The Histotechnology Group of Queensland Newsletter

Tissue Paper

Histotechnology Group of Queensland

"Bridging histology laboratories since 1982"

March 2013 – Volume 34

From the President – Anthony van Zwieten

Welcome all to 2013 and an exciting year ahead for the HGQ. I am honoured to be the new President of the group and I am eager to work with the committee and members to continually improve our representation and profile across as many Histopathology labs as possible in QLD.

Firstly on behalf of everyone associated with the group I would like to thank both Tony Reilly and Steve Riley for their efforts and expertise as previous HGQ President and Treasurer respectively. It is fortunate for the group that they are both continuing their association as committee members. Thanks also to Jerres Alcober and Emma Hughes for their continued hard work with this March 2013 edition. I look forward to working with them for the remainder of 2013 and beyond.

Since the end of 2012 the HGQ has been active. A $10,000 donation was made to the charity associated with Dr Brian Miller's work as a surgeon in third-world regions. Check out the letter in this edition for further details.

More recently, Jerres and I attended the QUT health awards night and presented Pauline Lim with the HGQ award for the best performance for the practical component of Histopathology subjects. Jerres took some great photos from the venue, the new Skyroom at the Gardens Point campus. As a Brisbane CBD venue it really appealed as a potential future HGQ conference and function venue. Check it out and let me know what you think.

We are in the process of organising our next Scientific meeting in May and I encourage all to log onto the group website www.hgq.org.au for further details. Remember that this site is now accessible for all Pathology QLD staff. I look forward to seeing as many people there as possible. Let everyone in your lab know about the HGQ! Anthony

P.S. The upcoming 6th National Histotechnology conference in Melbourne will be represented strongly as usual by the HGQ. Look out for Jerres and Tony on the dance floor ☺

Inside this issue …

Newsletter design:
Jerres Alcober

Executive Committee 2013

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Editor's Note – Emma Hughes

Hey guys, here we are again for the 1st Tissue Paper of 2013.

This issue includes a short article about the 2012 Annual General Meeting and the final Scientific Meeting for the year.

There is an article entitled Immunohistochemistry: An Introduction, which is a brief introduction to Immunohistochemistry for those of us who are not involved in this specialist area of Histopathology.

We also have a fantastic article from Andre Heiser about Fungi Staining and Anthony van Zwieten about p40 IHC at The Prince Charles Hospital.

I hope you enjoy this edition, and we will see you back in June for the 2nd edition of the Tissue Paper.

Secretarial Report – Jerres Alcober

Hello to all our readers. The HGQ hopes that everyone had a safe & fantastic festive season. 2013 promises to be a busy year with scientific meetings, conferences & social events already scheduled on the HGQ calendar.

Thank you to Pathology Queensland's The Prince Charles Hospital Laboratory for hosting the 2012 AGM & scientific meeting at Kedron Wavell Services Club. Members were treated to educational presentations from our guest speakers, a delightful 3-course meal and an entertaining Trivia Night. Thank you to all that contributed to organizing another successful event. The next scientific meeting will be hosted by Sullivan Nicolaides Pathology in May.

The Overseas Specialist Surgical Association of Australia Incorporated (OSSAA) has expressed their deepest gratitude for the HGQ's donation to their relief fund. Thank you again to our members for their contribution and their decision to donate $10,000 to OSSAA's efforts to assist medically disadvantaged countries such as Indonesia.

Members have unanimously granted "Free" membership from 2013 onwards. Membership will still include the same benefits & cover the calendar year (1st January – 31st December). Previous & prospective members are required to complete & submit a membership form each year.

Hope to see you at the upcoming national conference in Melbourne.

Happy reading. Enjoy!!

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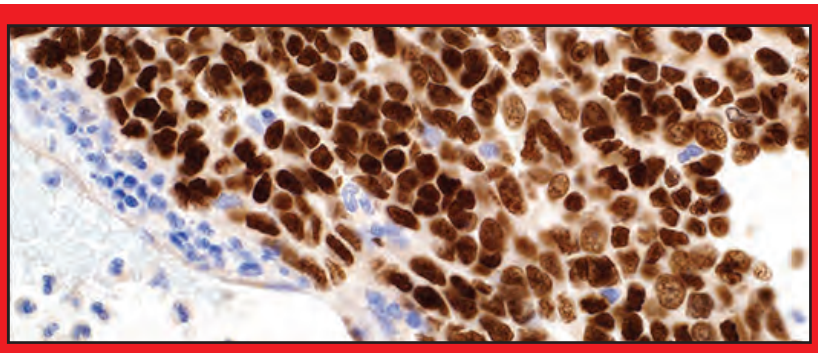

