



# HISTOGRAPH

## Editorial

Hi and welcome to the second issue of the Histogram for 2019. In this issue we have a few interesting articles for you to enjoy reading. Tony Henwood, former editor of the histogram has recently launched his book titled "Survival Guide for Laboratory Professionals". He has kindly allowed us to reproduce a sample chapter from his new book. Hope you enjoy reading it.

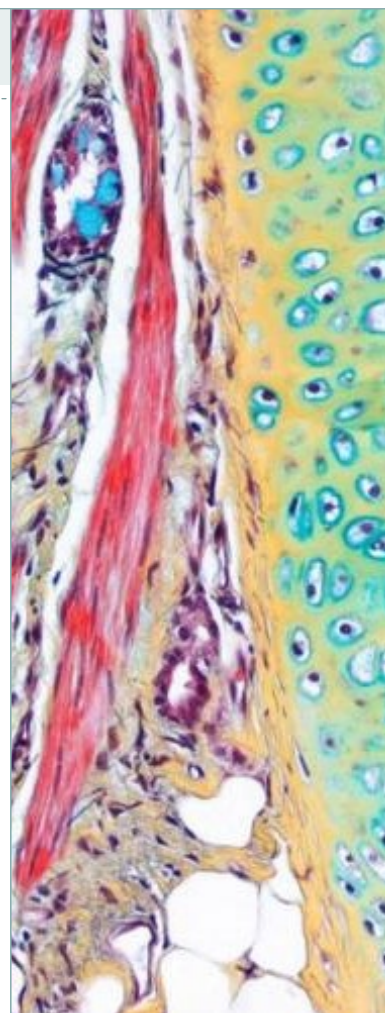
Remember to try your skills with our Test and Teach quiz. It is simple and highly educational. Try and identify the tissue and the pigment.

Thank you to Jerres Alcober and Kellie Vukovic committee members of the Histotechnology Group of QLD for allowing us to publish their newsletter coverage of the 9th National Histology Conference 2019 in Adelaide.

In recent years digital pathology is becoming an integral part of many laboratories. A number of histology labs are beginning to implement digital pathology to improve workflow and cut down on storage. Adopting digital pathology - An informative article written by Caroline Eddy.

Please enjoy reading this issue.

**Linda Prasad**



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**Histotechnology Society  
of NSW COMMITTEE  
MEMBERS are  
volunteers who work  
tirelessly to promote  
histotechnology and  
provide educational  
opportunities for  
continuing professional  
development. Thank you  
team for the GREAT  
JOB**

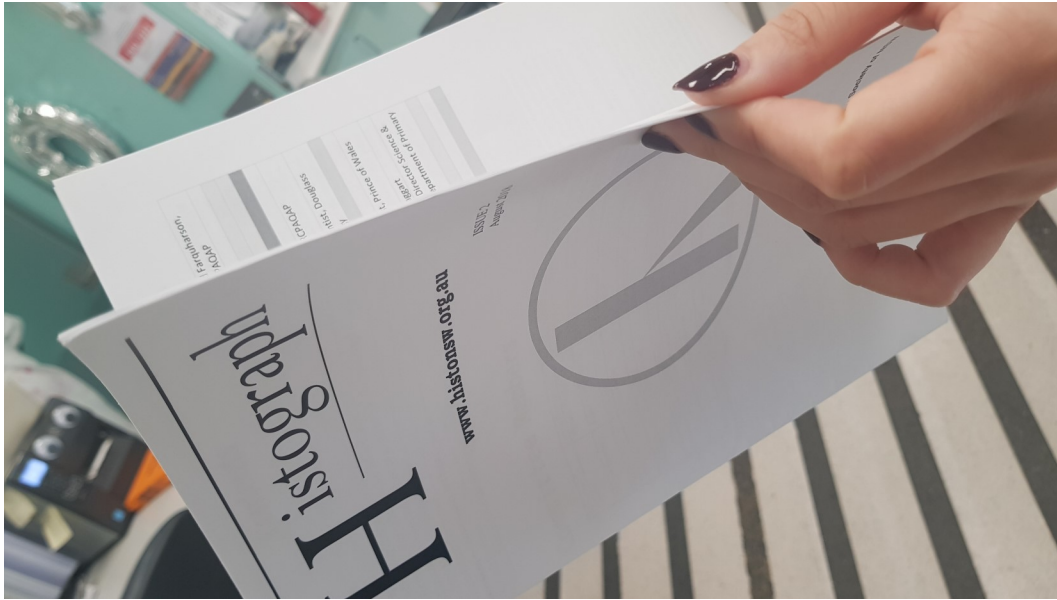
## COMMITTEE MEMBERS

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Committee Member	Tamara Sztynnda	UTS
Student Representative	Adrian Ureta	TAFE Ultimo
Student Representative	Andrew Da Silva	TAFE Ultimo

## Sub-committee 2018-2019 (Media & Newsletter)

Position	Name	Organisation
Editor- Histograph	Linda Prasad	Children's Hospital, Westmead
Sub-committee Member	Momoko Sakaki	Children's Hospital, Westmead
Sub-committee Member	Cristina Antonio	Douglass Hanly Moir Pathology





## Chairman's Report

The Adelaide Conference has come and gone. A very successful Conference. The feedback from delegates has been positive so well done to the Adelaide organising committee.

Several workshops have been held since the last Histograph:

In March we held a "Fixation" workshop at "The University of Technology". This was a dry Workshop with presentations by Dr Tamara Sztynka and Dianne Reader which involved discussions and interaction with the attendees. There were a few firsts with this. The first workshop at UTS and the first workshop aimed at supporting students. Despite it being a wet morning we had 56 attendees, mostly students from local Universities. The feedback was very positive and it is something we need to consider in planning workshops next year. There is a definite need for student support with basic histology techniques.

In July we held a "Wet Staining Workshop [Microorganism Staining]" again in the laboratories in UTS. Organising and the presentation by Dr Tamara Sztynka and Dianne Reader with some committee helpers on the day. Dianne prepared an excellent booklet. Twenty Eight participants, so it was well supported. A successful workshop and a few lessons learnt for next wet workshop at UTS. The facility is ideal for wet workshops.

Here is an update on the "Certification of the Med Lab Scientific Workforce" being prepared by Human Capital Alliance. At the Adelaide National Conference we held a meeting of State committee representatives. At this meeting Lee Ridoutt from Human Capital Alliance presented an update on the Certification scheme.

The other States now have updated information and there is general agreement that this project needs to be dealt with at a National level. Certification is due to commence the 1<sup>st</sup> of July 2020 with certification on a voluntary basis.

During the State meeting of committee representatives we also discussed the formation of a National Committee. This has been proposed for many years and for whatever reason has not progressed. It was pleasing to see a unanimous decision by those present to move forward with this. Mark Bromley has agreed to oversee its formation and we are well underway with the preparation of a Constitution. It has been agreed we will have two representatives from each State. The NSW committee recently agreed for Bharathi Cheerala and myself to be our representatives. This makes sense as both of us are heavily involved in the organising of the next National Conference.

