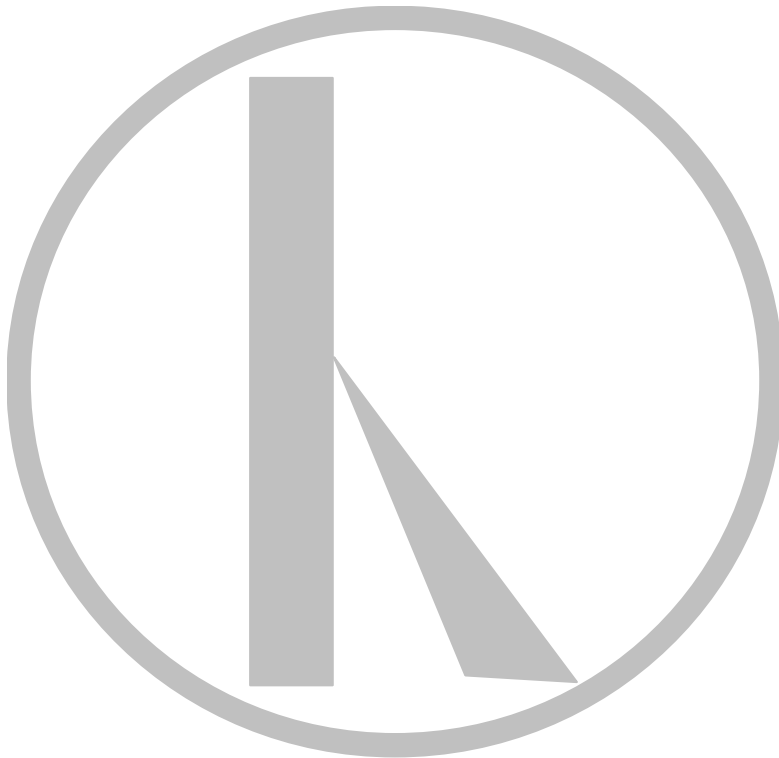


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# Histograph

**[www.histonsw.org.au](http://www.histonsw.org.au)**

ISSUE 3  
November 2010



**Newsletter of the Histotechnology Group of NSW**

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## Editorial

We are quickly approaching the end of the year and our Christmas Meeting at North Ryde is imminent. It has been a busy year and 2011 appears to be more intense. I have included several articles of current interest as well as answers to the Test and Teach challengers presented by Linda Gomes in the last issue.

The RCPA has also introduced Founding Fellowships of the Faculty of Science, an innovation that I hope will have a positive affect on our discipline.

Our National Meeting is next year at Rosehill Racecourse and the organising committee is galloping along like Americano in its plans. Any ideas for workshops/seminar sessions please contact the NSW Committee.

I hope to have a Christmas issue out but if unable, I hope you have a happy and restful holiday period.

Tony Henwood,  
Editor  
[anthonyh@chw.edu.au](mailto:anthonyh@chw.edu.au)

# Chairman's Report

Our Annual General Meeting was held in August in conjunction with a very interesting presentation on Lymphomas. Dr Ivan Burchett, a Pathologist at Douglas Hanley Moir, discussed lymphomas from diagnosis to treatment referring to specific cases.

Congratulations to the Committee Members who were elected at the AGM. We have a good group of hard working members who are dedicated to ensuring we provide interesting and informative meetings.

Our next function is our Xmas party on the 7<sup>th</sup> of November at North Ryde RSL. The guest speaker is Doug Roser, a volunteer with the Royal Flying Doctor Service. Doug has had an interesting career with the Royal Australian Air Force, Civil Aviation Authority and as a Project Director of Aids projects in the Philippines, Papua New Guinea and Indonesia. Do not miss this great night, all welcome.

An update on the 5<sup>th</sup> National Histology Conference. The format of the program has been finalised and we are in the process of discussions with potential speakers. A contract has been signed with Moreton Hire for the exhibition area; companies are in the process of being contacted with conference details. Information is about to be sent to members.

For information on future meetings and matters relating to the “ Histotechnology Group of NSW “, visit our website, [www.histonsw.org.au](http://www.histonsw.org.au).