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Alternative Fungal Stains in Histology

André Heiser – QML Pathology

There are a number of histological methods to demonstrate fungi. The traditional methods, such as PAS and Grocott's, follow the basic pattern of oxidising the glucose residues in the fungal walls into aldehydes then reacting the created aldehydes to demonstrate their presence. Here is a brief overview of some of the alternatives to the more commonplace methods of fungal staining in histology. While they all follow similar styles, the differences between them allow for balance when choosing a stain for convenience and efficacy.

All four alternative methods discussed (McManus, Chromic Acid and Schiff's, Gridley and the Permanganate and Alcian Blue) use the basic pattern of oxidation, aldehyde demonstration and counterstain.

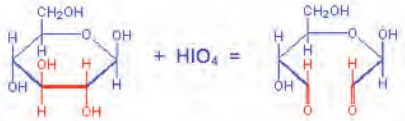
Oxidising Agents

Oxidation is the first step in all these methods. Reaction sites for the Schiff's reagent are created from glucose residues (found in fungal cell walls). Oxidants vary but the two main ones are periodic acid and chromic acid. Other methods described by Lillie (1951) and Prunieras (1960) uses permanganate. All three oxidants act in the same way (Lillie 1951).

The oxidation step converts glucose residues in the fungal walls (and other structures) into aldehydes which are then reacted using Schiff's or some other method to demonstrate their presence. A counterstain is used to contrast the positive and negative tissue elements.

"There are a number of histological methods to demonstrate fungi"

The safety and ease of periodic acid must be considered when comparing it to chromic acid. The PAS and CAS (described below) are good instances of this—the PAS will not be as specific but it easier and safer than the CAS. Permanganate is another option. It is stable but is difficult to use and methods must be standardised carefully.



Carbons 1 and 2 are converted to aldehydes (in this example, by using periodic acid) (Stains File 2005f).

Periodic Acid (HIO_4)

For our purposes, periodic acid is used in low concentrations and has the distinct advantage of being such a weak oxidant that over-oxidation is almost impossible. While this makes the PAS a robust stain, it also creates more false-positives when compared to chromic acid methods (see below). It is also

considered by some to be so weak that it can generate false-negatives. Periodic acid's major advantages over chromic acid-based methods are safety and disposal.

A good way to annoy the author is to pronounce periodic as *pee-ree-odik* instead of *per-eye-odik*.

Chromic Acid (CrO_3)

Chromic acid will *over*-oxidise glycol groups into aldehydes then acids.

Paradoxically, this is useful as fungal walls have more glycol groups than structures such as basement membranes. The greater number of glycol groups in the fungal walls means that there are more usable binding sites remaining after over-oxidation and the end result is better contrast between the fungal glycols (oxidised to aldehydes and acids) and background elements (over-oxidised into non-reactivity). The increased specificity is the reason that chromic acid is used also in the Grocott's Methanamine Silver stain (Speranza, 2005b). However, too much time in chromic acid will eventually render the fungal walls non-reactive.

A number of authors, including Carson (2010) and Speranza (2005a) have objected to a trend of using periodic acid to replace chromic acid as it is not powerful enough an oxidant and risks false negatives, especially of *Histoplasma* infections.

Some reasons for the switch are the problems of handling and disposing of chromic acid. However, if the slides are rinsed well of ethanol prior to the chromic acid step, the brown discolouration of the acid/ethanol reaction can be avoided and the chromic acid can be used indefinitely. This reduces the problems of disposal. It is up to the laboratory and pathologist to decide if the problems of handling chromic acid outweigh the advantages.

Permanganate (MnO_4^-)

Permanganate acts as an oxidant, is stable but it is non-specific. Nevertheless, there are a few fungal stains which use a permanganate oxidant. Like chromic acid, permanganate will eventually destroy the aldehydes it creates. Permanganate will give variable staining of fungi and the method needs to be standardised carefully before it can be used (Lillie 1951). An example, described by Prunieras, is described later.

Oxalic Acid ($H_2C_2O_4$)

Oxalic acid is used in commercial bleaching to remove colour and it is used this way to treat sections stained with permanganate in the melanin bleach and the Permanganate Alcian Blue stain (described below).

