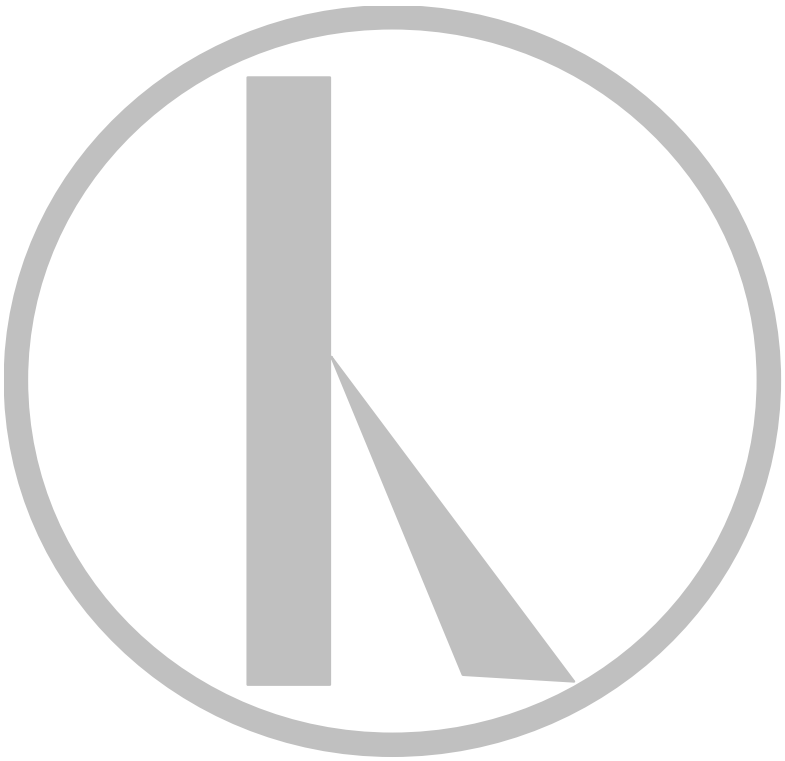
# Histograph

ISSUE 1
April 2005



NEWSLETTER OF THE HISTOTECHNOLOGY GROUP of NSW

## *Contents*

## Editorial

Welcome to the first issue of Histograph for 2005. 2005 promises to be a BIG year.

We have our National Histotechnology Conference in Melbourne in October, but before that, AIMS is holding its National Conference in Sydney from the 4th to the 8th July. I have been asked to organise the Histotechnology programme and have been privileged to obtain some excellent speakers. The aim of the sessions is to provide new and updated information on some of the areas of histopathology that will impact us in the future. More details in this issue.

The plans for the National Conference is well underway and the programme is looking SICK (according to Ian Thorpe, this means great). Further details to follow. So that I can ruin your weekend, I will see you all there.

Some of you may have wondered what happened to the story on "Histotechnology in the Desert" by Karen Appold. Unfortunately Karen wanted $200 to allow us to publish it and you can read it yourself on www.advanceweb.com .

The second part of the article "Amyloid Update", as well as abstracts from the literature, A gem from Histomail and our safety column rounds off this issue

Tony Henwood
Editor
Histograph

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## Chairman's Message

The current committee is looking at holding some interesting workshops and meeting during this year and you will soon be getting notification of these meetings.

Further through this newsletter you will find notification of future meetings, please remember to support your committee and group by participating in as many functions as you can through out the coming year.

The committee has decided to present the next state meeting at The Country Comfort Motel at Mudgee the venue for our very successful 2001 meeting. If you have an idea about anyone who you think may prove an interesting speaker with an interesting topic please feel free to contact myself or one of the committee members.

Yours in Histotechnology
Bill Sinai
Chairman.

# Amyloid Update – Part 2

## Alzheimer's disease

Alzheimer's disease is a degenerative disease characterized by the presence of numerous senile plaques and neurofibrillary tangles, primarily in the cortical areas of the brain. There is no known cause of the disease, although exposure to aluminium has been implicated by epidemiological studies and the finding of aluminium in the cerebral plaques and tangles (Mera 1991).

There is a genetic predisposition for early-onset disease, and Down's syndrome patients are particularly vulnerable. Many of the plaques contain amyloid, and a gene for amyloid precursor protein has been identified on the long arm of chromosome 21. It is possible that the amyloid plaques arise through over-expression of this gene. Since there is no specific marker for Alzheimer's disease, laboratory investigations are restricted to confirming the disease after the patient has died. Recent immunohistochemical studies have been directed towards showing the relationships between the neurones, senile plaques and neurofibrillary tangles, and the presence of amyloid and filament proteins such as tau, ubiquitin and neurofilaments (Mera 1991).

