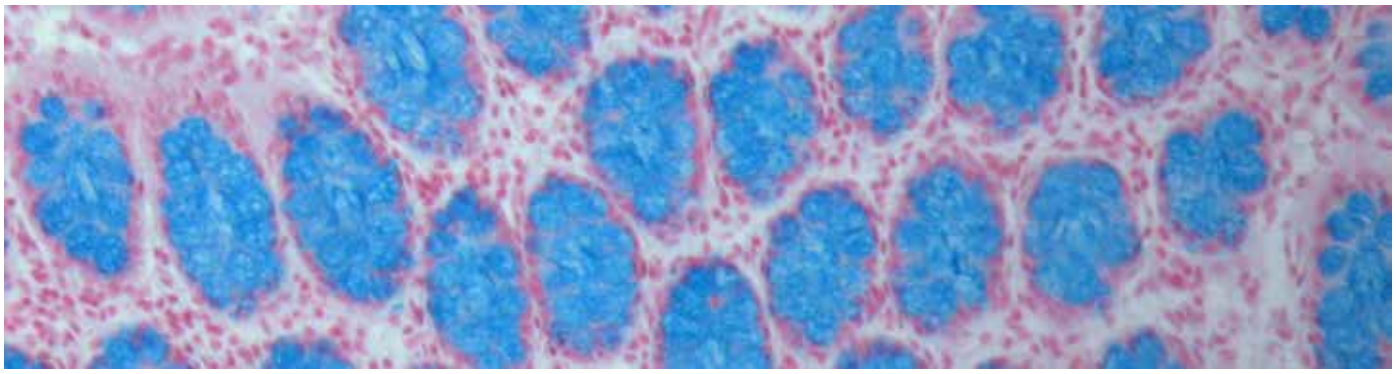
Tissue Paper

Histotechnology Group of Queensland

http://www.hgq.org.au March 2011 - Volume 28



"Bridging Histology Laboratories Since 1982"

Inside this issue:

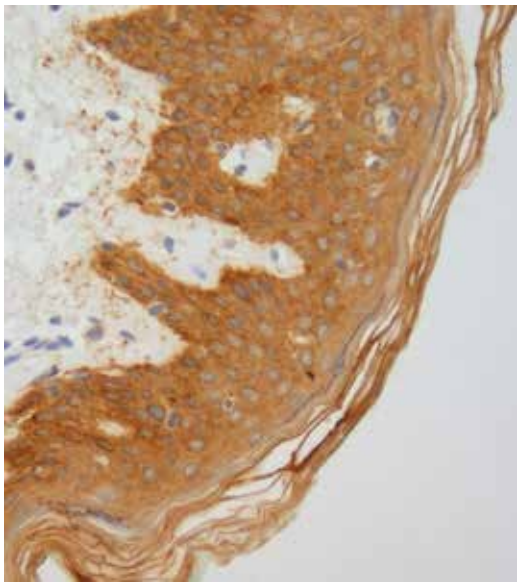
Newsletter Design by Jerres Alcober

From the President – Tony Reilly

Welcome to the first edition of the Tissue Paper for 2011. 2010 was a reasonable year for the HGQ. There were scientific meetings hosted by the Royal Brisbane Hospital and the Prince Charles Hospital and a very successful State Conference run in association with Tropical AIMS in Townsville. This was our first venture out of the south east corner of the state and it's success has given the committee food for thought with regard to holding future state conferences in more distant locations.

There are 3 scientific meetings organised for this year with the first to be hosted by S&N pathology in April. As per our usual practice there will not be a state meeting this year as there will be a National conference in Sydney in November.

One of the significant changes to occur in 2010 was the committee members. I would like to thank Greg bowley, Sharryn Rogers, Trish Laube and ..... for their contributions to the HGQ during their time on the committee. Joining are a host of new committee members ensuring that we now have representation from Queensland Medical Laboratory, Sullivan & Nicloaides, Princess Alexandra Hospital, Royal Brisbane Hospital, Prince Charles Hospital, MyLab, Queensland University of Technology, Healthscope, Forensic Sciences and the Department of Primary Industries. We have a new Tissue Paper editor and a new Secretary as well as the new website thanks to Jerres. We have only had one meeting this year to date and it was particularly noticeable that with the arrival of new people came a host of new ideas for our group. Most of all I am excited by the average age of the new committee members which augers well for the group's future.



Watch this space!

Brand new website www.hgq.org.au
Update member details
Pay annual subscriptions safely via PayPal

Want to target the right audience?

Advertise on the new & upgraded website.
Competitive advertisement packages are available.
Email – admin@hgq.org.au

Upcoming Social event in July 2011. Stay tunned!!!

"Bridging Histology Laboratories Since 1982"

Newsletter Correspondence

Anthony van Zwieten
Anatomical Pathology, Northside Pathology
Level 2 Clinical Sciences Building, The Prince Charles Hospital
Rode Road, Chermside 4032
anthony_van_zwieten@health.qld.gov.au (07) 3139-4379

Guidelines to Contributors
Please forward submissions in Microsoft Word or compatible program either via email and/or CD & DVD. For any attached photos, please also include these in a separate file. Include your name and address if required. Submissions can be in the form of a brief note, letter or as a complete article.

