to the nail bed.

Method:

Routine histopathological processing was performed on the sample. Following standard dissection techniques, the samples were processed overnight on a Leica Peloris Tissue Processor. Immunohistochemistry was performed using the Ventana Benchmark XT with UltraView Universal DAB Detection Kit.

Results:

Initial examination of the H&E sections suspected an apocrine adenocarcinoma. The histological presentation was extremely unusual and did not represent the sarcoma diagnosis made on the initial fine needle aspiration. The architecture did not suggest a typical apocrine adenocarcinoma but rather an atypical variation. Due to the similar histological appearance, one differential diagnosis was a Sertoli cell tumour.

Anti-cytokeratin (AE1/AE3) immunohistochemistry confirmed epithelial origin of the tumour. Novel use of anti-human cytokeratin (CAM5.2) antibody was performed on the canine tumour. This antibody, which is directed against cytokeratins specific to secretory epithelium such as apocrine and eccrine glands, stained positively confirming the suspected apocrine gland origin of the tumour.

Conclusion:

Novel use of anti-human cytokeratin (CAM5.2) antibody has positively confirmed this canine atypical apocrine adenocarcinoma.

Keeping your Tissue SAFE

David Butler

Supervising Scientist, Anatomical Pathology, Prince Charles Hospital, Chermside Brisbane 4032.

A Vacuum Sealing Apparatus (Milestone SrlTissueSAFE) was located in the Caboolture Hospital operating theatre suite for a period of 5 weeks. A variety of surgical pathology specimens were processed through this system and immediately refrigerated for transport to our pathology laboratory.

An evaluation of this pre-analytical process was undertaken to determine the suitability of this equipment for our laboratory group. The sealing process was successful in reducing exposure of theatre staff to formaldehyde & maintaining the integrity of the Surgical specimens for histopathological diagnosis.

Levelling Up With Levels

Victor Gonzales, Melissa Judd and Aaron Spasich.

Laverty Pathology, 60 Waterloo Road, North Ryde 2113

In histopathology, initial tissue sections may not show complete features of the lesion, and sections at different depths may be needed in order to make a final diagnosis. Sections can be done either serially (sections taken in consecutive order) or in steps (taking sections at regular intervals). Commonly referred to as taking levels, deeper levels are routinely taken to enhance both diagnostic sensitivity and accuracy. Even if a diagnosis is possible from the initial slide, deeper levels may be taken to look for additional histological features. Studies have suggested that deeper levels are very important in the diagnosis of skin cancer.

Apart from the diagnostic importance of taking serial sections, there are also technical reasons. At times, the technician needs to continue to cut levels through a paraffin block in order to see all surgical margins of the specimen to be seen (full-face). Sometimes deeper levels are also needed if the technician has not orientated the tissue correctly during embedding or the lesion cannot be seen. This can lead to superficial sections of a paraffin block being cut which do not include the lesion.

Here we give an example of the use of levels, and discuss the diagnostic importance that deeper levels provide in a variety of different specimens. We will show how levels are used where initial sections are nondiagnostic, and how further sections can find additional diagnostic features, where a proportion of these features can disclose additional pathological findings.

Altering pH for Easier Adenosine Triphosphatase (ATPase) Skeletal Muscle Fibre Typing

Dianne Reader¹ and Dr Tamara Sztynda²

¹ Department of Anatomical Pathology PaLMS, ² School of Medical and Molecular Biosciences University of Technology, Sydney

Objective: To devise a simpler and safer way of diagnosing skeletal muscle fibre types by varying the temperature, time and the pH of the ATPase stain and using a metachromatic dye.

Methods: The detection of myofibrillar ATPase under variable conditions such as temperature, incubation time and pH were tested. These trials also removed ammonium sulphide as the colourant and examined the use of two metachromatic dyes Azura A or Toluidine Blue as safer alternative.

Results: It was determined that the best temperature for the enzymatic reaction to be 37°C over a period of between 45 and 60 minutes incubation. It was also shown that a pH of 9.4 to a more alkaline 10.6 had the added advantage of showing the three skeletal muscle fibres (types 1, 2A and 2B) more clearly and could be done as one sample, thus saving time. Replacing the dangerous ammonium sulphide with Azura A (metachromatic dye) gave a clearer and equally reproducible result. Toluidine blue was found to be not as reliable and harder to replicate.

Conclusion: The number of myofibrillar ATPase techniques available is daunting; by changing the variables such as temperature, time and the pH a clearer alternative was found. The use of Azura A also makes this technique safer.

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Professor Anthony S-Y Leong 1945–2011

Professor Anthony S-Y Leong, passed away at his home in Kuala Lumpur in June of 2011. He left behind Wendy his beloved wife, a son, Joel, and daughter, Trishe, both of whom also entered careers in Pathology. Anthony Leong was born in 1945 in Singapore. He graduated MB, BS, among the first class from the University of Malaya in 1969. He completed a Fellowship at the University of Washington, and then obtained his MD at the University of Adelaide in 1981.