In hereditary cerebral haemorrhage with amyloidosis-Dutch type (HCHWA-D), there are no neuritic pathology or classical congophilic plaques. The amyloid is localised around blood vessels. Rozemuller et al (1993), using immunohistochemistry, were able to show that amyloid formation is different from that found in Alzheimer's disease.

## Variant Creutzfeldt-Jakob disease

Variant Creutzfeldt-Jakob disease (vCJD) is a novel prion disease in man, which was first described in 1996 in the UK. There is substantial evidence to indicate that vCJD represents the effects of the bovine spongiform encephalopathy (BSE) agent in man. The neuropathology of vCJD is characterised by the florid plaque, composed of a central amyloid core with a fibrillary periphery, surrounded by a rim of spongiform change in an intact neuropil. Unique patterns of Prion Protein (PrP) accumulation in vCJD are revealed by immunocytochemistry in the cerebral and cerebellar cortices, the basal ganglia, thalamus and brainstem. The neuropathology of the thalamus and midbrain is also characterised by severe neuronal loss and gliosis. vCJD is distinct from other human prion diseases in that disease-associated PrP accumulates within follicular dendritic cells in lymphoid tissue, and consistently in peripheral sensory ganglia. All vCJD patients so far have been methionine homozygotes at codon 129 in the PrP gene (Ironside 2000).

There is no evidence to indicate that cases of BSE infection have occurred in individuals in the UK who are MV or VV at codon 129 in the PrP gene. It is conceivable that BSE incubation periods in these groups may be longer than in methionine homozygotes, hence the precise numbers of future cases of vCJD are difficult to estimate at present (Ironside 2000).

## Gerstmann-Straussler-Scheinker Disease

Gerstmann-Straussler-Scheinker (GSS) disease is a familial neurodegeneration characterized clinically by adult-onset ataxia, postural abnormalities, and cognitive decline, and pathologically by amyloid deposits mostly localized in the cerebral and cerebellar cortices and the basal ganglia (Bugiani et al 2000).

The disease is due to mutations in the prion protein (PrP) gene. Processing of the mutant proteins originates the amyloidogenic fragments that accumulate in the tissue. PrP-immunoreactive amyloid deposits are the morphological hallmark of the disease. Hypertrophic astrocytes, activated microglia, and nerve cell loss are consistently associated with PrP-amyloid deposits, while spongiosis, diffuse PrP immunoreactivity, neurofibrillary tangles, Lewy bodies, and long fibre tracts

degeneration are occasionally associated. The clinical and pathological variability observed in GSS families is related to both mutations and the M/V polymorphism at codon 129 of the mutated gene (Bugiani et al 2000).

## Amyloidomas

Amyloid pseudo-tumour or "amyloidoma" is the local deposition of amyloid without systemic involvement. It is a rather rare and often causes clinical consternation. It is often misdiagnosed as a malignant tumour and it is only after biopsy and the demonstration of amyloid that its true nature is determined (Racanelli & D'Amore 1999).

The finding of amyloid deposits in the seminal vesicles has been known for many years. The deposits are usually localized and asymptomatic. In recent years seminal vesicle amyloidosis has been reported to simulate prostate and bladder cancer invasion on MRI (Maroun et al 2003).

Bornemann et al (1993) have described three cases of symptomatic neuralgia of the trigeminal nerve due to an amyloidoma in the gasserian ganglion. The correct diagnosis was not made prior to histological examination of the surgical biopsy specimens.

Medical history and clinical observation led to the diagnosis of a malignant process of the nasal cavities in the first patient; of an inflammatory dental focus in the second patient; and of multiple sclerosis in the third patient. CT findings were normal in cases 1 and 2; in case 3, a schwannoma was suspected from the CT appearances. In case 1, MRI had not been performed; in cases 2 and 3, MRI revealed a tumour mass that was also considered to be a schwannoma. Histologically, the tumours consisted of masses of amyloid deposits, which had largely replaced the pre-existing ganglionic cells and satellite cells. Electron microscopy confirmed the fibrillar structure of the deposits. Immunohistochemistry revealed the amyloid to belong to the AL-lambda subtype.