Advertisement Rates
Single A4 page - $50 Double-sided A4 page - $100
Please provide a copy of advertisement(s) in digital formats only.
All monies payable to:
HISTOTECHNOLOGY GROUP OF QUEENSLAND INCORPORATED
ABN: 81 375 702 241 (GST exempt)

TekMed

Ventilated Down Draft benches and cabinets

INTRODUCING THE LATEST IN STAINLESS STEEL VENTILATED DOWN DRAFT WORK BENCHES AND VENTILATED CABINETS FOR YOUR LABORATORY

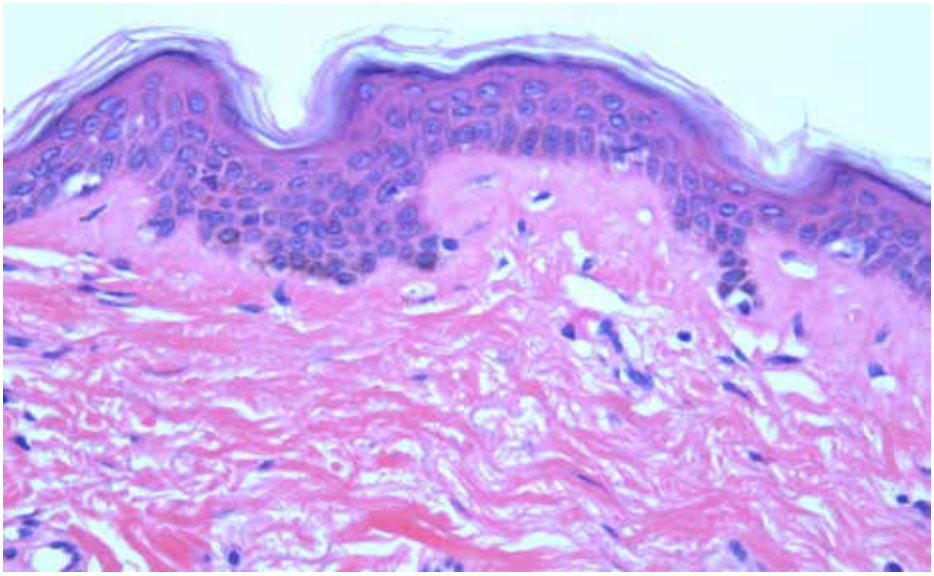
- ✓ Providing a laboratory environment free of pollutants
- ✓ Airflow travels over the entire working/storage area
- ✓ Fixed or variable height benches available
- ✓ Flexibility of fume extraction points or recirculating filters available providing installations suitable to your particular laboratory
- ✓ Available in standard sizes or fully customised solutions
- ✓ Benches and cabinets can be supplied in 304 or 316 Stainless Steel
- ✓ ISO 9001 certified company
- ✓ Australia wide planning, supply, installation and back up service

Call the Tekperts at TekMed and take advantage of our design service.
03 9487 9999 or craig@tekmed.com.au

TekMed

TekMed Pty Ltd
12 Bellevue Crescent
Preston Vic 3072

Tel: +61 (0)3 9487 9999
Fax: +61 (0)3 9484 8792
www.tekmed.com.au
info@tekmed.com.au



Tissue Paper

"Bridging Histology Laboratories Since 1982"

Secretarial Report – Jerres Alcober

This year is shaping up to be a promising year for the Histotechnology Group of Queensland.

The new HGQ website – http://www.hgq.org.au was launched on 15th March 2011. Since the launch there has been a lot of positive feedback from the community and members. Some features include: secure online membership payments, an interactive forum, "up-to-date" events calendar, individual user profiles & avatars, photo gallery and access to the latest HGQ newsletter - the "Tissue Paper". Over the upcoming months, the website will see additional upgrades and features which are set to benefit members even more.

2011 memberships have started to come in over the past few weeks with most contributions originating from the website. While renewals make up the majority of memberships, the current trend shows a rise in new applications. As of the start of 2011, membership coverage has changed from financial year (1st July – 30th June) to calendar year (1st January – 31st December). The previous membership period was extended to 18 months (1st July 2009 – 31st December 2010) to accommodate this change. Membership includes Tissue Paper subscriptions; website access; social events & state conference registration discounts; eligibility to vote; beverages & dinner covered at AGM. Renewal and new memberships can be completed online. See the website for more details.

There will be 3 scientific meetings this year with the last including the AGM. The first of these meetings will be hosted be SNP – Taringa Laboratory in early to mid May. This is set to be an exciting start to this year's schedule of scientific meetings. Additional information can be obtained from the HGQ website. All members and interested parties are welcome to attend.

The 2011 HGQ executive committee was determined and finalised by members at the 2010 AGM.