The next National Conference will be held at the “International Convention Centre” [ICC ], Sydney, 3<sup>rd</sup> to the 6<sup>th</sup> of June 2021. This will be a joint Conference with the Australasian Division of the International Academy of Pathology (IAP). Currently we are working closely with the IAP organising committee to ensure a successful Conference from both sides. With the venue booked and the dates set we are starting to look at topics and speakers. Early days although still a lot happening.

Future workshops and meetings:

We will be holding our first webinar workshop on the Monday the 26<sup>th</sup> of August at 6pm to 7.30pm. Topic, Microscope slides,

the “Old and the New”. A history of their development, types, handy tips in using them and the latest developments. For best results you need to match the slide to your application. Convened by Dr Tamara Sztynnda and presented by Trevor Hinwood and Alex Anderson. This is a free webinar open to everyone. Register through the Histotechnology Society of NSW website for the link to the webinar. [histonsw.org.au](http://histonsw.org.au). You will also receive a certificate of attendance.

At UTS we are holding another dry workshop on Advanced Tissue Recognition, navigating the Gastro Intestinal Tract (GIT) on the 14<sup>th</sup> of September. More information to follow on our website and Facebook.

Our Annual General Meeting will be held on Friday the 25<sup>th</sup> of October at North Ryde RSL. The guest speaker is currently being organised.

Information on workshops and seminars is being updated regularly so please review our website and follow our Facebook page.

Cheers,

Trevor Hinwood.

Chairperson.

Histotechnology Society of NSW.

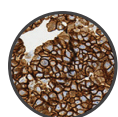




# HistoCyte Control Material

Laboratories often struggle for sustainable control material. **HistoCyte Laboratories** cell lines controls are standardized, developed, and manufactured to provide consistent results throughout the block.

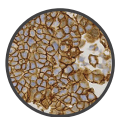
They have developed a range of controls, some with as few as two cell lines providing a positive and negative: Standard Controls. There is also a supply of more comprehensive products with as many as five cell lines, providing a range of expression and sensitivity: The Dynamic Range<sup>DR</sup>.



Breast Analyte Control<sup>DR</sup>



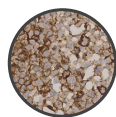
Estrogen Receptor Analyte Control<sup>DR</sup>



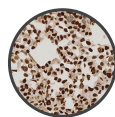
HER2 Analyte Control<sup>DR</sup>



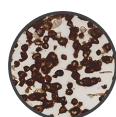
HPV/p16 Analyte Control<sup>DR</sup>



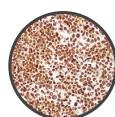
PD-L1 Analyte Control<sup>DR</sup>



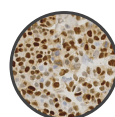
Progesterone Receptor Analyte Control<sup>DR</sup>



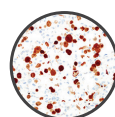
ALK-Lung (EML4-ALK) Analyte Control



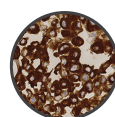
ALK-Lymphoma (NPM-ALK) Analyte Control



Breast Analyte Control (ER, PR and HER2)



HPV/p16 Analyte Control



ROS1 Analyte Control

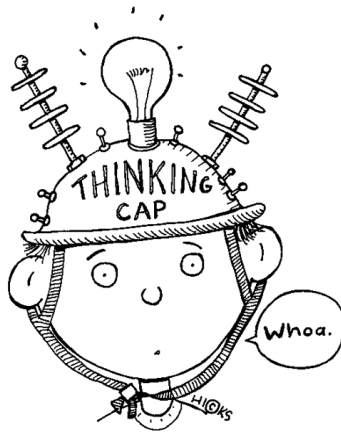
**HistoCyte now available in Australia.**  
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## TIME TO PUT ON YOUR THINKING CAP

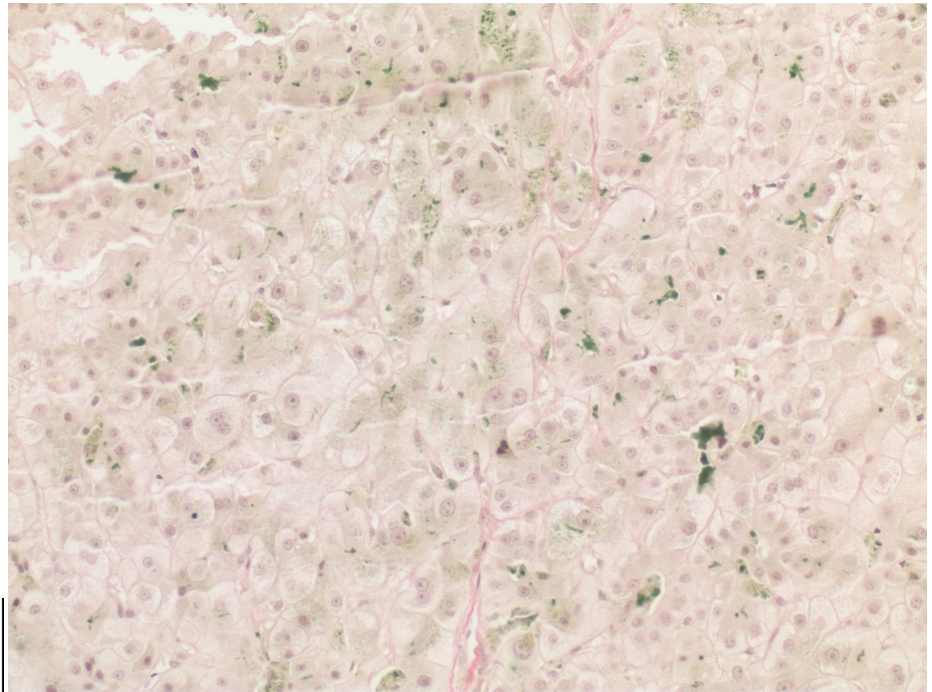
### QUESTIONS

1. What is the tissue
2. What is the pigment
3. What stain would you use to demonstrate it

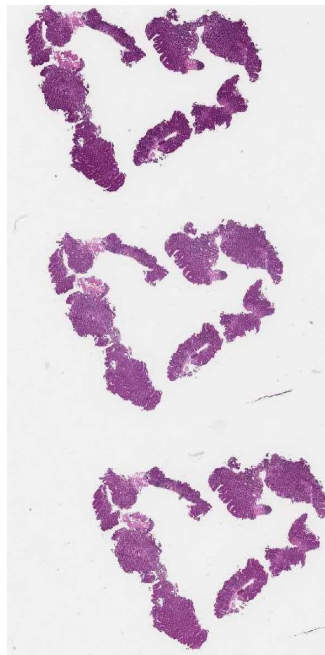


## TEST AND TEACH

By Tony Henwood



JOIN UP OR REMEMBER TO RENEW MEMBERSHIP



HISTO  
LOVE  
By Linda  
Prasad



# Strengthening International Relationships

In June 2019, Louie Cadao, Vice President of the Philippine Society of Histotechnology (PSH) visited Sydney and met with several members of the Histotechnology Society of NSW (HSNSW) (including Labby!)

It was great to establish a connection and friendship with an international histology society within our region.