Adams et al (2001) described two cases of pleural amyloidosis that radiologically mimicked malignant mesothelioma.

Tumoral amyloidosis (amyloidoma) of bone is a rare condition characterized by the massive destructive deposition of AL amyloid in bones. Pambuccian et al (1997) reported three cases where the radiological diagnosis included chondrosarcoma in the differential diagnosis. Microscopically, there were large, rounded deposits of amorphous eosinophilic material surrounded by numerous giant cells and a sparse lymphoplasmacytic infiltrate. The deposits proved to be composed of AL amyloid showing potassium permanganate resistant congophilia. Immunohistochemistry showed immunoglobulin IgG lambda, IgG kappa, and IgM lambda monoclonality of the plasma cell and (in one case) lymphoid infiltrate. The tumours were classified by morphology and immunohistochemistry as solitary plasmacytomas of bone (two cases) and plasmacytoid lymphoma (one case). An extensive review of the world literature showed 34 well-documented similar cases, occurring most often in the spine and skull, causing neurologic symptoms, tending to occur in middle-aged men and frequently progressing to generalized disease. Most if not all AL amyloidomas of bone represent solitary plasmacytomas of bone or plasmacytoid lymphomas.

### References


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Rozemuller et al (1993) American Journal of Pathology. 142(5):1449-57.

**Histotechnology Group of NSW**
**Future Meetings 2005-6**

| Topic | Presenter | Location | Date | Time |
|---|---|---|---|---|
| Laser Fusion of Blood Vessels | Mr Ted Sheedy Microsearch Australia | Douglas Hanley Moir North Ryde | 27th June 2005 | 7:00pm |
| | | | | |
| Silver Stains Workshop | HTG of NSW | Granville TAFE | July | TBA |
| | | | | |
| Tissue Microarrays and Pancreatic Cancer | Dr James Kench | Westmead Anatomical Pathology | 27th July 2005 AGM | 7:00pm |
| | | | | |
| Various | Dr Jim Scurry, Dr Ibrahim Zidawi, Mr Andrew Kennedy, Dominique | Mayne Health, Newcastle | 27th August 2005 | Saturday Limited numbers |
| | | | | |
| Cut-up for Technologists | Grant Taggart, Andrew Kennedy | Douglas Hanley Moir | September | 7:00pm |
| | | | | |
| Microtomy Workshop | HTG of NSW | Granville TAFE | October ? | TBA |
| | | | | |
| Many and varied | Histology Group of Victoria Inc. | National Histotechnology Meeting, Carlton Crest Hotel, Melbourne | 21st – 22nd – 23rd October 2005 | Friday to Sunday |
| | | | | |
| Museum Techniques | Andrew Kennedy | TBA | November ? | TBA |
| | | | | |
| Antibodies, Basic and Exotic | Dr Stephen Fairy | TBA | November ? Christmas Lecture | TBA |
| | | | | |
| IHC Retrieval Workshop | Penny Marr | Granville TAFE | TBA | TBA |
| | | | | |
| Various | Histotechnology Group of NSW | Mudgee | 17th – 19th March 2006 | Friday Lunch to Sunday Lunch |

# AIMS National Meeting Sydney 4-8 July

Sheraton on the Park, Sydney,

The Australian Institute of Medical Science is holding its National Conference in Sydney from the 4th to the 8th July. I have been asked to organise the Histotechnology programme and have been privileged to obtain some excellent speakers. The aim of the sessions is to provide new and updated information on some of the areas of histopathology that will impact us in the future. I have tried to structure a program that does not duplicate the Histotechnologist National Meeting in Melbourne and I believe you will find it both stimulating and innovative.

Dr Fiona Wood, the 2005 Australian of the year, will be opening the conference. Dr Wood was involved in the use of artificial skin for Burns patients and was acclaimed for her work following the Bali Bombings. The Saal Foley Lecture will be given by Dr Robyn Rodwell and will be entitled "Cell Therapy and Regenerative Medicine in the Treatment of Disease".