Schiff's Reagent

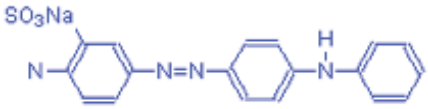
Schiff's reagent is used as part of a colour-change chemical test for aldehydes as it turns from clear to pink/magenta in water. This would make it ideal for histological practical jokes were it not for the fact that it is toxic and carcinogenic. It is used in histology to stain various aldehydes including those created by the oxidation of the fungal polysaccharides. Schiff's reagent is made from pararosanilin treated with sulphurous acid (Stains File 2005a). Excess Schiff's reagent can be removed by a sulphurous acid wash or thorough washing in water prior to the next staining step.

The other major method for demonstrating fungi is the silver step of the Grocott's Methenamine Silver stain. The PAB uses Alcian Blue but this is rarely used.

Counterstains

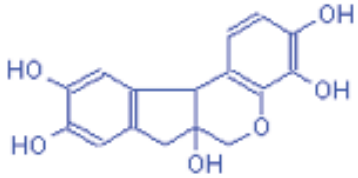
Counterstains are used to create contrast and definition between positive and negative tissue elements.

Metanil Yellow



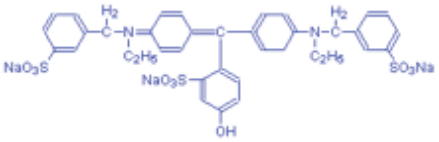
Metanil Yellow is a rarely-used stain for collagen. Its most common use is to add contrast to the red muscle in variations of the Masson's Trichrome. It is also used as a counterstain in the Gridley's method for fungi (Stains File 2005b).

Haematoxylin



Normally this is our prime stain but in fungal staining, haematoxylin is often relegated to a counterstain in the PAS (Stains File 2005c).

Fast Green



Fast green is used in the CAS and the McManus to provide contrast with the magenta of the Schiff's reagent (Stains File 2005d).

While there are a number of histological methods to demonstrate fungi, they all use the basic pattern of oxidation of the fungal glucose residue into aldehydes and then demonstrating their presence followed by a counterstain.

Of the six methods mentioned, the Grocott's and the PAS are the most common however other options exist and should be considered when searching for fungal stains.

Overview of Some Common and Uncommon Fungal Stains

Method		PAS	McManus	Grocott's	CAS	Gridley's	PAB
Oxidant	Periodic Acid	*	*	*			
	Chromic Acid			*	*	*	
	Permanganate						*
	Oxalic Acid						*
Aldehyde Demonstration	Schiff's Reagent	*	*		*	*	
	Aldehyde Fuchsin					*	
	Sulphurous Acid				*		
	Silver Precipitation			*			
	Alcian Blue						*
Counterstain	Haematoxylin	*					
	Metanil Yellow					*	
	Fast Green		*	*	*		
Advantages		Simplicity	Simplicity	Specificity	Specificity Sensitivity	Specificity, Intensity	
Disavantages		Non-specific	Non-specific	Safety, Difficulty	Safety	Difficulty	Non-specific

Note: A sulphurous acid step after the Schiff's reaction may be included or omitted as desired in any of the Schiff's-based methods but with sufficient washing with water, this step should not be necessary.

Of the stains I tried, I found the CAS to be the best. It was specific and sensitive and quite simple. The drawbacks were the long staining time (about two hours in total) and the dangers of chromic acid.

Chromic Acid and Schiff's Reagent Stain for Fungi (Carson & Hladi 2009, p. 236 – 238**)**

Of the stains I tried, I found this to be the best all-round stain, its only disadvantage being the use of chromic acid. The CAS is almost as simple as the PAS or McManus but gives more specific staining. While it takes a little longer than the Grocott's, it was a "low-maintenance" stain which could be largely ignored for most of the staining time.

Like the PAS, the CAS oxidises the glycols to create aldehyde binding sites. Sufficient time in chromic acid is necessary to over-oxidise the non-fungal elements into non-reactivity. The sulphurous acid removes unbound reagents from the Schiff's Reagent step (Carson & Hladi 2009, p. 238, Speranza, 2005b). Several older textbooks include this step to reduce unwanted staining from the Schiff's Reagent in the PAS and CAS reactions but it has fallen by the wayside as unnecessary.

The CAS method is similar to Gridley's method (described below) except Gridley's enhances the staining colour with an aldehyde fuchsin step (Speranza, 2005b). In older texts, the CAS is known as the Bauer reaction.