Our Committee extends our best wishes for Xmas and the New Year. Have a safe and enjoyable Holiday break.

Cheers,

Trevor Hinwood.

Chairperson.

Histotechnology Group of NSW.

## Committee Members

Chairman	Trevor Hinwood	<a href="mailto:trevor.hinwood@hdsscientific.com.au">trevor.hinwood@hdsscientific.com.au</a>	Product Specialist H D Scientific
Secretary	Kathy Drummond	<a href="mailto:kdrummond@dhm.com.au">kdrummond@dhm.com.au</a>	Senior Scientist Histology DHM
Assistant Secretary	Dianne Reader	<a href="mailto:dreader@nscchahs.health.nsw.gov.au">dreader@nscchahs.health.nsw.gov.au</a>	Senior TO Anatomical Pathology RNSH
Treasurer	Lin Parkes	<a href="mailto:evpoint2@bigpond.com">evpoint2@bigpond.com</a>	
Membership Secretary	Margaret James	<a href="mailto:megajam@optusnet.com.au">megajam@optusnet.com.au</a>	Dutta Path
Trade Rep			
Country Rep	Margaret McLauchlan	<a href="mailto:mclauchlans@bigpond.com">mclauchlans@bigpond.com</a>	Nextpath
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	Liam O'Donnell	<a href="mailto:liam@sydneyskinpathology.com.au">liam@sydneyskinpathology.com.au</a>	Sydney Skin Pathology
	Bharathi Cheerla	<a href="mailto:bcheerala@dhm.com.au">bcheerala@dhm.com.au</a>	IHC Histology DHM

# Review of Von Kossa's Stain

The Kossa method (1901), although widely used for localizing calcium in routine histology, actually demonstrates anions such as phosphate, carbonate, oxalate and fatty acids. In normal and pathologic deposits these anions are mainly combined with calcium, but to a smaller extent other metals may also be involved (1).

It is possible, though, to render this method specific for calcium by initial treatment with oxalic acid thereby converting calcium compounds into insoluble calcium oxalate and removing the other sorts of anions which otherwise co-react under the original Kossa method. In tissue sites containing small amounts of calcium the sequence of oxalate pretreatment and Kossa technique results in submicroscopic grains of metallic silver which can be enlarged up to microscopic dimensions using a physical developer (1). This results in a high

grade intensification of the original staining.

Gallyas & Wolff (1) have published the following method:

- 1) Treat frozen or paraffin sections with 1M oxalic acid dissolved in 50% acetone for 5 min.
- 2) Wash in distilled water 4 times for 30s each.
- 3) Immerse in 50% silver nitrate dissolved in 15% hydrogen peroxide for 5min (This solution can be stored in a refrigerator for months and used several times).
- 4) Wash with a solution containing 0.1% of silver nitrate, 0.2% of ammonium nitrate and 1% of acetic acid 4 times for 1 min.
- 5) Place a Petri dish containing the last washing solution (4) and the sections under an ultra-violet lamp for a period of up to 15 min, depending on the actual light intensity (e.g. 10 min using a Zeiss HBO-202 mercury vapour lamp placed at a distance of 80 cm from the horizontally placed sections covered by a 2 mm thick layer of the washing solution).  
Sections containing calcium turn pale yellow, whereas control sections remain colourless. The control sections are treated with 10% ethylenediamine tetraacetic acid (EDTA) di-sodium salt or 1M hydrochloric acid for 30 min before step 2.
- 6) Transfer the sections in a special physical developer and leave them until they have turned deep brown, but not longer than the time needed for the control sections to turn pale yellow (3-5 min depending on the calcium content of the section and temperature of the developer). The developer should be prepared immediately prior to use by slowly adding 10 ml of stock solution B to 10 ml of stock solution A under vigorous stirring.  
Stock solution A consists of 50 g sodium carbonate (anhydrous) dissolved in 1,000 ml of distilled water.  
Stock solution B consists of 2.0 g ammonium nitrate, 2.0 g silver nitrate, 10.0 g tungstosilicic acid and 1ml of 37% formalin dissolved in 1,000 ml of distilled water, added in this order.
- 7) Wash sections in 1% acetic acid for 30 min, dehydrate with alcohol, clear with xylol and mount in DPX

Technical note: Apart from the physical developer which works preferably between 20 and 25 °C, the temperature of the other solutions should be kept as low as possible, maximum 25 °C.