On the afternoon of the same day (Wednesday, 6th July), there will be two Histology sessions featuring speakers with expertise in new areas of Histotechnology as well as updates on existing Histotechnology issues:

| Title | Speaker |
|---|---|
| Problems in the Diagnosis of Melanocytic Tumours - Issues for Histotechnology | Dr Richard Scolyer |
| Review of Telomerase Immunohistochemistry - the Problem of Interpretation | Jeremy Henson |
| Australasian Biorepository Network-Oncology. Maximising a valuable tissue resource for researchers. | Dr Dan Catchpoole |
| Invertebral Disc Histology | Susan Smith |
| Histotechnology - New Challenges and Surprises | Tony Henwood |
| New directions in Renal Transplant Rejection Assessment | Dr Jeffrey Fletcher |
| The Histological Assessment of Burn Wounds - Correlation with Clinical Findings | Dr Jennifer Yuan |
| Neuroblastoma - Prognostic Markers Update | Andrew Gayagay |
| Laser Capture Microscopy - Applications in Histopathology | Jacqueline Mills |
| Purifying Nucleic Acids from Paraffin Blocks | Tony Henwood |
| Producing the Perfect H&E | Roy Ellis |

Other interesting sessions include "Doping" in Sport, Molecular Genetics of Tumours and Forensics. Please visit the AIMS 2005 NSM web site and register online if you are paying by credit card.
**www.aims.org.au**
For all enquiries please contact:

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AIMS 2005 NSM Secretariat
Conference Action Pty Ltd
PO Box 576 (Suite 104, 308 Pacifi c Highway)
CROWS NEST NSW 1585 AUSTRALIA
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Facsimile: +61 2 9901 4586
Email: emma@conferenceaction.com.au


Histology Group of Victoria Inc.

The committee of the Histology Group of Victoria has commenced working already on the program and format.

**Dates:** Friday 21st – Sunday 23rd October, 2005

**Venue**: Carlton Crest Hotel
65 Queens Road
Melbourne
(4.5 km SE of the GPO, overlooking Albert Park Lake)

A range of accommodation is available at the Carlton Crest, at both 3 ½ and 4 ½ star rating. Room rates are as follows:

| | |
|---|---|
| Standard Rooms | $134.00 |
| Congress Suites | $149.00 |
| Superior Rooms | $203.00 |
| Carlton Club | $233.00 |

Car parking is available beneath the hotel.

The city centre, Spencer Street transport terminus and Airport bus terminals are a short tram ride away, as are Southbank and St Kilda.
Alternative accommodation is also available nearby.

## Confirmed topics and/or speakers in the plenary sessions include:

- Mr Neil Hand — Queen's Medical Centre Nottingham UK
- Dr Cesar Wong — Chinese University of Hong Kong
- Lung morphometry — Women's and Childrens Hospital, Adelaide
- Non-clinical applications of histology — Anatomy Department University of Melbourne
- "Virgin Speakers"

**HGV website: http://home.vicnet.net.au/~hgvinc/**
**Contact the HGV: hgv@netspace.net.au**

# Calling all Histologists!

The HGV is in the process of organising the program for the 2nd National Histology Meeting. We are calling for submissions from all interested parties, from all states and territories in this wide, brown land. The topics we have thought of so far are:

- *A day in the life of a regional Histology lab*
- *Histology of Hirschprung's disease*
- *Enzyme histochemistry*
- *Immunohistochemistry case studies*
- *Overview of further education available to Histologists*
- *"Virgin" speakers – (the first time is always special…)*
- *Liver special stain panels*
- *Histology of Food*
- *Sentinel node protocols*
- *Specimen collection*
- *"Silver" theme Case studies*
- *New use for old Ab's*
- *C4d renal rejection marker*
- *Rabbit monoclonals experience*
- *Forensic*
- *Special stains*
- *Mortuary practices*
- *Museum techniques*
- *Veterinary histology*
- *Marine histology*

The list is by no means exclusive, but is more a list of topics that we would like to include in the program so if there is anything you would like to present in a National forum, now is the time to collect your ideas, get them down in a Powerpoint presentation (or other format by arrangement) and impart your knowledge and experience to an eager audience.

Poster presenters will not be required to deliver an oral presentation, but will be expected to attend at their poster display at a specified time each day.