During a trip to the Philippines in July, HSNSW Student Representative, Adrian Ureta, met with Louie and toured his lab at St Luke's Medical Center (Bonifacio Global City). Adrian also met with PSH President, Jomar Klee Z. Custodio and reaffirmed the relationship established during Louie's visit to Australia.

The Histotechnology Society of NSW would like to thank Jomar and Louie taking their time to meet with Adrian. We look forward to continuing our friendship with the Philippine Society of Histotechnology and sharing our knowledge and experience for many years to come.



Jomar Klee Z. Custodio and Adrian Ureta



Histology Lab, St Luke's Medical Center (BGC)



Louie Cadao and Adrian Ureta

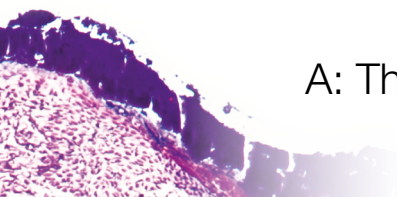


Histology Lab, St Luke's Medical Center (BGC)



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The Davidson Marking System is suitable for fresh and frozen tissue samples, and can be used pre- or post- formalin.

DMS dyes adhere to tissue throughout processing and are easily distinguished from each other under the microscope.



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The Davidson Marking System tissue marking dyes are available in two individual bottle sizes in eight colours, and a multi-colour set.

- Provided in 2 oz (59 mL) or 8 oz (237 mL) bottles.
- Available in green, yellow, red, black, blue, orange, violet and lime.
- Supplied as a multi-colour set of 5 x 2 oz tissue marking dyes (1 each of green, yellow, black, red and blue), with 50 applicator sticks and 1 plastic holding tray.

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## 9<sup>TH</sup> NATIONAL HISTOLOGY CONFERENCE 2019

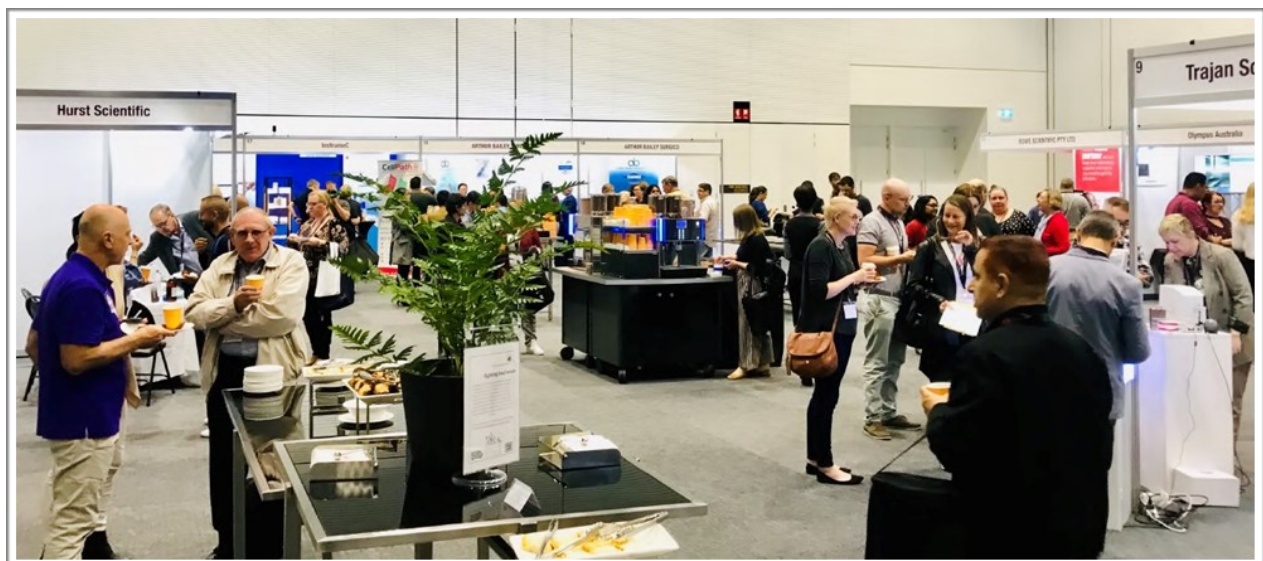
ADELAIDE • SOUTH AUSTRALIA  
24-26 MAY 2019

The 9th National Histology Conference was held at the Adelaide Convention Centre on 24-26th May 2019. The event proved to be a huge success with just under 300 registered delegates plus numerous trade exhibitors.

### FRIDAY

The conference began for many on the Friday with four pre-conference workshops to choose from. One of the morning options involved a complex cut-up workshop that was a close up inspection and hands on grossing tutorial of a complex kidney specimen. The second option was a troubleshooting workshop aimed to reduce errors in an anatomical pathology laboratory. The afternoon workshops included a repeat of the morning errors in the lab talk as well as a hands on comparative tutorial of IHC staining methods vs old school special stains to show Syphilis.

Although I didn't actually attend the Gin Cruise that was offered as a social event in the afternoon I only heard positive feedback from all those who attended. Others spent the afternoon wandering through the shops of Rundle Street Mall trying to find the famous Haigh's chocolate shop. The welcome function was held on Friday night and gave delegates an opportunity to meet with the Trade reps as well as their interstate colleagues. The food and drink was flowing which created a great atmosphere to open up the conference.



## SATURDAY

Saturday morning began with an official opening from the Governor of South Australia.

The actual hall where the majority of the conference was held at the convention centre, was decorated perfectly in purple with small touches throughout. There were balloons, tables up front and lecture style seating at the back. The SA Committee were a stand out in their purple t shirts and matched the purple theme throughout the whole weekend.



One of the key note speakers at the conference was Dr Arie Perry who is a Professor at the University of California where he serves as the Director of the Neuropathology Division and the Neuropathology Fellowship program. Dr Perry's talk on the practical utilisation of WHO2016 and cIMPACT-NOW in brain tumour diagnosis discussed the recent advances that have resulted in major diagnostic shifts. The new approach focuses on the integrated diagnosis which incorporates classic histopathology with specific molecular signatures. The presentation was very informative and concluded in a way I have never seen before— a 6 minute brain tumour parody to Bohemian Rhapsody where he got up with a microphone and showed off his excellent singing skills. I would highly recommend looking him up on YouTube <https://www.youtube.com/watch?v=FfP4HTuu6Vs>

The mid-morning session included an update from the RCPAQAP which discussed the changes that have gone on within the program including the new myQAP portal, new scanning hardware and imaging software. Changes to programs such as Electron Microscopy and Her2 Brish were also looked at.

Next was a presentation on Molar pregnancy which is an abnormal pregnancy that carries an increased risk of gestational trophoblastic disease. Correct diagnosis of complete mole is required so that the complete evacuation of the uterus and any further treatment can be undertaken. Given that genetic testing is an important part of the diagnosis of molar pregnancy, we learnt that fresh tissue should be kept in case genetic testing is required.