Tissue calcium is immobilized by acetic oxalic acid, which simultaneously removes the other sorts of anions capable of precipitating silver ions (e.g. phosphate, carbonate). The resulting submicroscopic grains of calcium oxalate are converted first into silver oxalate then into metallic silver by a treatment with silver nitrate followed by an ultra-violet irradiation (Kossa reaction). These submicroscopic metallic silver grains are enlarged up to microscopic visibility by means of physical development, which makes the staining highly sensitive. Co-staining of the argyrophil sites in the tissue is totally suppressed by various tricks, which render the silver staining selective for calcium.

## References:

1. Gallyas F, Wolff JR "Oxalate pretreatment and use of a physical developer render the Kossa method selective and sensitive for calcium" *Histochemistry* (1985) 83 : 423-430.

# AGM Chairman's Report

During the past year we held five meetings:

- The first being “Viruses- the truth is out there “. Presented by Sue Anderson, Clinical Virology, ICPMR. This evening was also our AGM being held at North Ryde RSL.
- We were all at sea with our Christmas Party at North Ryde RSL. Our speaker was Alan Edenborough who is involved in the restoration of Historic ships.
- The Central Coast 2 day meeting. This covered a range of topics presented by Surgeons. Pathologists, Researcher and a retired Laboratory Manager from ICPMR. Held at Toukley RSL. A great weekend organised by Marg and Steve McLauchlan.
- The Garvin Institute hosted a meeting on “ Biomarkers in Pancreatic Cancer “. Presented by Emily Colvin. The meeting was also supported by Dako.
- Douglas Hanley Moir hosted a presentation given by Dr Murray Killingsworth, Anatomical Pathology, Liverpool Hospital. The presentation covered “ Electron Microscopy in Anatomical Pathology- diagnostics to research “

We encourage members and friends to attend these meetings. We have interesting topics and speakers. It is also a great opportunity to meet and talk to fellow members.

Planning for the next National Histotechnology Conference is well advanced.

The date is the 4<sup>th</sup> to 6<sup>th</sup> of November 2011.

Venue is Rosehill Gardens Event Centre, Rosehill, Sydney. In 2009 this venue was awarded a National award, the “MEA Banqueting and Catering award”.

Accommodation is at Rydges, Parramatta, opposite the venue. A link has been established on our Website.

Costings are currently being finalised

Speakers, topics and the program are being worked on.

Note this in your diary and tell your friends, it will be a great conference.

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

Our website [[www.histonsw.org.au](http://www.histonsw.org.au)] has been further developed during the past year. Check it regularly as information on meetings and news items is being constantly updated. Membership information and renewal forms are also incorporated into the website. A section on the “National Conference” is currently under construction.

For those who have not renewed their membership, this is now due. Please do this as soon as possible. You are an important component of our Histotechnology Group.

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

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

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

Trevor Hinwood.

Chairperson.

Histotechnology Group of NSW.

## Christmas Meeting Notice



# Christmas Meeting & Party

*North Ryde RSL*

*26 November*

*Speaker is Doug Roser*

*Royal Flying Doctor Service.*

*Fully catered Christmas dinner.*

## Cryostat Decontamination- what a pain in the butt!

*Comments on the new proposed CAP standard by Jeff Silverman*

CAP is moving to more rigorous cryostat decontamination methods - mandating a weekly shutdown and wet chemical disinfection with a tuberculocidal agent for machines used regularly. Now, if a lab is doing 1-3 frozens a week, is that used regularly? We must lobby the CAP for more sensible and practical guidelines. The old wipe down with 70% ETOH without bringing the machine to room temperature for a full decontamination will become non-compliant and useful only as an interim measure. By the way, the UV lamps do not satisfy the CAP standard, I believe.