Yours sincerely

**HGV Organising committee.**

## PROGRAM DETAILS

| | | |
|---|---|---|
| Workshops | Friday 21st October 2005 | 0900 – 1200<br>1300 - 1600 |

| | | |
|---|---|---|
| Workshop 1 | **Basic Immunohistochemistry Techniques** | |
| Presented by: | *Dr Cesar Wong* | Associate Professor,<br>Chinese University of Hong Kong |

| | | |
|---|---|---|
| Workshop 2 | **Solvent Recycling** | |
| Presented by: | *Mr Andrew Kennedy* | Senior Science Officer |
| | *Mr Frank Quaranta* | Senior Technical Officer<br>Anatomical Pathology,<br>Concord Repatriation Hospital |

(Workshops 1 and 2 will run concurrently)

| | | |
|---|---|---|
| Workshop 3 | **Histology Mythology: Fact or Fiction?** | |
| Presented by: | *Mr Neil Hand* | Chief Biomedical Scientist<br>Histopathology Department<br>Queen's Medical Centre Nottingham UK |

| | | |
|---|---|---|
| Cocktail Party | Opening of the Trade Display | 1800 – 1930 |

| | | |
|---|---|---|
| Plenary Sessions | Saturday 22nd October | 0900 – 1030<br>Morning tea<br>1100 – 1230<br>Lunch<br>1330 – 1500<br>Afternoon tea<br>1530 – 1700 |

| | | |
|---|---|---|
| Conference Dinner | Saturday 22nd October | 1900 – late |

**THE POINT Albert Park Lake**

| | | |
|---|---|---|
| Plenary Sessions | Sunday 23rd October | 0930 – 1030<br>Morning tea<br>1100 – 1230<br>Lunch<br>1330 - 1500<br>**CLOSE** |

REGISTRATION DETAILS

**Full registration** (includes opening cocktail party, morning and afternoon teas and lunches) **$225.00**

**1 Day registration** (includes morning and afternoon tea and lunch) **$150.00**

**Workshops** (includes morning or afternoon tea) **$75.00**

**Student registration** (per day, includes morning and afternoon tea and lunch) **$50.00**
(Application for student registration must be accompanied by evidence of full-time enrolment in a recognized course)

**Cocktail Party** (extra tickets) **$45.00**

**Conference Dinner** **$85.00**

**Late Registration Penalty** (after August 19, 2005) **$75.00**

# Abstracts from the Literature

## *Determination of optimal rehydration, fixation and staining methods for histological and immunohistochemical analysis of mummified soft tissues*

Biotechnic & Histochemistry 80(1): 7-13, 2005

A-M Mekota and M Vermehren

During an excavation headed by the German Institute for Archaeology, Cairo, at the tombs of the nobles in Thebes-West, Upper Egypt, three types of tissues from different mummies were sampled to compare 13 well known rehydration methods for mummified tissue with three newly developed methods. Furthermore, three fixatives were tested with each of the rehydration fluids. Meniscus (fibrocartilage), skin, and a placenta were used for this study. The rehydration and fixation procedures were uniform for all methods. The stains used were standard hematoxylin and eosin, elastica van Gieson, periodic acid-Schiff, and Grocott, and five commercially obtained immunohistochemical stains including pancytokeratin, vimentin, alpha-smooth-muscle-actin, basement membrane collagen type IV, and S-100 protein. The sections were examined by transmitted light microscopy. Our study showed that preservation of the tissue is dependent on the quality and effectiveness of the combination of the rehydration and fixation solutions, and that the quality of the histological and histochemical stains is dependent on the tissue quality. In addition, preservation of the antigens in the tissues is dependent on tissue quality, and fungal permeation had no influence on the tissue. Finally, the results are tissue specific. For placenta the best solution combination was Sandison and solution III (both fixed with formaldehyde) while results for skin were best with Ruffer I (using formaldehyde and Schaffer as fixatives), Grupe et al. (using formaldehyde as a fixative) and solution III (in combination with formaldehyde and Bouin fixatives). Ruffer II (using formaldehyde as a fixative) and solution III (in combination with Schaffer fixative) gave the best results for fibrocartilage.

## *Comparison of Monoclonal Versus Polyclonal Calretinin Antibodies for Immunohistochemical Diagnosis of Malignant Mesothelioma.*

Granville, Laura A. MD; Younes, Mamoun MD; Churg, Andrew MD; Roggli, Victor L. MD; Henderson, Douglas W. MD; Cagle, Philip T. MD

Applied Immunohistochemistry & Molecular Morphology. 13(1):75-79, March 2005.