The committee consists of new and past members and are as follows: President - Tony Reilly; Secretary - Jerres Alcober; Treasurer - Steve Riley; Editor: Anthony Van Zwieten & Andre Heiser; Committee - Sue Bell, Amanda Russell, Dale Hyam, David Gan, Emma Hughes, Mitchell Wan, Mohammed Amigh, Helen O'Connor, Lloyd Blundell and Michael Doyle.

Looking forward to an exciting 2011. Enjoy!!!

"This year is shaping up to be a promising year for the Histotechnology Group of Queensland"

Editor's Note – Anthony Van Zwieten

This newsletter is for the histotechnologist to keep up-to-date with the latest news around the labs in QLD. I am very excited to be able to present this first edition for 2011, and any feedback and suggestions that you may have is welcomed.

Thanks to the new secretary in Jerres for setting up the brand new website, thrusting the HGQ into the social networking era. The new online forums will also be very useful for all to utilise when comparing methods and to allow open independent scientific advice for all things Histology.

The 2010 AGM hosted by the TPCH Lab was a success for the HGQ. Over thirty people attended and were treated with insightful presentations by both Lydia Mc Phee (Lung Transplant Histology) and Jerres Alcober (Prostate Adenocarcinoma and "Movember").

As Tony mentioned earlier on the opening page, it is great to see some new faces on the committee to add some enthusiasm to those more experienced.

As co-editor of this newsletter, I will hand the reigns to Andre for the next edition. Over to you Champ!

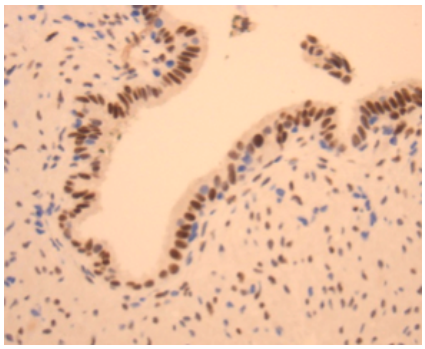
Comparison of antibodies for WT-1 Staining

Anthony Van Zwieten, Northside Pathology – TPCH

Wilm's Tumour-1 protein (WT-1) is a gene located at chromosome 11p13 which codes for a transcription factor, a DNA-binding nucleoprotein, 52-62 kDa. It has a role primarily in the development of genitourinary organs. There are at least eight isoforms ranging between 52 and 62 kDa produced by a combination of alternative splicing and RNA editing.

WT1 is synthesized and reside in the cytoplasm in an inactive form and translocates to the nucleus upon activation through phosphorylation. It influences cell proliferation by suppressing bcl-2 and regulating cadherin and p53.

The gene is expressed in mesenchymally-derived tissue, so is used clinically for distinguishing malignant Mesothelioma and ovarian serous carcinoma from non-serous carcinomas. The gene is also expressed in majority of acute leukaemia's and is applicable for the differential diagnosis of small cell childhood tumours. Wilm's Tumour is a paediatric malignancy
The following guide is used in our QC procedure for this marker. Only nuclear staining is deemed to be true staining. Fallopian tube is used as control tissue as suggested by an external QAP resource – NordiQC.



QC Criteria
Staining Pattern:
Nuclear
Control: Fallopian Tube
Expected Result:
Staining of epithelial and stromal cells

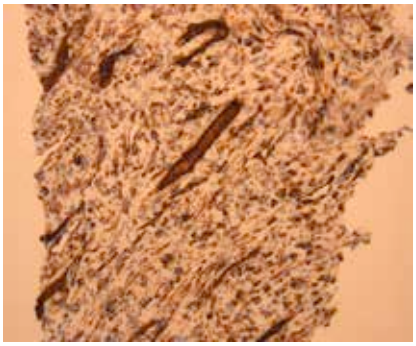
WT-1 at TPCH
In majority of cases, this marker is used as part of a panel of Abs to differentiate between malignant pleural mesothelioma and adenocarcinoma of the lung. Until recently the mAb 6F-H2 was the only clone used in publications and for diagnostic purposes.

However, this is a "Cross-reactive" mAb which stains other cell types such as endothelium, smooth muscle in a cytoplasmic pattern. As such this clone may not help with non-specific staining, especially with small biopsy specimens, where cellular morphology can be disrupted.

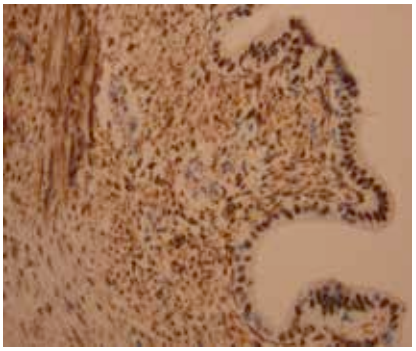
Heat induced epitope retrieval protocol using an alkaline buffer is recommended.

Our lab uses HIER of ER2 (pH 9) for 20 minutes @ 100°C using Bond Max with Polymer Refine Detection Kit.