Tony was Professor at Adelaide (almost 20 years), the Chinese University of Hong Kong, followed by 10 years as Director of Anatomic Pathology at John Hunter Hospital in Newcastle. He assumed the role of Director of Pathology at Sunway Medical Center in Kuala Lumpur in December 2010. Over the course of his career, Tony published more than 350 articles in journals, and chapters in more than 20 books. He was active on the Editorial Board of 30 journals and held visiting professorships at universities in China, Korea, Japan, Thailand, and the United States (Appl Immunohistochem Mol Morphol Volume 19, Number 5, October 2011 p 387-388).

I have known Tony since 1984 when I was the Principle Scientist at the Repatriation General Hospital in Adelaide. He was my supervisor for my Masters Research Degree at Adelaide University. Tony was always passionate about Histopathology and Histotechnology and was always willing to research and develop new techniques. Showing him my in situ hybridisation results always caused a twinkle to appear in his eye and his enthusiasm to become infectious.



Tony, the Histotechnologist Friend, will be missed.

Lipofuscin Review

Lipofuscin (age pigment) is a brown-yellow, electron-dense, autofluorescent material that accumulates progressively over time in lysosomes of postmitotic cells, such as neurons and cardiac myocytes. The exact mechanisms behind this accumulation are still unclear. Lipofuscin (LF) is also called `age pigment' or `wear-and-tear pigment'. 'Ceroid', another commonly used term, usually specifies a very similar, or perhaps even identical, material formed under pathological conditions, such as lysosomal malnutrition, diseases, stress, atherosclerosis, tumors, etc. The use of the terms 'LF' and 'ceroid' is not yet generally consistent, as demonstrated by use of the term 'neuronal ceroid lipofuscinosis' for Batten's disease (1).

There is still no generally accepted opinion as to the influence of LF on cellular functions. Since LF-accumulation observed together with several other manifestations of senescence, such protein modification, it is difficult to identify its specific role in age-associated disorders. It is well known, however, that in lysosomal storage diseases, when accumulation of ceroid pigment is rapid and pronounced, numerous functional disorders are observed (1).

LF is characterized by a number of morphological, physical and chemical properties. It represents a mixture of different chemical substances, and, thus, is not a homogeneous substance with a chemical composition that may be specified. Therefore, the properties of LF are variable

within certain limits. The same is true for ceroid (1).

Light microscopically, LF-inclusions are yellow-brown, with variations in the amounts, shade and intensity (1).

granules Lipofuscin often autofluorescent; however, when they contain iron or copper the fluorescence can be quenched (3). The fluorescent spectra of lipofuscin generally show excitation maxima at about 360 nm and a yellowish emission maxima at 540-650nm (4). Although the nature of the fluorophores responsible for autofluorescence is not completely clear, evidence suggests that various autofluorescent substances can be formed as a result of reactions between carbonyls (mainly aldehydes, produced by lipid peroxidation) and amino compounds (1).

Histochemically, LF is usually Sudan black and PAS positive, indicating the presence of lipids and carbohydrates. The pigment granules are also Schmorl's positive, and, to a lesser extent acid-fast and argyrophilic. LF also exhibits the activity of several lysosomal enzymes including peroxidase, acid phosphatase and \(\beta\)-glucuronidase and the presence of metals (1,3).

Ultrastracturally, LF is observed as an electron-dense material surrounded by a typical lysosomal three-layer membrane. These inclusions also often contain electron-lucent vacuolar components. The fine structure of LF varies between different types of cells, and LF-granules have been

classified into four groups on the basis of their ultrastructural characteristics: granular, homogeneous, lamellated and compound (i.e. granular-homogeneous, homogeneouslamellated, and granular-lamellated) (1).

LF is formed from worn-out or damaged cell components. This type of material is mainly subjected to intralysosomal degradation by autophagocytosis, but it can also be formed by heterophagocytosis, as in the case of retinal pigment epithelial cells. Initially, sequestered material such within is autophagosomes (macroautophagocytosis). These structures are usually surrounded by membranes and do not contain two lysosomal enzymes. They subsequently fuse with primary or secondary lysosomes to form secondary lysosomes of the autophagic type, in which the material is degraded (1).

The neuronal ceroid-lipofuscinoses (NCLs) collectively constitute the most common type of inherited neurodegenerative diseases in childhood. Their incidence in the US has been estimated at 1:12,500 and they usually show an autosomal recessive mode of inheritance. The age of onset varies from infancy to late adult. Most childhood forms are clinically characterized by progressive mental and motor deterioration, blindness, epileptic seizures, and premature death, while the rare adult-onset forms dominated by dementia. Despite the varying ages of onset and clinical course, all forms of NCL share unifying pathomorphological accumulation features. There is autofluorescent, periodic acid-Schiff (PAS)and Sudan black B-positive granules that are resistant to lipid solvents in the cytoplasm of most nerve cells and, to a lesser degree, of many other cell types. Many of these granules are also Luxol Fast Blue positive.

The storage process is associated with progressive and selective neuronal loss and gliosis with secondary white matter lesions. The ultrastructure of the storage deposits varies between different forms of NCL and has provided the basis for the traditional classification, along with the age of onset (2).