Of putative specific markers for diffuse malignant mesothelioma, nuclear staining with Zymed polyclonal calretinin antibody has shown the best specificity to date for epithelial diffuse malignant mesothelioma versus adenocarcinoma. We compared specificity and sensitivity of this polyclonal antibody for diagnosis of diffuse malignant mesothelioma with a new

monoclonal antibody from DAKO. One hundred eighteen adenocarcinomas and 111 diffuse malignant mesotheliomas-70 epithelial, 22 sarcomatous, and 19 biphasic-were immunostained with calretinin antibodies from Zymed (polyclonal rabbit, prediluted, PAD:DC8) and DAKO(monoclonal mouse, 1:100, clone DAK Calret 1) using manufacturer-recommended procedures. Cases were blinded and assessed for nuclear versus cytoplasmic staining, percent positive cells, and background. Both antibodies showed similar positive predictive values for diffuse malignant mesothelioma by nuclear staining (Zymed = 95%; DAKO = 97%). False positives in 4 (3.4%) and 2 (1.7%) adenocarcinomas, respectively, stained greater than 10% of cells. Sensitivity for epithelial malignant mesothelioma was slightly less for DAKO antibody (Zymed = 80%; DAKO= 73%). Neither antibody performed well on sarcomatous malignant mesothelioma (Zymed = 2/22; DAKO = 1/22). Both antibodies are useful in the diagnosis of epithelial malignant mesothelioma, although monoclonal antibody is slightly less sensitive.

## *A bright field/fluorescent stain for aluminum: its specificity, validation, and staining characteristics*

JR Walton

Australian Institute for Biomedical Research NSW 2204 Marrickville (Sydney) Australia

Biotechnic & Histochemistry 79(5-6):169 – 176, 2004

A sensitive bright field/fluorescent histochemical staining method has been developed that reveals endogenous aluminum in subcellular structures. The method, achievable within 30 min, is based on phloxine B and phosphotungstic acid, with ethanol differentiation. Hematoxylin is used for nuclear and fast green FCF for cytoplasmic counterstaining. To test the method's specificity, we incubated living neuroblastoma cells overnight in culture media containing aluminum, calcium, iron, copper or zinc, or no added metal ions. After fixing the cells and applying the staining method, only cultures exposed to aluminum stained magenta. Applying the method to paraffin embedded tissue sections pretreated with one of two chelating agents that remove aluminum demonstrated less magenta staining in the chelated sections than in adjacent unchelated sections. Immersing sections overnight in solutions containing exogenous aluminum had no observable effect on staining for endogenous aluminum; therefore, it is unlikely that any exogenous aluminum present in histological reagents would alter the method's staining results.

## *An improved formulation of the zirconyl hematoxylin stain for acidic mucins*

JM Mcnulty and AA Smith

Biotechnic & Histochemistry 79(5-6): 191 – 196, 2004

Zirconyl hematoxylin stains acidic mucins darkly and specifically using a solution of 100 mg hematoxylin, 5 ml ethanol, 5 ml 0.5% sodium iodate, 400 mg zirconyl chloride octahydrate, and 30 ml 25% aqueous glycerol. The stain is especially advantageous for studying goblet cells and Paget cells.

## *HistoChoice as an alternative to formalin fixation of undecalcified bone specimens*

MA Kacena , NW Troiano , CE Coady , MC Horowitz

Biotechnic & Histochemistry 79(5-6):185 - 190, 2004

We compared histochemical and immunohistochemical staining as well as fluorochrome labelling in murine bone specimens that were fixed with 10% neutral buffered formalin to those fixed with HistoChoice®. We showed that sections from undecalcified tibiae fixed for 4 h in HistoChoice® resulted in enhanced toluidine blue and Von Kossa histochemical staining compared to formalin fixation. HistoChoice® produced comparable or improved staining for alkaline phosphatase. Acid phosphatase localization was better in formalin fixed specimens, but osteoclasts were visualized more easily in HistoChoice® fixed specimens. As expected, immunohistochemical labelling was antibody dependent; some antibodies labelled better in HistoChoice® fixed specimens while others were better in formalin fixed specimens. Toluidine blue, Von Kossa, and alkaline phosphatase staining of sections fixed for 12 h produced sections that were similar to 4 h fixed sections. Fixation for 12 h preserved acid phosphatase activity better. Increasing fixation to 12 h affected immunolocalization differentially. Bone sialoprotein labelling in HistoChoice® fixed specimens was comparable to formalin fixed samples. On the other hand, after 12 h formalin fixation, osteocalcin labelling was comparable to HistoChoice®. For most histochemical applications, fixing murine bone specimens for 4 h with HistoChoice® yielded superior staining compared to formalin fixation. If immunohistochemical localization is desired, however, individual antibodies must be tested to determine which fixation process retains antigenicity better. In addition, there was no detectable difference in the intensity of fluorochrome labelling using either fixative. Finally, fixation duration did not alter the intensity of labelling.