An unusual result? Stained with clone 6F-H2



Left: *Test*



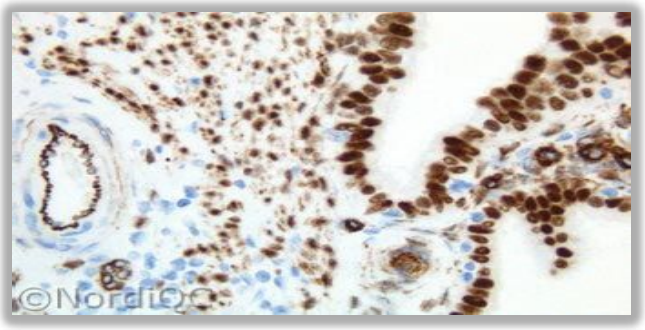
Right: *Control*

This case of spindle cell lung adenocarcinoma showed prominent staining of skeletal and smooth muscle. This non- specific pattern was deemed non-contributory, as WT-1 was employed as part of a mesothelioma panel including Calretinin which was negative.

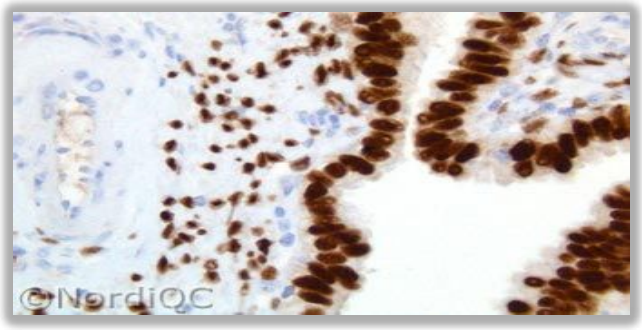
In this pleural biopsy, mesothelioma was being considered but turned out to be metastatic lung adenocarcinoma. Note the cytoplasmic staining of endothelium and smooth muscle in the control tissue.

During the investigation of this marker, we identified a new mAb for WT-1. The information from the NordiQC website was utilised by our laboratory for IHC Quality Control purposes. [Continue next page]

The following images are from the NordiQC IHC website: http://www.nordiqc.org/Run-28/Assessment/assessment-28-WT1.htm



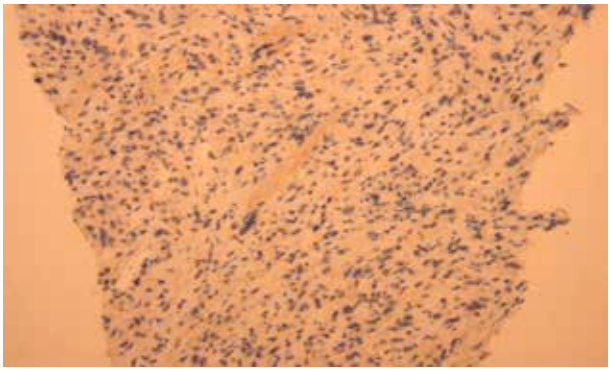
***Clone:** 6F-H2 (Dako)*



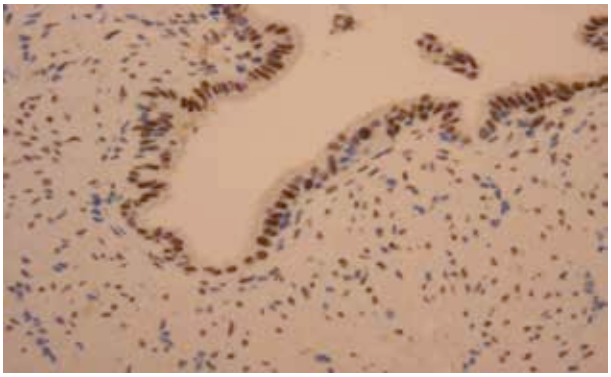
***Clone:** WT49 (Leica)*

Note that with the new mAb clone WT49, cytoplasmic endothelial staining is lost whilst nuclear staining of epithelial and stromal cells is observed.