Our system has gone to a commercially available tuberculocidal, virucidal, and broad spectrum bacteriocidal moistened wipes.

Here is a skeletonized basic procedure form what CAP will require:

1. Remove all utensils and brush out and collect section debris disposing of this according to regulated medical waste protocol.
2. Bring the instrument to room temperature.
3. Wipe all working surfaces with the tuberculocidal wipes, visibly moistening all surfaces. Surface must remain wet for 2 minutes. Use multiple wipes as needed. Instruments can be similarly disinfected.
4. Carefully dry all surfaces with gauze. Dispose of all wipes and gauze as biohazardous.
5. Dry and lubricate the microtome as per manufacturer's instructions.
6. Turn on cryostat and bring to working temperature.
7. Document procedure in your maintenance log.
8. Look forward to doing it again next week.

Look, this is a necessary procedure, but weekly??? Perhaps some workload- based formula or an alert system- like pathologists alert the lab when a case with granulomas or caseating necrosis is sectioned. Every lab will have to bring a tech in on weekends or at night, to do this, or have two cryostats to compensate for the fully one working day most machines will have to be down to be cleaned in this manner.

# *Surgical Cut-Up Workshop*

## **Are You Interested in Our Surgical Wet Cut-Up Workshop 2011?**

- Gain practical skills with ethically collected tissue
- Held on Saturdays 5th March, 7th May, 2nd July and 3rd Sept 2011.
- Each session runs 10:00am to 3:00pm, morning tea and lunch included
  
- 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
- 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**

Maximum intake 12 only so be quick!

Cost: \$110 per session or a total of \$400 if paid before first session.

Contact: Anne Prins 02 6125 4644 [anne.prins@anu.edu.au](mailto:anne.prins@anu.edu.au)  
Penny Whippy 02 6244 2874 [penelope.whippy@act.gov.au](mailto:penelope.whippy@act.gov.au)

# **Treasurer's Report: Annual General Meeting**

Report for Financial Year 1/07/2009 – 30/06/2010

1) Sydney Credit Union Account # 94099

Opening Balance @ 1/7/2009	\$ 33,833.86	Cr.
Receipts 1/7/09 – 30/6/10 ..	10,680.78	
Include: Memberships; advertising revenue–Histogram; Bank interest; scientific meeting fees; ATO refunds on BAS	<hr/>	
	44,514.64	

Payments 1/7/09 – 30/6/10	14,704.97	
Include: Function venues; Insurance; storage facility; BAS; Maintenance of web-site; stationery; costs of producing Histogram; Financial support for scientific meetings etc; speaker gifts; Stationery; replacement lap-top computer, software & warranty	<hr/>	

Closing Balance @ 30/06/2010	\$ 29,809.67	Cr.
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Held in: Sydney Credit Union

S4 Business A/c # 94099..	\$ 6,934.00	
Term Deposit # 94099 I79..	\$ 22,875.67	
	<hr/>	
	\$ 29,809.67	Cr.

2) Sydney Credit Union Account – National Conference 2011, Separate Float A/c

14/10/2009	Received Float from S. Aust Histo Group	\$ 15,000.00
	Interest	275.70

	<hr/>	15,275.70
Payments 14/10/09 – 30/6/10		1,201.51
Include: deposit on venue Rosehill Gardens Event Centre, incidentals	<hr/>	

Closing Balance @ 30/6/2010	\$ 14,074.19
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Held In: Sydney Credit Union

S4.1 Business A/c # 94099	\$ 4,074.19	
Term Deposit # 94099 181	\$10,000.00	
	<hr/>	
	\$14,074.19	Cr.

Deposits Paid on venues (included in above) in advance of events:

\$ 200.00	Nth Ryde RSL	HTG NSW 20/8/10 - AGM
\$ 500.00	Nth Ryde RSL	HTG NSW 26/11/10 – Christmas Function



As Hon. Treasurer for the past 12 months I offer you my annual Report for your consideration.