## *Improving histopathology turnaround time: a process management approach*

Laurence Brown

Current Diagnostic Pathology 10(6):444-452, 2004

This article describes the use of business process management to re-structure the Histopathology Service at the Leicester Royal Infirmary. Process mapping showed that 56% of the time spent in preparation and 16% of the time in the laboratory was wasted. Up to 99% of the time in the reporting phase of the process contributed nothing to the finished product. Overnight waits for fixation, reporting, typing and authorization were major sources of delay. Further delays were caused by duplication of stages, slide labelling, batching and inappropriate quality control. Alterations to the methods of working in Leicester resulted in an improvement in the turnaround time allowing 80% of cases to be reported within 24 h of receipt. The main changes included trimming fresh specimens, fixation during the process schedule overnight, removal of unnecessary steps, laboratory reorganization, team-based reporting and extending the working day. The improvements to the turnaround time have allowed same-day clinics and provision of reports to Multidisciplinary Team Meetings within 24 h of major surgery.

# Mike Rentsch Column

The Best from Histomail
Question:-

I am curious as to whether many labs in Australia are still using B5 fixative for bone marrow trephines and Lymph Nodes. I am also curious to know from labs that no longer use B5 how they made the change and whether the impetus for change came from the lab or from environmental regulations. Has this question been posed recently at all on Histomail? I know it comes up on a regular basis on the Histonet list.

Reply No:1
MMC histo did away with all mercury- containing products several years ago on OH&S grounds.

Reply No:2
Dorevitch, Heidelberg hasn't used B5 for over 5 years. The change was driven by occupational health and safety issues primarily, and it is of course more environmentally-friendly.

Reply No:3
ICPMR have not used B5 fixative containing mercury for over ten years for both environmental and OH&S issues. We tried everything else that was recommended and have stopped using them all. We very occasionally use Z-fix for lymph nodes. We have been using FAA for Bone Marrows since we stopped B5 use.

Mike Rentsch Comment:-
Those who could tolerate having to coat their metal cassettes with wax etc. or having a maintenance programme to replace stainless steel valves and seats in pumps and valves etc were well rewarded. B5 and other Mercury Based fixatives were popular because of their sharp and intense staining with many

techniques esp.H&E and trichromes. The days of pouring Mercuric and other Hazardous solutions down the sink are long gone!!

While I will probably be corrected on what I am about to say, it is my belief that B5 retained a place in labs mainly because of two reasons. The first being the excellent detail provided in thin sections of Lymph Node for differentiating lymphomas, and secondly because the reference methods in the US and original papers on various antigens for PAP and APAAP methods specified B5 and NBF was considered inferior for markers. This attitude has changed with improvements in Monoclonals and HIER. Workers have tried using other metal based fixatives esp. Zinc Sulphate. It is true that a Zinc Sulphate Modified Susa Fixative can produce results not unlike mercury based fixatives and with lysis of red cells but it is very complex and tedious in its preparation. Zinc Sulphate prevents formalin from cross-linking and fixation is most likely occurring in the alcohol of the tissue processor rather than in the "Jar".

The FAA fixative mentioned above is very good for bone marrows and all bloody tissues including curettings. Red cells are lysed giving a clearer picture and fixation is rapid with some decalcifying. Caution:- If you prepare pre-filled biopsy jars with FAA for BM Kits or trays, make sure you use polypropylene jars and not polystyrene, as sometimes "Ethers" are produced (Not at a dangerous level) that will soften the jar and start to dissolve it. Specimens should not be left more than 48 hrs in FAA as some RNA lysis may occur after this time-transfer contents to 70-80% Alcohol.

The F13 fixative of Adrian Warmington, Clyde Riley et al is certainly worth having a look at for antigen preservation. It is a NBF replacement fixative based on Alcohol and Polyethylene Glycol. The paper makes for very interesting reading and includes the results of a blind trial. A major benefit appears that the need for HIER is reduced or eliminated for some antigens. Anyone wanting the article name etc. let me know.

Mike Rentsch

# Oxalic Acid – Safety Issue

Oxalic acid is one of the most common reagents used in histotechnology. It is a reducing agent that often follows Potassium Permanganate in several stains. It is used in such techniques as: Reticulin Silver stain, Shikata's Orcein Stain, the removal of melanin, iron and formalin pigments, Amyloid AA demonstration using Congo Red, and the Gelatine Silver stain for nerve fibres.