Unusual case stained with new WT49 Ready to Use (RTU) clone

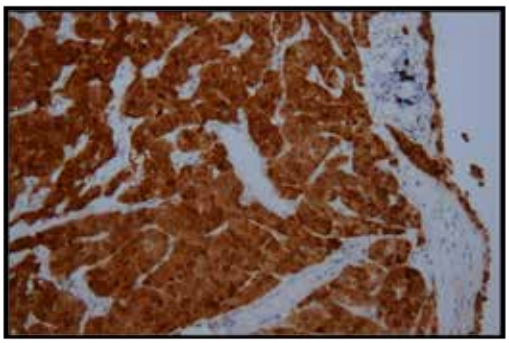


Case

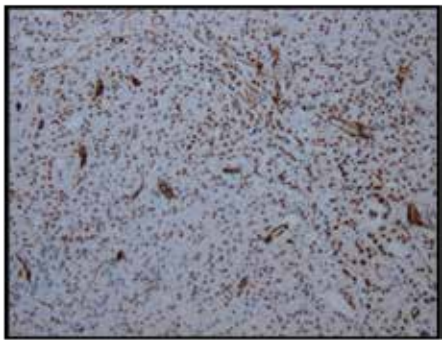


Control

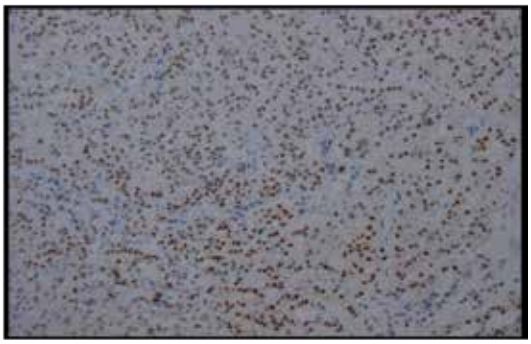
The same case repeated with new mAb WT49, note the absence of staining within the biopsy specimen, with specific nuclear staining of epithelium & stroma within the Fallopian Tube control. This staining pattern was of use to the reporting Pathologist as a true negative reaction is observed, compared to the non-specific cytoplasmic staining seen with clone 6F-H2.



A case of Calretinin IHC staining of Epithelioid Malignant Mesothelioma (note the prominent Cytoplasmic and nuclear staining)



The same case stained with mAb 6F-H2 (Note tumour nuclear staining with endothelial cytoplasmic staining)



The same case stained with WT49 RTU clone – note specific nuclear staining

[Continue next page]

Why cytoplasmic staining?

Cytoplasmic staining is considered to be an artefactual cross reaction associated with some clones of WT1. WT1 is synthesised and reside in the **cytoplasm** in an inactive form Translocates to the **nucleus** upon activation through phosphorylation.

This may be a possible reason why both nuclear & cytoplasmic staining occurs with clone 6F-H2 (A cross-reactive WT-1 Ab)

Conclusion

WT49 clone is more sensitive Ab for WT-1 staining in targeted tissue and cell types here at TPCH. This clone will be useful in IHC of small biopsy specimens to detect specific nuclear staining.

The mAb clones 6F-H2 and WT49 are recommendable clones for WT1. For both clones HIER seems mandatory to achieve optimal staining. However HIER will induce a cytoplasmic reaction for the mAb clone 6F-H2. Fallopian tube is an appropriate control for WT1: Virtually all the epithelial cells and the smooth muscle cells shall show an as strong as possible nuclear reaction with only a minimal cytoplasmic reaction if using clone 6FH2.

Acknowledgements

Dr Andrew Dettrick – Pathologist, Northside Pathology TPCH

Mr David Butler – Chief Scientist Anatomical Pathology, Northside Pathology TPCH

Ms Lydia McPhee – Scientist Anatomical Pathology, Northside Pathology TPCH for microphotographs

NordiQC website - http://www.nordiqc.org

"WT49 clone is more sensitive Ab for WT-1 staining in targeted tissue and cell types here at TPCH"

Histotechnology in the 1980s

David Butler, Chief Scientist Anatomical Pathology, Northside Pathology - TPCH

Well Anthony if you pass me a beer I'll tell you what the 80s were like.....

The day begins as usual with embedding the overnight runs, taking cassettes off the rotary tissue processors and putting under vacuum for 30min to remove air bubbles, this time they've all behaved, next week our new Shandon vacuum processor arrives so we look forward to that. No more finding tissue stuck between solutions or left in one solution all night. Yahoo!

The manual tomes are in use and we have our own steel knives, dreaming of one day of having a self-sharpening knife. Early in the decade we are rewarded with disposable blades and blade holders adapted to our existing microtome's! Until then each afternoon sharpening sessions and regrinding the glass plates took place. One of the scientists is trying acrylic resin sections and cutting them on wide glass knives but the work on the automatic microtome is time consuming and not suitable for our routine work. Stains needed to be adapted to the resin sections, but morphology of the thinner section was superb and examination of bone marrow tissue was enhanced. These plastic sections were proposed as the missing link between light and electron microscopy.

Further down the lab as I cut my small biopsies, the cutup rages, for ventilation we open the windows and the paperwork goes flying in the breeze. They cutup all day and have several boards prepared for the frozen sections booked on the white board. Fortunately we have stepped out of the freezing microtome era. When they're not busy they help in cytology and filter urine specimens onto Millipore filters, which stand in xylene overnight to clear. All the prep is done on the bench. Slowly the numbers of FNA specimens rise and needles are prodded into any and all solid specimens to create teaching sets, and better under- stand the artefacts induced by this technique.

Still further along the dozens of racks of H&E slides are stained in butter dishes and cover-slipped by hand, again the windows are opened to vent the fumes. Specials are cut and stained as the last of the morning's H&Es are done. Labels are bought from Printers, and in a variety of colours, slides are etched with diamond pencils and the best adhesive is Elmer's glue or Poly-L-lysine. Articles on section adhesives indicate hydrolysis of polymers offers a possibility – maybe. Automated strainers are of linear or rotary type and with due care and attention work well.