Personally, I found the fungal staining to be a little pale but still very clear. I skipped the sulphurous acid step and added a sodium thiosulphate step after chromic acid. I tried using the microwave to accelerate the chromic acid step (as per the QML SOPs for the Grocott's Methanamine Silver stain) but this did not work, presumably because the chitin was over-oxidised.

I had assumed that glycogen would also stain positive in the CAS and I was pleased to see that this is the case.

Reagents

- 5% Chromic Acid
- Schiff's Reagent
- Fast Green
- Sulphurous Acid Solution (mix just before use):

300mL Distilled Water

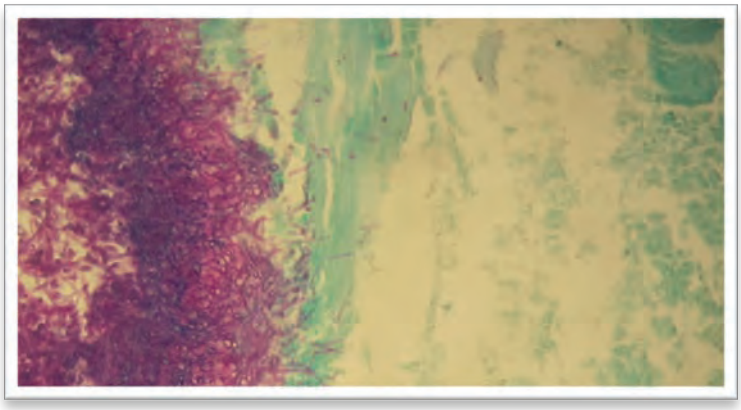
15mL 10% Sodium Metabisulphite

18 mL 1n Hydrochloric Acid

Method

1) Bring the slides to water.
2) Wash in deionised water.
3) *5% Chromic Acid*:

   60 minutes @ Room temperature.
4) Wash in deionised water.
5) *Schiff's Reagent*: 15 minutes.
6) 3 x *Sulphurous Acid Solution*: 2 minutes (I omitted this step).
7) Rinse in tap water: 15 minutes.
8) Counterstain with *Fast Green*: 1 minute (I used *Light Green* and *Methylene Blue* with success with both).
9) Rinse in water
10) Dry, clear and mount.



The CAS showed the fungal walls clearly with high specificity. The counterstain provided a good contrast. Of all the methods, this was the best. The power of chromic acid meant that longer time is needed in Schiff's Reagent and water to create the magenta seen in the PAS.

McManus Stain for Fungi

(Carson & Hladi 2009, p. 235 – 236)

The McManus Method for fungi is simply the PAS reaction with a light green counterstain to contrast with the pink/magenta of the fungal walls. As the McManus uses periodic acid rather than chromic acid, it is less-specific than the CAS and there will be increased staining of PAS-reactive background elements such as basement membrane and connective tissue. The lack of specificity is contrasted by the speed and simplicity of the stain.

Like the CAS, some versions of the McManus use sulphurous acid after the Schiff's reagent. From my own trials, there was no difference in the staining.

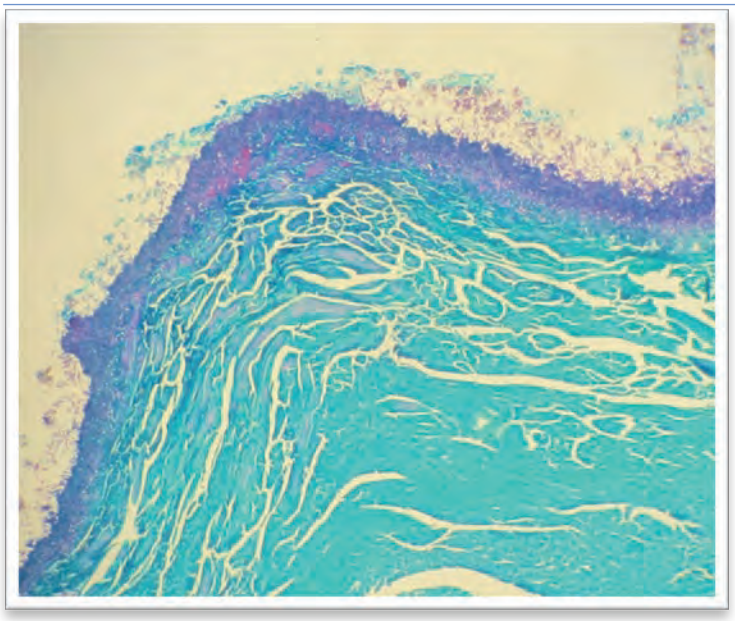
The McManus acted as expected. There was non-specific staining (which lead to an aesthetically ugly slide where the magenta clashed with the green) but the fungal staining was clear and strong. Also, the magenta was far easier to see with the green counterstain than it is with haematoxylin used in the PAS. Another advantage is that it takes a little less time than the traditional PAS.