Not a lot to comment on with the exception of Receipts being down over the previous year. We did have a generous sponsorship by Leica for our Christmas function in 2008 which of course helped but not this past year. However with additional revenue received from advertising in the Histogram this year, we are travelling okay. The 2010 year had a strength of 110 paid-up Members, including 5 Trade Memberships and of course our several Life Members. Our aim would be to increase Membership for the 2011 Year if possible.

Outgoings increased this year with the additional cost of 'hosting' our web-site. This amounts to an extra \$600 annually so therefore would like our members to make use of it to the max! Liability insurance as well as our Storage facility have also increased in cost. In addition we had the need to up-grade our lap-top complete with the necessary software and warranties. This outlay was just short of \$2,000. We also had a loss on the Central Coast Meeting in April this year due to lack of support and on our annual Christmas Function. In all cases, an increase in numbers supporting these arranged events would help to turn the figures around. Hint: please speak with your fellow Histotechs!! The ones who do support us always have a great time!

As we had more funds leaving our bank account than was received the last three quarters of the BAS (GST reporting) have been refunds to our Histo Group.

On saying that we are still in a financially healthy position carried forward from the State Conference held in Canberra in 2007. As Trevor will mention the committee is working hard on the next National Conference to be held in Sydney in November, 2011, we being the host Group. We trust we will come out of that satisfactorily – either at a small profit but hopefully no loss – all depends on numbers supporting it!

Now, those EFT Memberships and the like. We still have an unknown Member from last year who would not have received a receipt nor any paperwork from us due to NOT adding his/her name for the Reference! NETBANK (Commonwealth Bank) do not display your name when it appears on our Credit Union statements unless you add your own name at "Reference". Most are catching on now however 'someone' on 28/7/2010 sent from NETBANK \$38.50 with a Reference as "Histo"! Any idea who this may be????????? I don't!!!!!! No Membership form received by us, no receipt sent from us and no future paperwork will be available to him/her until identified! Ahhh!

Emailing Tax Invoices/Receipts to you is my preference. Upon your receiving it please check "Balance Due" before sending us another \$38.50 for the year! Some people are doubling up and I either need to do a refund or credit them for the following year – all really unnecessary extra work. I will endeavour to get my IT person to change the "Tax Invoice" to "Tax Invoice/Receipt" and that may help as well.

Once more, our thanks go to Grant Taggart and Douglass Hanly Moir Pathology for their very generous offer of allowing the committee the use of a meeting room at their Giffnock Avenue premises, free of charge.

Finally, thanks to all of you for supporting us in your separate ways and let us continue and make it a very successful 2011 Year!

The Financials for the 12 months ended 30 June, 2010 have been produced in detail and are available for your perusal tonight.

Lin Parkes  
Hon. Treasurer

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# EDTA in Decalcification

EDTA (ethylene diaminetetraacetic acid) can be found in several in-house and commercial decalcifiers. Gayle Callis remarks that the purpose of EDTA in an acid decalcifier is probably NOT performing the actual removal of calcium (or very little) from the bone but chelating the calcium ionized from the bone by the acid which then settles to the bottom of the container. People who have developed acid decalcifying solutions, with either formic or hydrochloric acids tout that the bone section looks very good, better than if EDTA is present in the solution.

The acid is going to ionize the calcium from bone at a faster rate than the EDTA can chelate the calcium. But most importantly, EDTA does not work in a low pH environment. The chemistry of how EDTA acts at different pHs is well documented by chemists, and found in book chapters on EDTA. Gayle reports that the pH of formic acid is about pH 3, and if so, EDTA only begins to chelate calcium at around pH 4, and when fully protonated at pH 8, decalcifies faster at that pH

than at pH 4, or below pH7. The working pH for most EDTA solutions is commonly 7 to 7.6, but when the pH becomes more alkaline (pH 8) then alkaline sensitive protein linkages can be damaged.