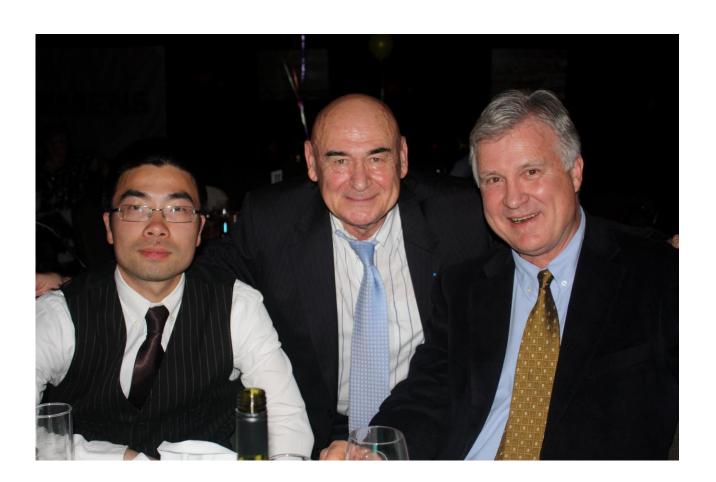
The concept of NCL is based on a relatively morphological phenotype, uniform by the accumulation in characterized neurons and, to a much lesser extent, in cells of intracytoplasmic many other with autofluorescent deposits cytochemical properties and ultrastructural patterns. Despite ubiquitous storage, only the neurons of the CNS are selectively destroyed. The storage material is largely proteinaceous and, depending on the identity of its main protein component, the NCL can be divided into 2 broad categories, those storing subunit c of the mitochondrial ATP synthase and those storing saposins. High saposins concentrations of exclusively associate with granular osmiophilic deposits, suggesting ultrastructure that the determined by the stored protein. Immunoreactivity of the intraneuronal deposits for amyloid beta has also been reported. However, the mechanisms of accumulation of these highly hydrophobic proteins and their relation, if any, to clinical symptomatology and neuronal death remain unsolved (2).

The morphological uniformity of the NCL contrasts with their newly discovered genetic heterogeneity. More than 115 mutations in at least 7 different genes (CLN1–3, CLN5–6, CLN8 and cathepsin D) underlie different forms of NCL in man and animals. The products of most of these

genes are ubiquitously expressed and not neuron-specific (2). All the mutations that have been associated with this disease have been linked to genes involved with the neural synapses metabolism - most commonly with the reuse of vesicle proteins.

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Surgical Cut Up Workshops at NHM

Two half day Surgical Cut Up Workshops were held at Granville TAFE on Friday, 4th November 2011 as part of the 5th National Histology Meeting in Sydney and presented by Penny Whippy and Anne Prins.

The workshops were quickly filled with a maximum of twelve per workshop. All states were represented as well as three from New Zealand. The format of the workshop simulates the cut up in a routine Histology laboratory. Samples are received with completed request form, allocated a laboratory identifier and described and sampled for processing. The attendees worked in pairs with one describing, cutting and placing tissue in cassettes and the other scribing. Roles are reversed so that both perform both tasks.

A comprehensive workbook was provided with general instructions re checking patient details, descriptions, diagrams, inking etc as well as instructions for the organs and tissues provided. At these workshops, five samples were provided for each attendee – a simple transfer of TURP, uterine curette, core, gastric or cervical biopsy, a simple unorientated skin, an oriented, more complex skin, a segment of small bowel and a kidney. Extra samples were provided in case attendees needed them – a wedge biopsy of lung, wedge of ear and a cone biopsy..

Penny gave a brief talk to outline what was to be done before work started. The attendees were all very enthusiastic and most completed all five samples. Only our Kiwi attendees wanted their blocks which Anne processed and mailed to them as the unprocessed cassettes would not have gone through Customs.

We are indebted indeed to the efforts of Sandra Pitchfork and Karen Stockton who made our day run so smoothly. From the well organised (and scrubbed & polished) laboratory, the registration and lunch rooms provided, to their help and generosity with time and helpers when we needed them. All in all, a very satisfactory day.





Histo hypotheticals (I wish)

Tony Henwood Histopathology Westmead Childrens Hospital



Scientific Histopathology has developed greatly in the last ten years. We have seen an exponential growth in proteins demonstrable using immunohistochemistry. We have developed more rapid processing methods, and expected a higher quality in our microscopic product. As well as this we have been made aware of more stringent occupational, health and safety requirements. AND Accreditation is always just around the corner.

We are confronted with problems caused by pre-analytical, analytical and post-analytical variations. This will tax our scientific knowledge (and dare I say prowess?).

How are we to meet these challenges?

This interactive workshop presents problems that affect the quality of our results and participants will be encouraged to advice as to a suitable course of action. With each case, the current research and developments

Cost: \$40 (Limited Places)

Date: 12th May Saturday 2012

Time: 9am - 12pm

Venue: Doreen Dew Lecture room

4th floor Children's Hospital Westmead

Hawkesbury Rd Westmead

RSVP: 4th May Friday 2012

Contact: Bharathi Cheerala, Douglass Hanly Moir Pathology Ph: 02 9855 5434 (bcheerala@dhm.com.au)

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