Reagents

- 1% Periodic Acid
- Schiff's Reagent
- Fast Green

Method

1) Bring the slides to water.
2) Wash in deionised water.
3) *1% Periodic Acid* 5 minutes.
4) Wash in water.
5) *Schiff's Reagent*: 15 minutes.
6) Rinse in tap water: 15 minutes.
7) Counterstain with *Fast Green*: 1 minute (I used *Light Green*).
8) Wash in water.
9) Dry, clear and mount.



The McManus showed its non-specificity in the ugly clashes between the magenta and the green but the fungal elements are clear—indeed it is easier to make them out in the McManus than in the PAS.

Permanganate and Alcian Blue Stain (Prunieras 1960)

This was described by Prunieras as a way to highlight connective tissue, keratin and fungi. While it is not intended for fungi *per se*, I mention it as an example of a permanganate oxidant stain.

So far, I cannot get this to work. While the Alcian Blue tries its best, the staining is, at best, erratic and non-existent at its worst. Also, the (barely) blue fungal elements were often masked by the Davidson Blue marker dye used on the tissue during dissection.

This was expected as all the sources I read on this stressed the temperamental nature of the permanganate step and the need to standardise the method carefully.

Reagents

- Permanganate Solution

  100 mL 2.5% $KMnO_4$

  100 mL 5% H_2SO_4

  700 mL Distilled Water

- 1% Alcian Blue in 3% Acetic Acid
- 1% Alcian Blue in 10% Sulphuric Acid
- 1% Alcian Blue in distilled water
- 2% Oxalic Acid
- Preferred counterstain

Method

1) Bring the slides to water.

2) Oxidise with *Permanganate Solution*: 10 minutes.

3) Bleach in *Oxalic Acid*: 30 seconds.

4) Wash in running water, then distilled water.

5) Stain one slide in each of the three *Alcian Blue solutions*.

6) Wash in water.

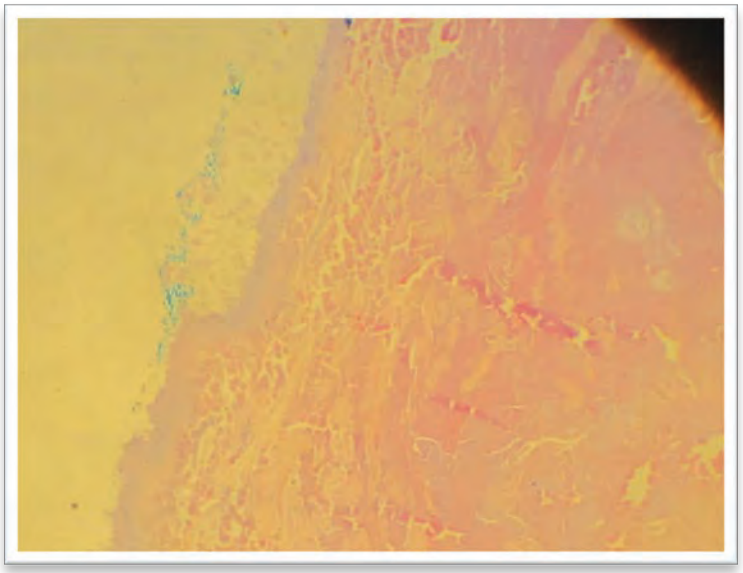
7) Counterstain as desired.

8) Dry, clear and mount.

The PAB. The staining is variable and often non-existent. Even though I knew where the fungi were in the slide, I had difficulty finding it. Also, the marker dye on the tissue obscured the pale blue of the Alcian Blue.