Dr Rajiv Patel began the third session with an introduction to the grossing of skin specimens. Dr Patel is a Professor of Pathology and Dermatology at the University Of Michigan School Of Medicine and completed some of his studies at Flinders University in Adelaide. His presentation discussed the need for proper grossing techniques of skin specimens which are essential in our workplaces. Errors in this area are serious and sometimes difficult to rectify. A number of different skin specimens were discussed from punch biopsies to complex skin specimens.

Michael Bushe-Jones from Path West in WA presented an interesting find in their lab, where a CMV control was accidentally used to stain a TTF-1 slide. It was discovered that this tumour marker seemed to stain the CMV infected cells on the aberrant control. To determine if this was a legitimate phenomenon, a number of known CMV positive cases were stained with the TTF-1 antibody. Close analysis showed that TTF-1 not only reliably stained CMV infected cells but it also seemed to stain some infected cells which had not been picked up by the CMV antibody.

The last presentation of the day was an in-depth discussion of the diagnosis, collection and prognosis of renal biopsies. The kidney biopsy is considered the most valuable tool in providing nephrologists with information regarding the pathological causes of intrinsic renal disease. This session outlined the indications, complications and procedural aspects of a kidney biopsy.



A special presentation was made to honour two invaluable histology figures who have contributed so much to our profession over the years. Laurie and Sue Reilly from Townsville were presented with a trophy as a thanks for all they have done in the Histology world.

The Conference Gala Dinner sponsored by Agilent was themed “Through the Looking Glass” and was attended by 194 delegates. The night was absolutely amazing with an Alice in Wonderland inspired room and a great band that kept people up dancing all night. The highlight of the evening was definitely a fireworks display over the river at the beginning of the evening which was an unexpected surprise for all.





## SUNDAY

At 9am on Sunday morning many bleary eyed delegates turned up for the first presentation of the day by Ian Olver who is a medical oncologist, bioethicist and researcher. The presentation titled “The Evolution and Revolution in Cancer treatment” discussed the shift in cancer treatment towards more targeted therapies rather than using cytotoxic drugs. We learnt that genomic analysis will become more



important than histological subtype in selecting treatments and may be achieved by liquid biopsies.

The RCPA QAP then discussed the approach for the assessment of Her2BRISH gastric technical and diagnostic proficiency. The presentation provided an overview of the Her2BRISH Gastric program, discussed the assessment program and highlighted results from previous surveys.

The second session of the day consisted of three different presentations. Bronwyn Christiansen from the Royal Children’s Hospital looked at the combination of C4d and C5b-9 staining in the diagnosis of Gestational Alloimmune Liver Disease. The project demonstrated that a combination of the two markers can be used to improve the sensitivity and specificity of a diagnosis of GALD. Jean Mitchell from the NHS followed with a presentation on the History of Haematoxylin. She explored the pathologists and scientists that lend their names to different types of haematoxylin and the techniques they incorporated into our all-important diagnostic nuclear stain. Finally, Jacqui Simmonds from Lismore showed us a case study on a patient that presented with possible DCIS in her breast after calcifications were found. The biopsy interestingly revealed calcified *Schistosoma japonicum* eggs which is a parasite that is usually passed through the body.

The final presentations of the conference included the use of archived FFPE tissues for research purposes by Dr Lauren Thurgood. We learnt that the process of FFPE induces numerous chemical changes and degradation to DNA, RNA and protein that can hamper its usefulness for research purposes. Clare Loudon from The Children’s Medical Research Institution concluded the conference with a presentation on the cryosectioning of cancer tissues for proteomic analysis.

The Histology Group of South Australia’s Committee needs to be congratulated for putting on an excellent event. We also need to thank the many Trade representatives who spent the whole weekend showing us the latest technologies and gadgets they have ready for us to try. Without the support of the Trade, events like this would not be possible. The Conference concluded with the announcement of the 10th National Histology Conference that will be held in Sydney on the 4-6th June 2021.

**Kellie Vukovic**





## Artisan Link Pro

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The Artisan Link Pro Special Staining solution is designed to provide pathology labs with consistent staining results, optimal user safety and significantly improved efficiency. Automate your special stains with Artisan Link Pro and experience an easy and uncomplicated process for consistent results, slide after slide.

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- 28 high-quality Ready-to-Use standardized stain kits
- 30 optimized, validated protocols that are editable for easy, yet flexible, results



# 2019 – 2020 MEMBERSHIP RENEWAL NOTICE

Dear Member,

This is a friendly reminder that your membership is up for renewal. If you have already renewed, thank you! If you still need to renew, just visit our website [www.histonsw.org.au](http://www.histonsw.org.au). Once you're logged into the site, click My Account in the top-right. In the My Subscriptions section there should be a Renew button if your membership expires.

Please note the 1 year membership and Industry/Company/Trade membership have been changed to variable subscriptions, with 1-5 years available as options. Also a 30 day expiry notification and an automatic email to notify members their subscription has ended is in place. Both have a link to the My Account page to make it easy for members to renew. Please don't hesitate to contact us if you encounter any issues.

## **As a member you will have the top 3 benefits:**

1. Networking & Communication with experts
2. Professional Development
3. Learning Best Practices.

## **Also, have access to top 5 programs and services:**

1. Support of continuing education (NATA Requirement)
2. Discount on Workshops & Conferences
3. Free Scientific Talks
4. Support of Faculty
5. Free Newsletter & Information

## **Upcoming events:**

**26th August:** Webinar on Glass slides

**14th September** at UTS: Workshop on Advanced Tissue Recognition & Navigating GIT Tract"

**25th October** at North Ryde RSL - Annual General Meeting

Next National Conference combined with International Academy of Pathology (IAP) Australasian Division will be held in Sydney from 4th June to 6th June 2021 at the International Convention Centre  
Further details will be sent closer to the events.

Thank you to all members and companies for your continuing support and participation. We look forward to seeing you all at the upcoming events

Organising Committee

Histotechnology Society of NSW



# Introducing, someone you already know.

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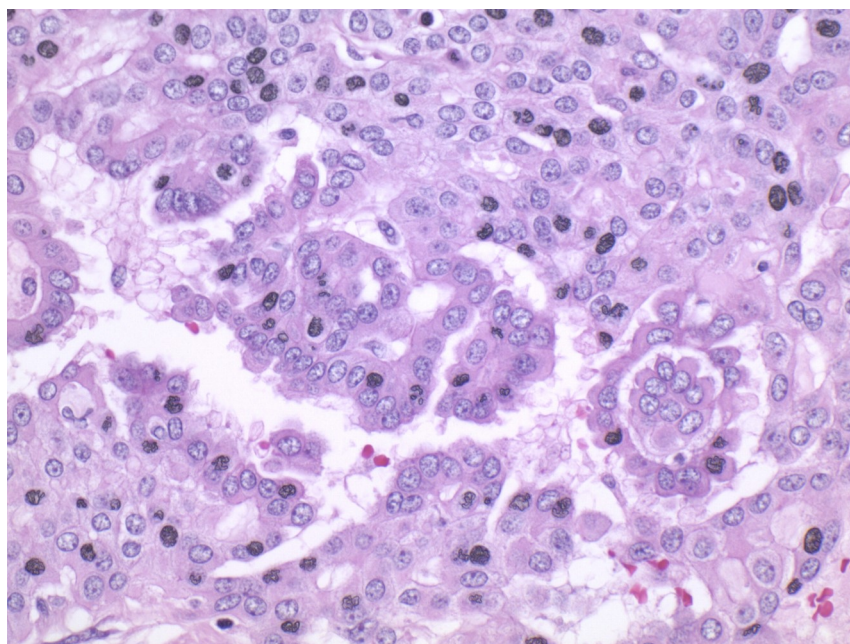
## QUESTIONS