[Continue next page]

Right: *Typical Microscope in the 1980s Histology Lab*

Left: *Leather Strops were used to sharpen steel micotome knives*

The IHC is done by hand and speeded up with the use of heated stain-plates, this makes the work frantic and requires skill to get right. PAP complex is replaced by ABC and ABC by labeled streptavidin. Kits start off small and develop to large volume ones with purists preferring to dilute their own antibodies and linking reagents. DAB is the preferred Chromogen although AEC has a small following, with the Europeans preferring the APAAP system.

Antigen retrieval is by trypsin and pepsin or proteinases, although some papers have expounded the use of sucrose and guanidine solutions. Many fixatives are proposed as formalin replacements but the balance between preservation of cellular detail and immunoreactivity teeters from one to the other.

Many different views are published but formalin prevails as fixative of choice for routine work and soon companies develop antibodies to work on formalin fixed material. Hybridoma technology produces monoclonal antibodies and the range of products explodes beyond all imagination. But automation of the process is slow.

The décor of the lab is usually drab but frequently punctuated from volcanic eruptions of dyes not watched closely enough during preparation, purple from Harris Htx, pink from schiff's reagent and red from carbol fuchsin. Fixation if Formaldehyde based and Helly's, Zenkers, and B5, are used for core biopsies, Bone Marrows and Lymph nodes, respectively. Researchers develop their own fixatives, mixing and tailoring buffers to suit their projects and publish accordingly.

Carsons fixative is recommended for those wanting one suitable for EM and LM. The literature is abound with modified stains as people try to adapt stains to suit their research or shorten methods to produce quicker clinical results. Combinations of stains produce some dazzling results. AIDS as it was known brought with it a range of opportunistic infections not previously encountered in humans, and the use of microwaves to speed up staining (not all with the safest outcomes) was regularly touted.

Manuals were often a mix of textbooks and hand written notes from scientific observation. You didn't time reactions as much as watch for the endpoints through the microscope, a type of art and science hybrid.

Through the decade Laboratory Information systems invade Histopathology labs and staff relent to looking at black screens run by DOS programs as often as they look down microscopes, data entry is mostly the domain of typists but that slowly changes as scientists come to grips with technology based equipment. Mechanical timers are lost to electronic beeps. Dishwashers replace porcelain crocks of chromic acid, intricate special stains are replaced by IHC, and carcinogenic chemicals are replaced by less toxic ones (where they can be), autopsy numbers decline (if you do them), shifts start before dawn and finish after dusk in private labs, managers complain of the cost of new technology, and safety played a more prominent role in our work.

And then came NATA……

Right: *Slee Cryostat used in the 1980s*

Roll of Honour

- Grimelius
- Sirokeen knife sharpener
- Millipore Filters
- Gram-Weigert
- Dieterle
- Masson-Fontana
- Picro Mallory trichrome
- Orcein-Giemsa
- Martius Scarlet Blue
- Slee cryostat
- B5 Fixative
- Leather Stropps
- Bees wax in Parraffin wax
- Mechanical Processors (no Fuzzy logic)
- Microscopes that you need a truck to shift

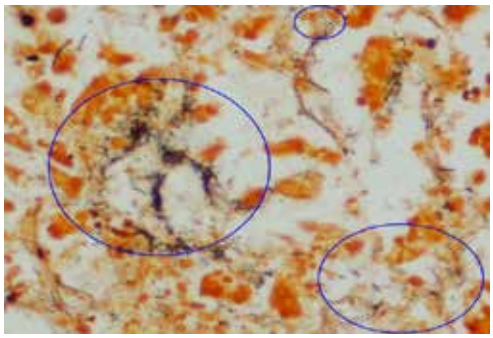
If anyone wants to fill the gaps they can catch-up with me at the next scientific meeting!!

Catching Syphilis

David Gan, QML

Why do we stain multiple slides at different incubations when we are looking for syphilis?

Syphilis is caused by the spirochete Treponema pallidum and is by far the most common spirochete to infect humans. It is a sexually transmitted disease which can affect most organs and can mimic many other diseases. It was described as early as the 15th century and before penicillin was discovered, was a major cause of death. It is a thinly coiled, double-membraned motile bacterium 6-15um long and 0.1-0.2 um wide. It can sometimes look a little like a stretched out slinky. Serology is by far the most common test for syphilis but histological stains are sometimes helpful on paraffin sections.



Left: *Control slide of T.pallidum demonstrated by Warthin Starry technique (note the spiral morphology of the individual organisms)*

Warthin-Starry, Steiner and Dieterle are methods often used to demonstrate Treponema pallidum and Helicobacter pylori. These bacteria are very difficult to see in a Gram stain. Because of their size, when using silver stains, syphilis will take longer to develop than Helicobacter and other bacteria. Syphilis are much thinner than other bacteria, Treponema pallidum 0.1um - 0.2um wide vs other bacilli approximately 0.5um wide. Be- cause of this, it is important when staining for spirochetes to use a known spirochete control (if you have one) and to use a variety of impregnation or developing times to ensure optimal staining.