EDTA comes in several molecular weights depending on whether it is EDTA without attached sodium, EDTA disodium or EDTA tetrasodium. Tetrasodium is very soluble in water or PBS, but has a very alkaline pH that requires adjustment down to pH 7 to 7.6 otherwise the high pH will damage alkaline sensitive protein linkages. EDTA and disodium EDTA are not as soluble, usually no more than a 10% solution, requiring heat or addition of sodium hydroxide in order to dissolve. EDTA is also expensive. EDTA is not affected by heating up to 60°C but only if the bone is totally fixed with formalin. Gayle would not advise using lengthy 60°C EDTA decalcifications, due to possible damage to heat labile antigens. There are publications on using EDTA to decalcify fresh bone samples, then snap freeze, cut frozen sections, then acetone fix the

sections for murine CD markers that were compromised by both NBF and acid decalcification.

EDTA is a slow decalcifier but it does not cause damage to antigens, nor does it affect nucleic acid or other tissue component staining. It does affect the enzyme histochemical stain for alkaline phosphatase, and magnesium ions (chelated by EDTA) must be replaced in the staining solution. Most clinical laboratories prefer to use formic or hydrochloric acid decalcifying solutions instead of EDTA when a rapid diagnosis is needed.

If you use EDTA alone, rinse the bone well (after decalcification) with running tap water since residual, excess EDTA in tissue precipitates in the presence of alcohol, and the precipitate makes the tissue difficult to section. Endpoint determinations for complete removal of calcium with EDTA cannot be done chemically, although there is a weight gain/weight loss method that can be used or use a Faxitron.

## Histogram Advertising Charges

A4 PAGE - Printed Copies Supplied By Advertiser	1 or 2 sides..... \$75.00
A4 B&W PAGE - Printed In The Journal	1 A4 page ..... \$100.00
A4 COLOUR - Printed In The Journal	1 A4 page .....\$300.00

# Founding Fellowships of the Faculty of Science, RCPA

An interesting innovation has been instituted by the Royal College of Pathologists of Australasia. On 10 December 2009, in an historic vote, the membership of the College agreed to the inception of The Faculty of Science under Article 57c of the Articles of Association. Earlier this year the First Faculty Committee was tasked with the establishment of the Faculty. This Committee is responsible for recommending to the RCPA Council, appointment of Founding Fellows from among the ranks of distinguished scientists and pathologists who have made significant contributions to science in pathology and who demonstrate a desire and commitment to promoting science in pathology through the College into the future. The Founding Fellows will form the basis of the new Faculty and will have the opportunity to nominate and vote for the Faculty Committee at the inaugural Annual General Meeting to be held in March 2011 at the Pathology Update in Melbourne.

Successful admission to Fellowship will be accompanied by the ability to participate in many College activities, awarding of the post-nominal FFSc (RCPA) and will require payment of an Annual fee. Founding Fellowships are available until 31 December 2011. Beyond this date, new criteria will be applied for application to FFSc (RCPA) which may require participation in College education and assessment programmes.

The RCPA Council approved the following Criteria for Admission of Founding Fellows (By-law 8):

1. Qualifications (By-law 7.1):

- a) Fellowship of the College or possession of an MBBS, BSc or BAppSc, or equivalent undergraduate degrees, from a university recognised by Council and
- b) Possession of a research doctoral degree (MD (Research), PhD, DSc), in a discipline of pathology, from a university recognised by Council or Fellowship, by examination, of a professional body recognised by Council (for example, FAACB, FAIMS, FASM, FHGSA).

AND

2. Significant distinction and ongoing contribution and excellence in the science relating to pathology (By-law 8.2). It is expected that successful applicants would ordinarily demonstrate this by:

- a) Publication of a substantial body of original scientific work in a discipline of pathology, and at least one of the following
- b) Possession of a senior scientific leadership position or role within pathology,
- c) Award of significant peer-reviewed grant funding for research into the science relating to pathology,
- d) Evidence of innovation, such as a significant scientific discovery or invention of importance to the practice of pathology,
- e) Significant involvement in teaching and training in pathology.

AND

3. Place of work (By-law 7.1(1)(a)(i): Founding Fellows should be entitled to work as a pathologist or scientist in his or her country of domicile.
4. Nomination: Non-Fellows require nomination by two Fellows of the College.
5. Exemptions: Council, at its discretion, is able to award Founding Fellowships of the Faculty of Science to applicants who do not meet all of the above criteria.