1. What is the stain?
2. What is the artefact?
3. How would you fix it?

## ANSWERS

1. H&E
2. Black nuclear artifact—xylene—part dry coverslipping
3. Remove coverslip, dehydrate, clear and recoverslip

## TEST AND TEACH FROM LAST ISSUE



Explanation of Test & Teach continued by Tony Henwood on pg 27

# Histo Lab Bingo

<b>B</b>	<b>I</b>	<b>N</b>	<b>G</b>	<b>O</b>
<b>Slide dropped and rolled onto with chair</b>	<b>Accidental stain on face from Special Stains</b>	<b>Equipment breakdown (completely unable to use)</b>	<b>Block gets reprocessed</b>	<b>Rare specimen received (rare for your lab)</b>
<b>Visit from company rep</b>	<b>Tissue on slide looks like an animal</b>	<b>Microscope blows a bulb</b>	<b>Jar contains no specimen</b>	<b>≥ 30 separate IHCs ordered for one case</b>
<b>NATA inspection</b>	<b>New employee starts</b>	<b>Specimen received unlabelled</b>	<b>Two coverslips on one slide</b>	<b>H&amp;E staining issues</b>





# Delphic AP

- Eliminate the risk of error with bar-code driven, single-piece workflow, enabling full traceability of every specimen and item.
- Complete interfacing to cassette writers, slide writers, label printers and auto-stainers.
- Advanced pathology reporting with integrated RCPA reporting protocols.
- Meets all Australian standards and billing requirements.
- Improve customer service and quality with optional electronic orders module and online/mobile access to histopathology reports.
- Compatible with digital pathology systems enabling a seamless workflow for lab staff and pathologists

A book has recently been launched titled:

“Survival Guide for Laboratory Professionals”.

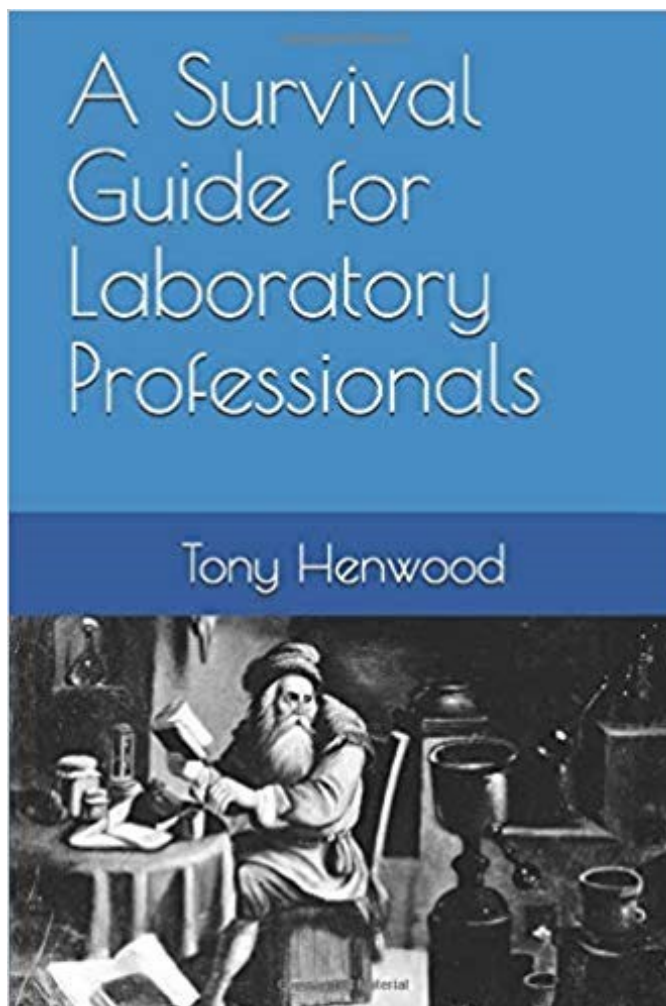
Authored by our former editor of Histogram, Tony Henwood, he has allowed us to reproduce a sample chapter. Tony presents a resource that makes our professional life easier and the range of chapters included in this book presents the information in an easy to read and understand format.

Both the hardcopy and epub versions are available on the US Amazon site (the epub version is also available on Amazon Australia):

[https://  
www.amazon.com/  
dp/1074504933](https://www.amazon.com/dp/1074504933)

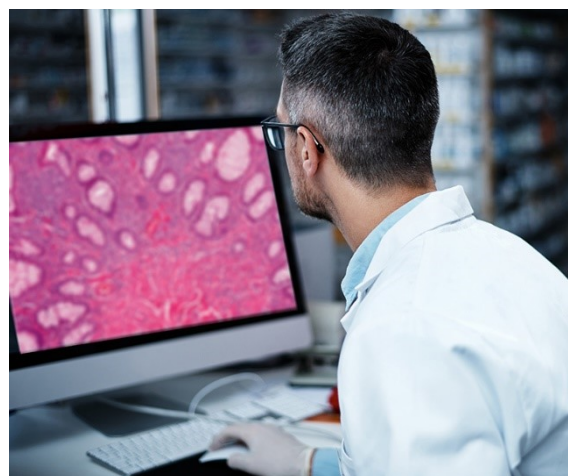
Continued on page 23

## “A Survival Guide for Laboratory Professionals”



## Adopting digital pathology

Digital pathology encompasses the replacement of glass slides in the histology laboratory with digital slide images, as well as automated image analysis. Over the last decade, technological advances have led to improvements in digital slide scanner quality and a reduction in the cost of digital image storage. Because of this an increasing number of histology labs are now beginning to<sup>3</sup> implement digital pathology to improve workflow efficiency and enable easier sharing of slides for collaboration or remote consultation.



### The right IT capability

When considering how to transform and future-proof their practices for the introduction of digital pathology, lab managers and pathologists need to consider not only the slide scanners and other hardware required to ensure that digital slide images are suitable for clinical use, but also their supporting IT systems. The two main systems are the anatomical pathology laboratory information system (APLIS) and the digital pathology system (DPS). Most histology labs are already using an APLIS to manage the lab workflow and pathologist reporting. The DPS is required to manage the digital slide images, and usually offers features to support collaboration, case management and automated image analysis.

### System integration

Key to the success of digital pathology implementation is integration between the APLIS and the DPS. This has been highlighted in reports by labs from a range of countries<sup>1</sup>. For example, a study from the Department of Pathology at the University Medical Center, Utrecht, The Netherlands, found that integration is needed to provide a better user experience<sup>2</sup>, while the Department of Pathology at the University of Pittsburgh Medical Center, USA, concluded that the integration of the DPS and APLIS “streamlined [the] digital sign-out workflow, diminished the potential for human error related to matching slides, and improved the sign-out experience for pathologists”<sup>3</sup>.