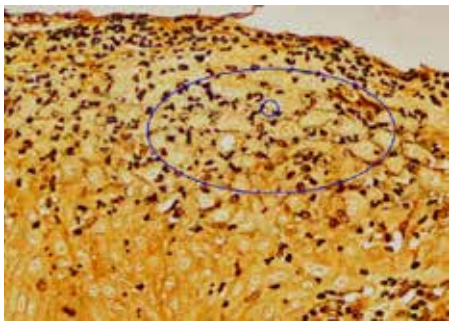
We had a case recently which showed the need to follow these steps.

We received tissue from an inflamed tonsil from a 53 year old male, querying lymphoma or infection. It was later revealed that syphilis serology had been reported as positive but to make it more "interesting" for the pathologist, this information was not given in the clinical notes.

The usual panel for microorganisms was performed as well as a Warthin Starry and some IHC stains including our relatively new Treponema pallidum antibody. The PAS, Gram, Grocott, Wade-Fite and Ziehl-Neelsen stains were all negative. The darkest Warthin-Starry showed a small number of spirochetes with reduced intensity and contrast compared to our spirochete control slide.

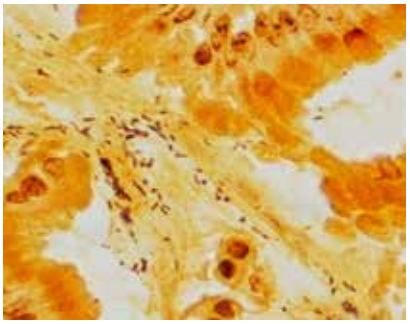
For interest, we repeated the Warthin-Starry with extended time in the developer. A Helicobacter pylori control and the test were also run using minimal time in the developer.

The test slide that was developed for less time, showed no spirochete staining although the Helicobacter pylori control stained up quite well.



Left: *Warthin Starry technique on patient slide shows a small number of spirochetes with reduced intensity and contrast)*

Left: *H.pylori control slide stained with Warthin Starry*

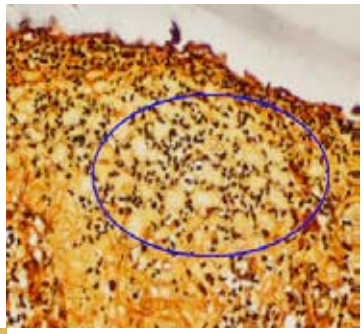


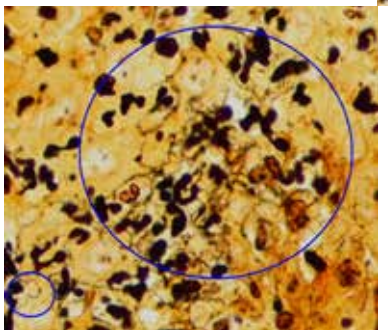
The slides that had extended time in the developer stained up more spirochetes than the original slides but with increased background staining from inflammatory cells and lymphocytes. This small trial seems to fit in with the expected staining for spirochetes and Helicobacter pylori but even with a syphilis control and multiple slides, care must still be taken. A test slide was stained up using an automated modified

[Continue next page]

Right: *Extended development improves spirochete staining*

(Low Power)



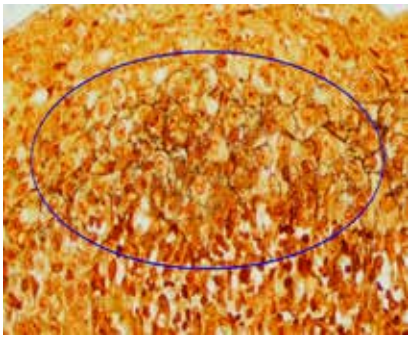


Left: *Extended development improves spirochete staining*

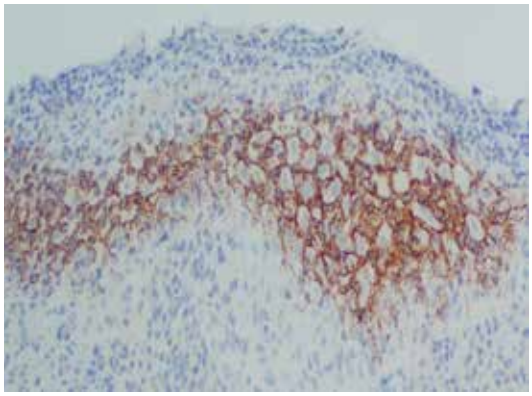
(High Power)

Steiner technique (Ventana) which stained the spirochetes much better and with less background than our Warthin-Starry. This may be due to the lack of a sensitiser in our Warthin-Starry technique (Some sensitisers are quite hazardous).