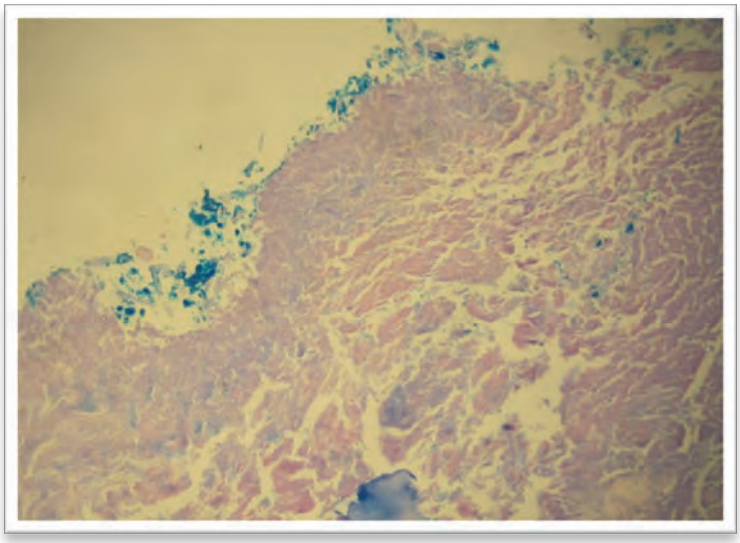
PAB Using Acetic Acid

The fungi are the faintly blue areas on the upper left edge. Note the overpoweringly strong blue of the marker dye at the far left.



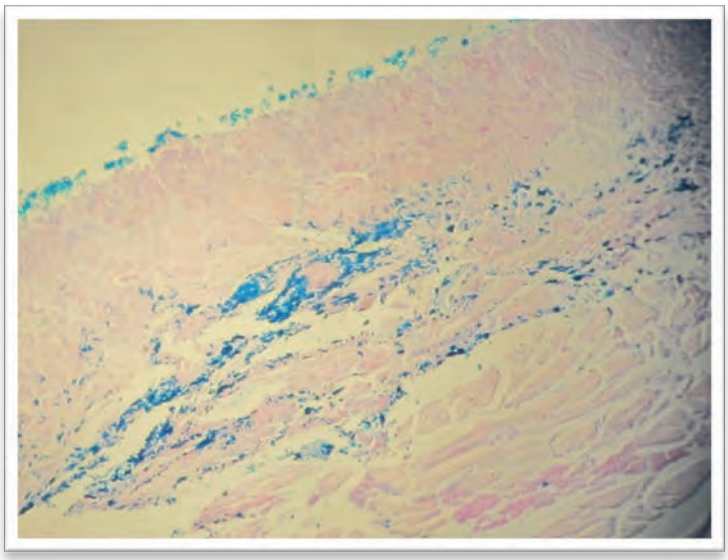
PAB Using Water

The fungi are along the upper left edge of the tissue. If I didn't know where I should look, I would not have found

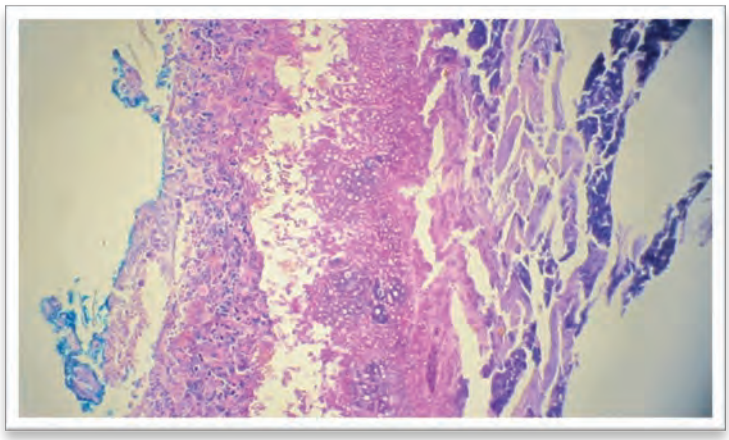


the fungi at all. Again, the marker dye overwhelms the faint blue of the fungi.

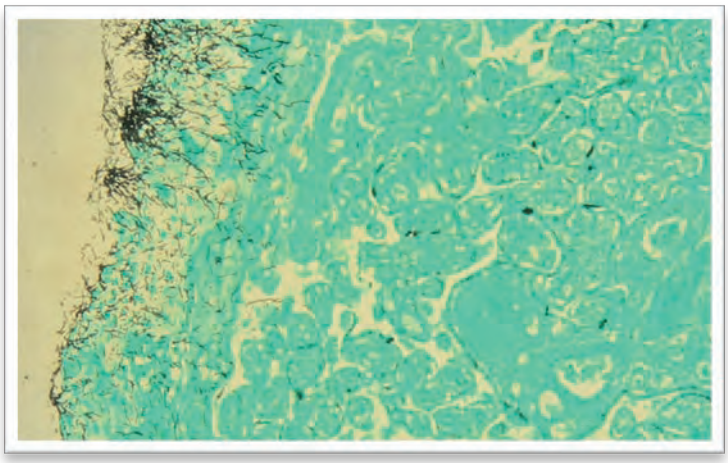
PAB Using Sulphuric Acid



As before, the fungi stains faintly and is masked by the tissue dye.