If you have any queries, regarding Founding Fellowship or the Faculty of Science, please contact Dr John Burnett, Chair, First Faculty Committee, Faculty of Science, RCPA via email at [john.burnett@health.wa.gov.au](mailto:john.burnett@health.wa.gov.au) or Prof Paul Waring, Vice-Chair, First Faculty Committee, Faculty of Science, RCPA via email at [pwaring@unimelb.edu.au](mailto:pwaring@unimelb.edu.au).

# Nailing Onychomycosis

With a prevalence of 2 to 18% in world's population, onychomycosis is the most common nail disease accounting for up to 50% of all nail diseases. Although not life threatening, it can cause pain, discomfort and disfigurement, and may produce serious physical and occupational limitations. Dermatophytes are the most common cause with *Trichophyton rubrum* and *Trichophyton mentagrophytes* being the most common pathogens (1).

Traditionally, potassium hydroxide preparation and fungal culture have been the preferred methods used for the diagnosis of onychomycosis. However, with the low sensitivity of both methods and the time needed for growing the organisms in culture, periodic acid-Schiff (PAS) staining of the suspected nail specimens has been studied, and a number of investigators found this stain to be more sensitive and to have higher negative predictive value (1).

In Grocott's Methenamine Silver (3) method, oxidation of a polysaccharide component of fungal cell walls with chromium trioxide (chromic acid) generates aldehyde groups. In the second step of the method, the aldehydes reduce a methenamine-silver

complex, depositing dark brown or black colloidal silver. The aldehydes are detected with Schiff's reagent in the related Bauer chromic acid-Schiff methods (3).

D'Hue et al (2) compared GMS to PAS and concluded that GMS is superior to PAS for the diagnosis of onychomycosis, being both more sensitive and qualitatively better. In their study, they were able to detect fungal organisms on GMS stained slides in 5 out of 51 originally PAS-negative cases.

Reza Kermanshahi & Rhatigan (1) performed both PAS and GMS stains on 30 clinically suspected onychomycosis cases and found twenty-two cases were positive with PAS, staining three cases more than GMS, which stained only 19 cases. The difference between the two stains was not statistically significant. Repetition of GMS stain on these three cases (cutting in a deeper level in the paraffin block) resulted in positive results. Four out of 30 cases were negative with original PAS stain, but stained positive both with the repeat PAS and GMS.

Reza Kermanshahi & Rhatigan (1) noticed that when the organisms were abundant in the

specimen, they had no difficulties in identifying them by either stains. However, when they were few in number and inconspicuous, the greater contrast of the GMS stain in comparison to the light green background made identification easier, an observation made by D'Hue et al (2). This feature of GMS stain is a result of using chromic acid, a strong oxidant, which reduces the staining of background tissue structures, such as reticulin, collagen and basement membranes. Whereas when using PAS stain, the difficulty in identifying the organisms is in part secondary to using periodic acid, a weak oxidant, that results in staining not only polysaccharide-laden wall of fungal organisms, but also all polysaccharides wherever they appear (1).

Freda Carson (3) also reports that chromic acid is the preferred oxidizer for fungi to be stained with Schiff's reagent, because it oxidizes many tissue carbohydrates to aldehydes, then to carboxylic acids, which do not stain, so the fungi stand out on a paler background than after oxidation with periodic acid. The periodic acid-Schiff (PAS) procedure is notoriously poor for staining *Histoplasma* owing to incomplete oxidation.

## References:

1. Reza Kermanshahi T, Rhatigan R. "Comparison between PAS and GMS stains for the diagnosis of onychomycosis" J Cutan Pathol 2009.
2. D'Hue Z, Perkins SM, Billings SD. "GMS is superior to PAS for diagnosis of onychomycosis" J Cutan Pathol 2008; 35: 745-747.
3. Carson F "Periodic acid cannot replace chromic acid in Grocott's method for fungi" Biotechnic & Histochemistry 2010, 85(4):270.