With integrated systems, communication via an interface is triggered by certain events in the workflow. For example, once slide preparation is completed in the APLIS, the interface passes patient, request and slide details to the DPS, where a new case is created. Then, once the corresponding slides have been scanned into the DPS, they are automatically associated with the correct case. Without integrated systems, lab staff would be required to re-enter the

the patient and case details into the DPS, introducing the risk of human error.

Likewise, updates to request or patient details in the APLIS should be automatically communicated to the DPS, ensuring that case information in each system is synchronised. This dynamic communication between the two systems also eliminates the need for duplicate data entry.

With digital pathology, the pathologist's reporting workflow is driven by cases assigned in APLIS, rather than slides on the workbench. When the pathologist opens a case to report in the APLIS, the digital pathology image viewer is automatically launched on a second screen. This means that the pathologist is presented with patient and request details from the APLIS on one screen, side by side with the corresponding slide images from the DPS on another screen. Without integrated systems pathologists would need to search the DPS to find the correct images for each patient case at the time of reporting.

Given the importance of system integration in the transition to digital pathology, labs need to ensure that their APLIS is capable of interfacing to their chosen DPS. Interoperability and the use of technology standards is essential to ensure that systems are compatible. HL7 v2.x is the most widely used standard for exchanging clinical data between different systems<sup>4</sup>, so an interface designed using HL7 messaging provides a standardised and flexible platform. It is also important that the interface is bi-directional, to ensure systems remain synchronized.

### Specimen tracking and unique slide identification

Many labs are now introducing full specimen tracking systems into their APLIS to ensure that the patient's specimen, including every block and slide created for the case, is uniquely identified through barcode labelling and tracked throughout the lab workflow. This is an essential foundation for labs to have in place in their APLIS before the introduction of digital pathology<sup>5</sup>. Without unique, barcoded slide labels the DPS is not able to identify every image as a single entity that belongs to the patient case. It also means the pathologist can systematically review and track each slide for the case. The labelled slides must be of high quality to ensure accuracy of scanning and minimise barcode read error rates.

### Regionalisation

When moving to digital pathology, laboratories may also be considering the benefits of regional consolidation to reduce duplication of resources and benefit from economies of scale. For example, specimens may be processed in a central laboratory, and the scanned slides made available to pathologists based in different locations across a geographical region.



In this case it is important to ensure that the APLIS supports a multi-site environment, i.e. multiple laboratories operating from a single laboratory system.

### Remote pathologist reporting

Going digital may also open the possibility of remote reporting. If supported by the DPS and APLIS, pathologists can connect remotely from any location to view digital slides and create reports, facilitating off-site consultation or more flexible work arrangements.

### Conclusion

An increasing number of histology labs are moving towards the adoption of digital pathology. To fully realise the benefits of going digital, labs need to ensure they have the right platform in place in their APLIS. The ideal LIS should:

enable unique slide identification, achieved through an APLIS specimen tracking system

be capable of interfacing to the lab's chosen DPS to provide end-to-end synchronisation of both systems and eliminate duplicate data entry

support a multi-site installation to allow pathologists to work across different locations, if labs are moving towards regional consolidation

provide remote pathologist reporting functionality, if required.

### Author

Caroline Eddy, Marketing Communication Specialist at Sysmex

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4 [http://www.hl7.org/implement/standards/product\\_brief.cfm?product\\_id=185](http://www.hl7.org/implement/standards/product_brief.cfm?product_id=185)

5 Cheng, Chee Leong, Injecting Digital Pathology into the Diagnostic Laboratory – is it possible to Integrate Painlessly? Oct 2014

## Embedding

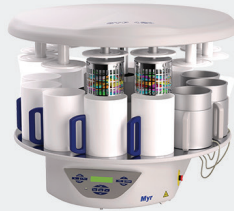


EC 350



EC 500

## Processing



STP 120

## Sectioning



M-240

## Staining



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- Tissue Embedding Centre EC 350
- Tissue Embedding Centre EC 500
- Automated Slide Stainer Myreva SS-30
- Semi-Automated Rotary Microtome M-240

# Chapter 2 Water

## WATER TYPES

The composition of Tap water will vary depending on the location and season of the year. It is usually a very dilute solution of the carbonates and chlorides of calcium, sodium, and copper. Its pH is usually a little over 7, so it acts as a very weak base. Both distilled and deionized water contain so few ions that neither one will act as a weak base.

Treatment chemicals are added to drinking water mainly to reduce or eliminate the incidence of waterborne disease, for other public health measures, and to improve the aesthetic quality of the water. Such chemicals include aluminium sulphate and ferric sulphate (coagulant), ammonia (disinfection), calcium hydroxide (hydrated lime) (pH control and softener), and copper sulphate (algaeicide). The primary use of coagulant and flocculent chemicals is in the removal of suspended and colloidal solids such as clays. Coagulation is particularly important in the treatment of surface waters. Removal of the solids is achieved by aggregating fine suspended matter into larger flocs. Coagulant and flocculent chemicals will also remove some natural organic matter, colour and microorganisms (e.g. bacteria, viruses and algae).

Distilled water is produced by heating water to evaporation and then condensing it on a cold surface. During this process, most impurities are either evaporated off ahead of the water (in the case of most organics) or left behind (in the case of minerals). The water is also effectively deionized because the salts are left behind. It is fairly pure water. To get very pure water it needs to be re-distilled several times.

Deionized water is produced by passing water through a salt bed or ionized resin bed

that captures the mineral ions (ie, a “water softener”). The water is not necessarily pure, however, especially in regards to organic chemicals. Reverse osmosis is also used to deionize water.

There can be issues with both distilled and de-ionised water. It has been recorded that distilled water obtained through improperly constructed distribution lines has added 50 to 100µg of lead per litre to reagent water when the source water from public supplies contained less than 5µg of lead per litre. The use of repeated distillation has generally been replaced by highly efficient ion-exchange processes. However, if the deioniser is not used regularly, organisms may propagate on the ion-exchange resin (Zief & Michelotti 1971).

To produce pure water, boil 600 ml of de-ionized water and add 6 mg of potassium permanganate to it and distil the water. Discard the first 100 ml of distillate. The next 300 ml will be very pure water.

## TESTING WATER

There are several tests that can be used to gauge the quality of laboratory water:

- Test for Halides (chlorine, bromine, iodine): Add a few drops of 10% silver nitrate to 10ml of water to be tested. Good water should not show any cloudiness. The colour of the precipitate indicates the halide present:
  - o White precipitate = chloride
  - o Cream precipitate = bromide
  - o Pale yellow precipitate = iodide
- Test for Sulphates: Add a few drops of 10% barium chloride to 10ml of water to

be tested. A precipitate indicates the presence of sulphates.