Right: *Spirochete staining with automated modified Steiner technique*



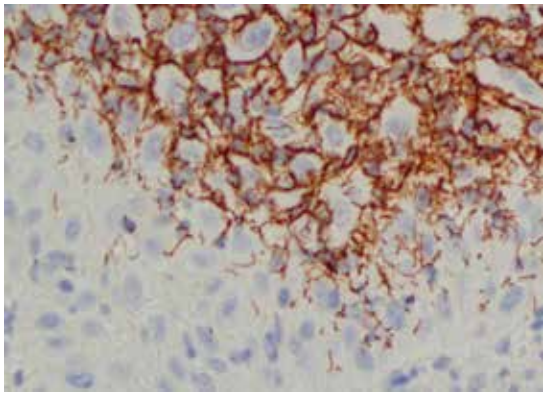
The IHC stain for Treponema pallidum (Biocare) stained the bacteria up very strongly and was by far the most sensitive and specific. Areas with only a few bacteria were very obvious even on low magnification.



Treponema pallidum IHC staining

Left*: Low power*

Right*: High Power*



With the growing popularity of this IHC stain and with excellent IHC stains for H pylori available, will the challenges/frustrations of the Warthin-Starry and Steiner be a thing of the past?

Some people are like slinkies, relatively useless but bring a smile to your face when you push them down the stairs.

References: Pathology of Infectious Diseases (Conner, Chandler) NSH Teleconference Spirochete Staining January 2011

Do you have any feedback about this newsletter?

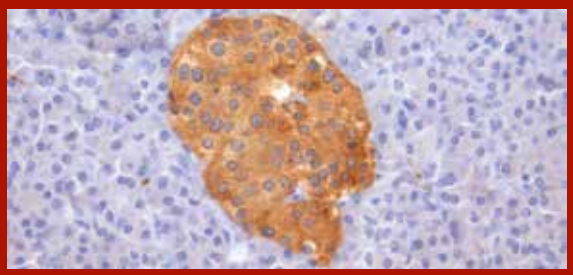
Your feedback is welcomed.

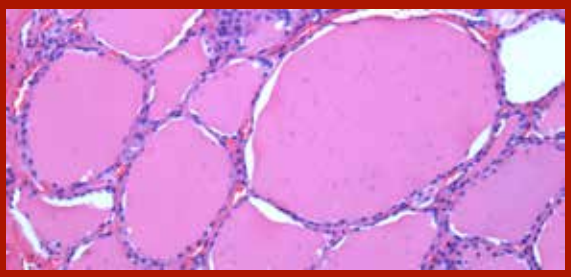
Hope to see everyone at the next scientific meeting.

Date: Early to Mid May 2011

Venue: Sullivan Nicolaides Pathology

Address: 134 Whitmore Street, Taringa





Check out the new and improved website for further details and to RSVP for events

Histotechnology Group of Queensland

http://www.hgq.org.au

Improve quality

Increase productivity

Deliver better patient care

Total Consumables Solutions

Leica Microsystems' Surgipath® Consumables Range

Find the ideal consumables for your laboratory with Leica Microsystems' new Surgipath range. From cassettes and paraffin to slides and stains, Surgipath products have histology covered.

- BROAD RANGE – solutions for every step of the histology workflow
- IT'S YOUR CHOICE – multiple options mean you get the solution that is right for your laboratory
- TOTAL SOLUTIONS – quality Leica consumables join world-class Leica instruments

TOTAL HISTOLOGY is a broad range with tailored solutions to help you deliver better patient care.

www.leica-microsystems.com

Living up to Life

Leica
MICROSYSTEMS

Histotechnology Group of Queensland

Membership Application Form

To complete membership online go to: http://www.hgq.org.au

Please indicate by ☑:

☐ New Membership ☐ Renewal of Membership ☐ Change of Details

Name: _____

Employer: _____

Mailing Address: (Work Address Preferred)

_____

_____

_____

Email: _____

Phone: _____ Fax: _____

Class of Membership:

☐ Full - $25 per year

☐ Student (Full-Time Only) - $10 per year with proof required

For New Members Only: New members must be proposed and seconded by current financial members. Should you not know of any members, the executive committee can purpose and second a new member's application.

Proposed by: _____ Signature: _____

Seconded by: _____ Signature: _____

Applicant's signature: _____ Date: _____

Please return completed form via (options below):

Postal Address: Jerres Alcober
Anatomical Pathology Department,
Pathology Queensland
The Prince Charles Hospital
Level 2 – Clinical Sciences Building
Rode Road, CHERMSIDE, QLD 4032

Email Address: admin@hgq.org.au

Fax number: (07) 3139 4546

Please make all payments via (options below):

Direct Deposit: Acc name: Histotechnology Group of Queensland Incorporated
BSB: 084009
Acc number: 198048439

Cheque: Make payable to Histotechnology Group of Queensland Incorporated

Paypal: http://www.hgq.org.au - "Membership" tab

Cash: In person - Stephen Riley/Jerres Alcober