# Answers to Last Issue's Test and Teach



## Stains Questions

1.	Favourite nuclear stain	Haematoxylin
2.	Congo red stains this	Amyloid
3.	This should be neutralised with NaCl before discarding	Ammoniacal Silver
4.	A good stain for elastic fibres	Verhoeff's Elastic Stain
5.	What substance does Fouchet's stain	Bile
6.	A good fat stain	Oil Red O
7.	A stain for fungi ( 3 words)	Groccotts Methenamine Silver
8.	Staining process for mast cells.	Azure A
9.	Gordon and Sweets stain these fibres	Reticulin
10.	Stain for positive and negative organisms.	Gram

## TRUE or FALSE

Ref. Choice Health Reader

1. Circadian rhythm has an effect on the effectiveness of cancer treatments..... **True**  
(depending on the time of day of administration of chemotherapy or radiotherapy it can affect the potency of the treatment)
2. Eating carrots will protect your eyes against the glare of computer screens..... **False**  
(Leafy green are rich in lutein a powerful antioxidant which may protect your eyes against the glare of computer screens)
3. Indigo naturalis is a red plant-based powder used to treat psoriasis..... **False**  
(it is a blue plant-based powder common in traditional Chinese medicine.)
4. "French paradox" is a term referring to the French enjoying regular consumption of red wine and experiencing low rates of heart disease ..... **False**  
(It is generally thought the red wine consumption protects the French against heart disease but the paradox is the high fat diet and the low incidence of heart disease)
5. Diverticular disease is a disorder effecting one-third of the American population over 60. **True**
6. Eating meat, fish and dairy products and drinking alcohol could protect the brain from shrinkage in old age..... **False**  
(Vitamin B12 found in meat, fish and dairy products is important in cognitive function and drinking tea, which contains polyphenols are powerful anti-oxidants.)
7. Cardiorespiratory fitness can reduce the risk of dying of cancer associated with being overweight..... **True**  
(1 hour of moderate exercise most days of the week, reduces the risk of dying of cancer associated with fatness)

8. Human breast milk is a sterile food source for babies..... **False**  
(Human breast milk contains a diverse range of bacteria which may play an important role in the baby's immunity).
9. It is no longer necessary for you to see your GP to get a medical certificate; your pharmacist can do instead..... **True**  
(Changes to workplace legislation in 2008 issued a list of "registered health practitioners" which included pharmacists. Guidelines for issue is quite restrictive, the greater majority being for colds and flu)
10. Smokers are more likely to have a restless night than non smokers..... **True**  
(Scientists from John Hopkins University USA found smoking tobacco may contribute to sleepless nights)

## Match the invention with a year

1.	Ball Point Pen	1938 Laszlo and Georg Biro
2.	Concrete	133BC Romans
3.	Mercury Thermometer	1714 Gabriel Fahrenheit
4.	Magnifying Glass	1250 Roger Bacon
5.	Telescope	1608 Hans Lippershy perfected by Galileo
6.	Tea Bags	1919 Joseph Krieger
7.	Bicycle	1839
8.	Microprocessor	1965 IBM
9.	Compound Microscope	1590 Hans & Zacharias Janssen
10.	Ink	2500BC China

## Anatomy questions

1. Located between the capsule and medulla of the kidney. **CORTEX**
2. Islets of Langerhans are found here. **PANCREAS**
3. Parathyroid glands help regulate this mineral. **CALCIUM**
4. These dramatically increase the surface area of the intestine. **VILLI**
5. The pineal gland is here. **BRAIN**
6. The most common molecule in the body. **WATER**
7. Which is most superficial: dermis, epidermis or hypodermis? **EPIDERMIS**
8. This lung has 3 lobes. **RIGHT LUNG**
9. This organ plays a key role in T cell differentiation. **THYMUS**
10. The white of the eye is called this. **SCLERA**

## Notice of National Scientific Meeting 2011

*Histotechnology Groups of Australia*

# National Scientific Meeting

Where:

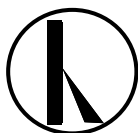
**Rosehill Gardens Event Centre, Rosehill, Sydney**

*In 2009 this venue was awarded a National award, the "MEA Banqueting and Catering award".*

When: **4-6<sup>th</sup> November 2011**

Accommodation: **Rydges Rosehill**





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