- Test for Nitrate: The brown-ring test can be used. To 1ml of the water sample, add 1ml saturated Iron(II) sulphate solution. Carefully add drops of concentrated sulphuric acid. A brown ring (of  $\text{Fe(NO)SO}_4$ ) indicates the presence of nitrates. Unfortunately, other ions can interfere with the test, giving false positive tests including thiocyanate, iodide, bromide, and chromate.
- Test for Metal ions: Add 1M sodium hydroxide solution dropwise to the metal ion solution. The colour of the precipitate indicates the metal ions present:
  - o Light blue precipitate - copper II
  - o Dirty green precipitate - iron II
  - o Rusty brown precipitate - iron III
  - o White precipitate which dissolves in excess ammonia solution or sodium hydroxide - zinc
  - o White precipitate which dissolves in excess sodium hydroxide (only) - aluminium
  - o White precipitate that is not soluble in excess solutions - magnesium or calcium
- Pure water has a high resistance to electricity (see below) and a resistance of about 1.5 to 2mOhm per cm is acceptable.

## pH OF WATER

Pure water is poorly ionic with minimal hydrogen ions ( $10^{-6}$ – $10^{-7}$  mol/L of  $\text{H}_3\text{O}^+$ ). As such, accurate pH measurement is difficult. There are not enough ions to enable electron transport between

the measuring and reference sides of the pH electrode (Riché et al 2006).

Adding the slightest amount of acid or base to pure water will change its pH significantly. Pure water readily absorbs carbon dioxide when exposed to the atmosphere, and this forms carbonic acid. Carbonic acid dissociates into bicarbonate that is in equilibrium with carbonate. Dissolution of  $\text{CO}_2$  in water ultimately leads to a pH of approximately 5.8 (Riché et al 2006). If you work alone, the pH of pure water will probably be 6.9; if there are several people in the lab, the pH may fall to 6.8.

## CONDUCTIVITY OF WATER

Conductivity measurement is used as an alternative to the pH measurement of high-purity water. Conductivity measures the flow of electrons through a fluid, which is proportional to the concentration of ions, their charge, and mobility.

Conductivity and resistivity are inverses of each other. Both reflect the number of ions in the water. The more ions there are, the faster electricity can be conducted through the water, so the less resistance there is. Conversely, the fewer ions there are in the water, the slower the electricity is conducted through the water, so the greater the resistivity is.

The SI unit of conductivity is siemens per meter (S/m). The siemens is the unit of electric conductance, electric susceptance and electric admittance in the International System of Units (SI).



Conductance, susceptance, and admittance are the reciprocals of resistance, reactance, and impedance respectively; hence one siemens is equal to the reciprocal of one ohm.

When only pure water is present, a conductivity value of  $0.055 \text{ S.cm}^{-1}$  at  $25^\circ\text{C}$  is measured. This results from the water dissociation into hydroxide and hydroxonium ions. Therefore, at  $25^\circ\text{C}$ , a conductivity of  $0.055 \text{ S.cm}^{-1}$ , or a resistivity of  $18.18 \text{ M}\Omega.\text{cm}$ , implies that the water is ultrapure and that the pH is inherently 6.998 (Riché et al 2006).

## INTERNATIONAL STANDARDS

### NCCLS/CAP

Specifications for Reagent Water	Type I	Type IIA	Type IIB	Type III
Maximum bacterial content, colony forming units per ml (CFU/ml)	<10.00	10	1000	N/A
Minimum resistivity (Mohm/cm @ $25^\circ\text{C}$ )	10	1.0	1.0	0.1
Maximum silicate, mg/l $\text{SiO}_2$	0.05	0.1		
Particulate matter	0.22 $\mu\text{m}$ filter	N/A	N/A	N/A
Organic contaminants	Activated carbon	N/A	N/A	N/A

### ASTM

Laboratory Grade Water	Type I	Type II	Type III	Type IV
Max. Conductivity Micromhos/cm	0.06	1.0	1.0	5.0
Minimum resistivity (Mohm/cm @ $25^\circ\text{C}$ )	16.66	1.0	1.0	0.2
pH	-	-	6.2–7.5	5.–8.

In general, type I water can be used for washing glassware, but the final rinse must be in the same type of water as required by NCCLS for the type of test to be performed in that glassware. Type II water is used for all stains/procedures done in histology, IM, EM, enzyme histochemistry and cytology. Type III water is required for clinical chemistry, electrophoresis and molecular biology.

#### List of Chapters:

Chapter 1	A Chemistry Resource
Chapter 2	Water
Chapter 3	Mixing Things
Chapter 4	Desiccants
Chapter 5	Calculations
Chapter 6	pH Measurement
Chapter 7	Buffers
Chapter 8	Specific Gravity
Chapter 9	Laboratory Glassware and Plasticware
Chapter 10	Glass Slides and Coverslips
Chapter 11	Filter Paper
Chapter 12	Gloves
Chapter 13	Laboratory Balances
Chapter 14	Centrifugation
Chapter 15	Micropipettes
Chapter 16	Disinfection
Chapter 17	Laboratory Cleaning
Chapter 18	Autoclaving
Chapter 19	Safety Cabinets
Chapter 20	Safety Data Sheets
Chapter 21	Cryogenic Liquids
Chapter 22	Static Electricity in the Laboratory

## Black Nuclei Artefact

Tony Henwood Histopathology, the Children's Hospital at Westmead, NSW

This illustrates a technical artefact. These dark nuclei lack detail and can appear when we are tardy in coverslipping sections. It is caused when excessive time is taken between removing the slide from the xylene bath and applying the coverslip. The section starts to dry and minute bubbles become trapped over the nuclei when the coverslip is applied (Rolls et al 2008). Removing the coverslip, either with xylene or freezing the slide at -70°C (Cozma & Henwood 2019), rehydrate with alcohol and re-clearing with xylene, followed by rapid coverslipping will rectify this artefact.

A similar artefact has been noted in PAP smears, where it is known as “Cornflaking”. Gary Gill (1997) notes that this artefact, similar to black nuclei, is common when the coverslipping of PAP stained smears is done under a fume hood. Fume hoods draw air at 30 meters/minute. This artificial draught accelerates the evaporation of xylene from the surface of preparations about to be coverslipped, promoting the incidence of the “brown artefact,” or “cornflake” cells. These are superficial squamous cells covered with air locked in the ridges that course over their surface. This artefact can be obscuring, and unless it is removed prior to microscopic examination, it can hide abnormal cells. It is possible to prevent the brown artefact by coverslipping slides behind a transparent chemical splash shield set at the front edge of the fume hood. The shield diverts air around the local workspace and reduces the rate of xylene evaporation (Gill 1997).