The PAS using the same tissue.



The traditional Grocott's fungal stain (different tissue)

Gridley Fungus Stain (Carson & Hladi 2009, p. 238 – 239).

The Gridley stain is similar in principle to the CAS except for an added aldehyde fucshin step which binds any remaining free linkages of the Schiff's reagent to enhance the reaction. This gives deeper staining than in the PAS or CAS (Speranza 2005b).

I have not tried this one yet as I am having trouble sourcing the reagents.

Reagents

- 5% Chromic Acid
- Schiff's Reagent
- Aldehyde Fuchsin

Pararosaline	1g
70% Ethanol	200mL

HCl conc	2mL
Paraldehyde	2mL

Mix and let stand for 2 – 3 days. Filter and store at 4°C.

- Metanil Yellow

Metanil Yellow	0.25g
Distilled Water	100mL
Glacial Acetic Acid	0.25g

Method

1) Bring the slides to water.

2) Wash in deionised water.

3) *5% Chromic Acid*:

60 minutes @ Room temperature.

Or

8 – 10 minutes @ 60°C.

4) Wash in water: 5 minutes.

5) *Schiff's Reagent*: 15 minutes.

6) Rinse in several changes of 70% ethanol.

7) *Aldehyde Fuchsin*: 30 minutes.

8) Rinse in 95% ethanol.

9) Rinse in distilled water.

10) *Metanil Yellow*: 30 – 60 seconds.

11) Rinse in distilled water.

12) Dehydrate (from 95% ethanol) and clear quickly and mount.

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30 Slide 10 Bath Linear Stainer!
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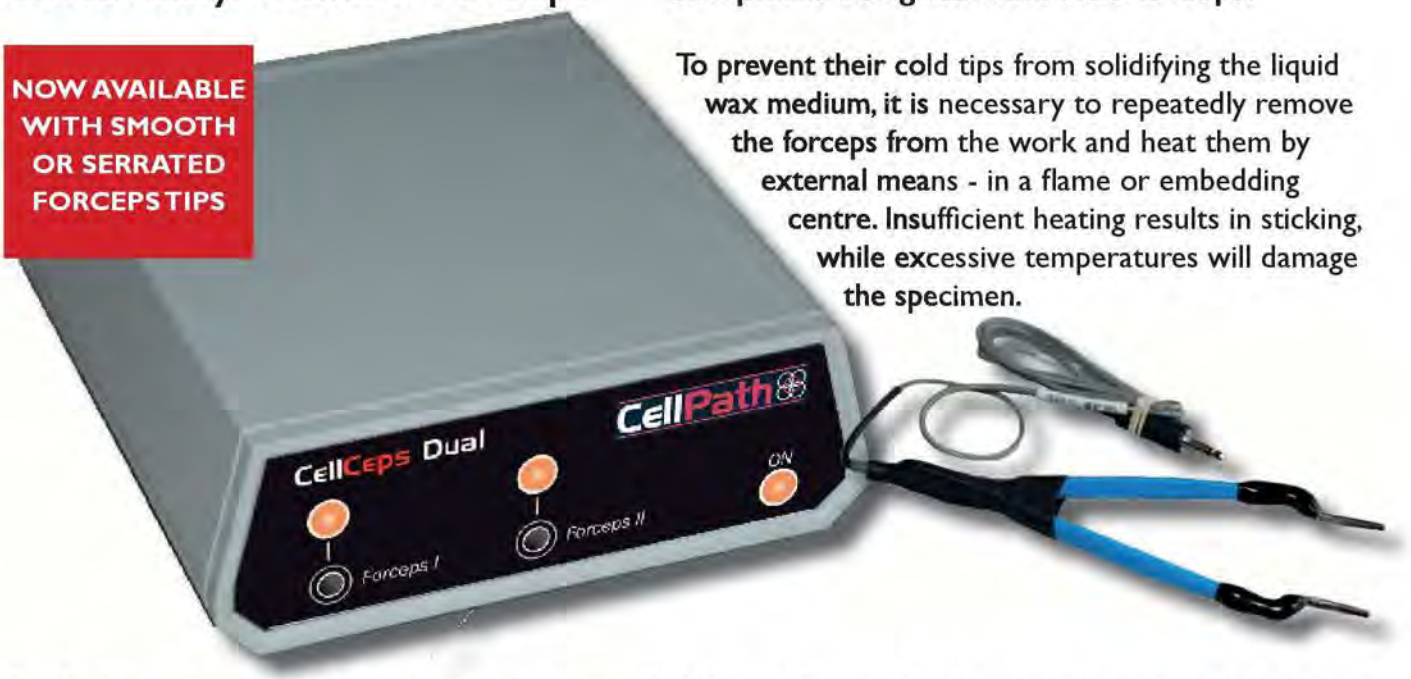
Electrically Heated Forceps