Oxalic acid is a dangerous chemical. One MSDS states (1):

> POISON! DANGER! MAY BE FATAL IF SWALLOWED. CORROSIVE. CAUSES SEVERE IRRITATION AND BURNS TO SKIN, EYES, AND RESPIRATORY TRACT. HARMFUL IF INHALED OR ABSORBED THROUGH SKIN. MAY CAUSE KIDNEY DAMAGE.

Oxalic acid is routinely used as a cleaning agent in the bottle and insulator industries. Dissolving the white crystals in water results in a mildly acidic cleaning solution. As it will not immediately burn the skin as a mineral acid or caustic solution will do, a user may think that the use of chemical-resistant gloves is an unnecessary precaution. Besides being corrosive, oxalic acid is extremely toxic and can be absorbed directly through the skin, possibly resulting in symptoms similar to those seen from acute ingestion of the compound (2).

Local and/or systemic effects of this toxin can be severe and can result in death. The compound complexes with calcium in the body. Kidney or brain damage could result from the formation of calcium oxalate deposits if sufficient amounts are absorbed or ingested. Oxalic acid is also found as an ingredient in some commercial powdered cleansers (2).

2% Oxalic acid is a useful glass cleaner in the laboratory. Waste Permanganate and oxalic acid solutions from stains can be used to remove many dye and silver stains from glassware. Treat soiled glassware with aqueous permanganate for a few minutes followed by reduction with oxalic acid. Unfortunately oil red O stains still refuse to be removed with this technique. Does anyone have a good technique for this most stubborn of stains (Gee this sounds like a commercial!). During World War I, rhubarb leaves were recommended as a substitute for other vegetables that the war made unavailable. Apparently there were cases of acute poisoning and even some deaths. Ingesting the leaves has also poisoned some animals, including goats and swine (3).

Oxalates are contained in all parts of rhubarb plants, especially in the green leaves. There is some evidence that anthraquinone glycosides are also present and may be partly responsible. It is not clear as to the exact source of poisoning from rhubarb, possibly a result of both compounds. The stalks contain low levels of oxalates, so this does not cause problems (3).

The biodynamic (toxicity) mechanism by which oxalic acid works is somewhat different from organic poisons and is more analogous to heavy metal poisoning. Organic poisons often work through at the biochemical level, e.g. cyanide by interfering with respiration at the cellular level, strychnine by screwing up inter-synaptic transmission. There are many molecular substances

in foods that offer no nutritional benefit, and must be processed and excreted. Oxalic acid, for example, is excreted in the urine, and its crystals are commonly found in microscopic urinalysis. Too much oxalic acid in the urine will result in kidney or bladder stones (3).
Calcium combines with oxalic acid to form the less soluble salt, calcium oxalate, which is also found in kidney stones. Plant leaves, especially rhubarb, cabbage, spinach, and beet tops, contain oxalic acid. Oxalic acid is also found in potatoes and peas. Vitamin C is metabolised to oxalic acid. It contributes to over-saturation of the urine with crystals and possibly to stone formation (3).

From an MSDS (Material Safety Data Sheet) for Oxalic acid, LD50 (LD50 is the Median Lethal Dose, which is the dose of a drug or chemical predicted to produce a lethal effect in 50 percent of the subjects to whom the dose is given) in rats is 375 mg/kg. So for a person about 145 pounds (65.7 kg) that's about 25 grams of pure oxalic acid required to cause death. Rhubarb leaves are probably around 0.5% oxalic acid, so that you would need to eat quite a large serving of leaves, like 5 kg, to get that 24 grams of oxalic acid. Note that it will only require a fraction of that to cause sickness (3).

So what are the symptoms of Oxalic Acid poisoning?
On the body as a whole one might experience weakness, burning in the mouth, death from cardiovascular collapse; on the respiratory system - difficulty breathing; on the eyes, ears, nose, and throat - burning in the throat; on the gastrointestinal system - abdominal pain, nausea, vomiting, diarrhoea; and on the nervous system - convulsions, coma (3).
Safety Precautions? Wear goggles, lab coat, and gloves. When using the solid, use a chemical hood.

**References:**


1. Mallinckrodt Baker, Inc. MSDS Number: O6044 --- Effective Date: 02/08/01
2. http://www.fohbc.com/FOHBC_References2.html
3. http://www.rhubarbinfo.com/rhubarb-poison.html

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**2005 - 2006**

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