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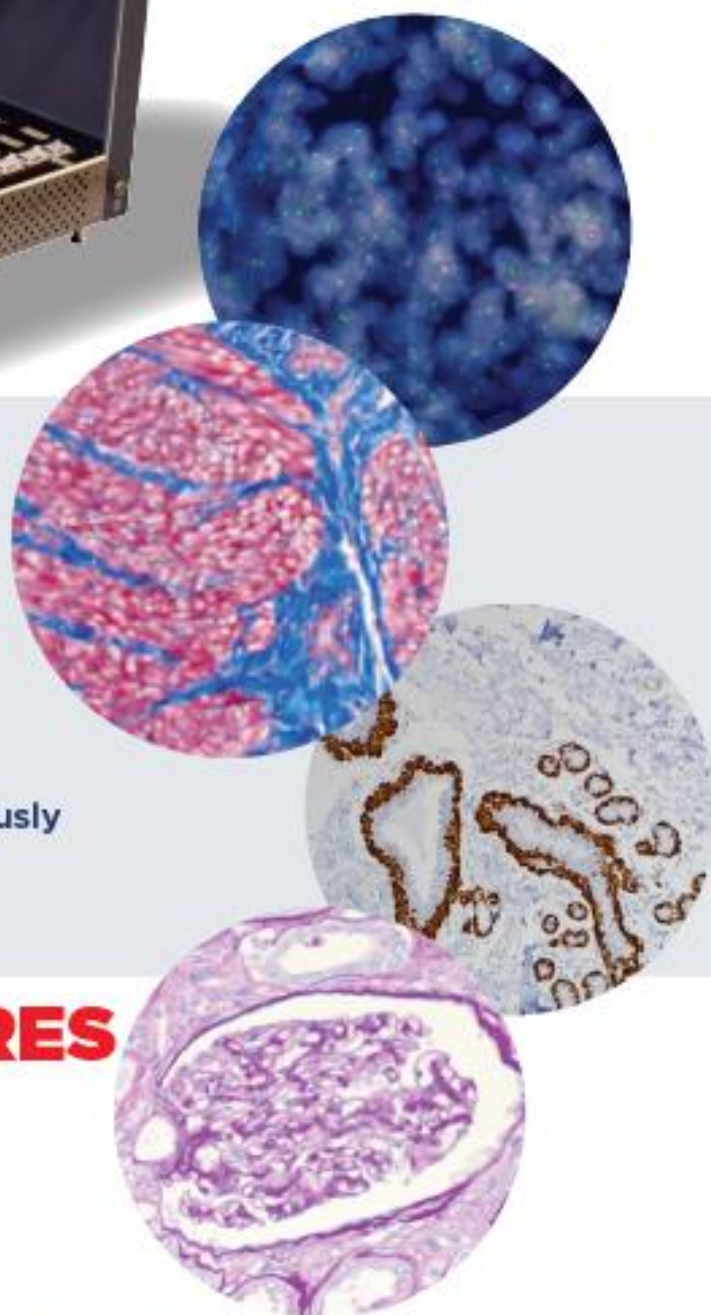
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# MICROORGANISM STAINING

**WHICH STAIN WORKS THE BEST FOR WHICH BUG.  
PRESENTED BY**

**Dr Tamara Sztynka (UTS)  
and Dianne Reader (RNSH)**

The Microorganism Wet workshop which ran on Saturday, 27<sup>th</sup> August at the University of Technology Sydney hosted by POCD and the Histotechnology Society of NSW, was to get the delegates to think outside the box and compare two methods for the identification of four different microorganisms.

The day was full on and I hope all who participated enjoyed the experience.

Below some feedback on the different methods is provided.

1. The comparison for the hot Ziehl-Neelsen (Ziehl-Neelsen (ZN) Technique for tubercle Bacilli by Ziehl (1882) and Neelsen (1883) to the cold method (Ziehl-Neelsen (ZN) stain for Mycobacterium bacilli (Kinyoun 1915)). Safety wise the cold is better. The results showed the acid-fast bacilli stained strongly red and contrasted well the methylene blue. When comparing this with the same tissue which was stained on a special staining machine, the results were paler by comparison. Also, the hand method was more time efficient being 40 minutes whereas on the machine it took over an hour.
2. The Periodic Acid Schiff's (PAS) technique was contrasted against the Chromic Acid Schiff's (CAS) method and allowed everyone to see the difference between the two procedures when staining fungi. We all should remember that the PAS stain is a histochemical reaction in that the periodic acid, oxidises the carbon to carbon bond forming di-aldehydes which react to the fuchsin-sulfurous acid to form the magenta coloured product. Chromic acid, however, is a stronger oxidiser than periodic acid and will tend to over-oxidise tissue components to the point of them not staining after Schiff's reagent. However, in the case of fungi it stains only the fungi and not the rest of the tissue. Compared to the automated method, PAS stained manually showed all the fungi components including spores which was clearly defined as magenta coloured where the machine stained slides which were weaker, paler and time consuming.

3. The Alcian Blue/ Periodic Acid Schiff's (AB/PAS) compared with the Mucicarmine staining methods were contrasted. They both stain the cryptococcal membranes equally as well. Visually they are both very beautiful stains.
4. The two different Gram stains were juxtaposed, both stained very well and there is no comparison as they both showed the gram negative and positive bacteria equally and identically well. Choice of technique really depends on which method pathologists prefer.

Hopefully, Tamara and I have managed to have everyone thinking that maybe the protocols in their laboratories could do with some artistic flare and helped highlight safety as a component of methods to remember.

We would like to thank John Lee and Alex Cross without whom this workshop could not be possible as they supplied the reagents and the food and to Bill Sinai for the paraffin tissue blocks.

To all of those from the Histotechnology Society of NSW who showed up to help a big thankyou as both Tamara and I could not have done this without your support.

Dianne Reader

#### Upcoming Workshops

Monday 26<sup>th</sup> August at 18.00hrs. A webinar on Glass Microscope slides presented by Trevor Hinwood (Chairperson of the HTS NSW) and Alex Anderson (Trajan)

Saturday 14<sup>th</sup> September At UTS the Tissue Recognition workshop on "Through the Gastric Intestinal Tract, from Oesophagus to Rectum"

Watch the website for details.

## MICROORGANISM STAINING WORKSHOP

### By Momoko Sakaki

Thank you to everyone who attended the Microorganism Staining Workshop today. It was a fun and informative day and it was great to see so many Histo Professionals and students spending their Saturday on continuing education!

Big round of applause for Tamara Sztynka and Dianne Reader for organising this fantastic workshop. It is a huge task to organise a wet workshop and they did a fabulous job.

Thank you also to the com-

mittee members, Trevor Hinwood, Ewen Sutherland and Linda Prasad for assisting with the workshop and to the staff at UTS for providing the equipment and clean up.

Special thanks to John Lee from Point of Care Diagnostics (POCD) for providing the reagents for the workshop and sponsoring morning tea and lunch. We could not run these workshops without the support of amazing companies such as POCD

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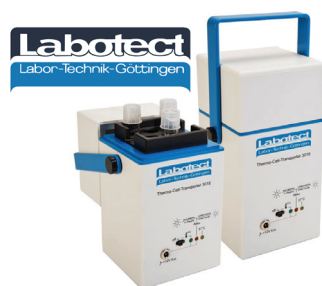
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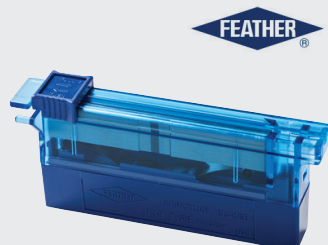
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### **What's new?**

The Histotechnology Society of NSW has a **Discussion Group**. Join the group today to ask questions, share your knowledge and connect with your fellow Histotechs all around NSW and other states.



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