CellCeps are electrically heated forceps designed to assist in the preparation of histological slides. Orientation of a specimen during wax embedding has traditionally been completed using stainless steel forceps.

NOW AVAILABLE WITH SMOOTH OR SERRATED FORCEPS TIPS

To prevent their cold tips from solidifying the liquid wax medium, it is necessary to repeatedly remove the forceps from the work and heat them by external means - in a flame or embedding centre. Insufficient heating results in sticking, while excessive temperatures will damage the specimen.



CellCeps incorporate safe, low-voltage, energy controlled heating elements which constantly maintain their tips at the optimum temperature for manipulation of specimens in histological wax. They may be used continuously, without adjustment, and without influencing the temperature of the medium or tissue. Eliminating the task of keeping the forceps at a usable temperature enables the operator to concentrate on orientation of the tissue, and results in a significant increase in productivity. The unit allows 2 pairs of forceps to be used simultaneously. The use of smooth tipped forceps minimises tissue carry over during the embedding stage.

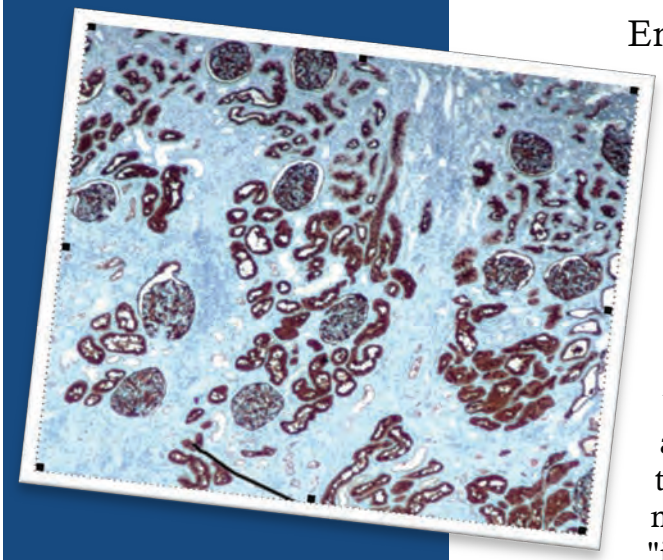
- Easier handling of tissue during embedding
- No sticking of forceps to the tissue
- Safe to use
- Forceps available in a choice of smooth or serrated tips.
- Will not damage the tissue
- Speeds embedding
- Supplied complete with 2 sets of forceps

Part No.	Description
GZB-0100-00A	HEATED FORCEPS - UNIT (CONTROL & SUPPLY + 1 x 1mm & 1 x 2mm SERRATED)
GZD-0100-00A	HEATED FORCEPS - UNIT (CONTROL & SUPPLY + 1 x 1mm & 1 x 2mm SMOOTH)
GZA-0100-00A	HEATED FORCEPS - 1mm SERRATED (RED)
GZC-0100-00A	HEATED FORCEPS - 1mm SMOOTH (RED)
GZA-0200-00A	HEATED FORCEPS - 2mm SERRATED (YELLOW)
GZC-0200-00A	HEATED FORCEPS - 2mm SMOOTH (YELLOW)
GZA-0300-00A	HEATED FORCEPS - 4mm SERRATED (BLUE)
GZC-0300-00A	HEATED FORCEPS - 4mm SMOOTH (BLUE)
GZB-0200-00A	HEATED FORCEPS - CONTROL UNIT
GZB-0300-00A	HEATED FORCEPS - POWER SUPPLY FOR CONTROL UNIT

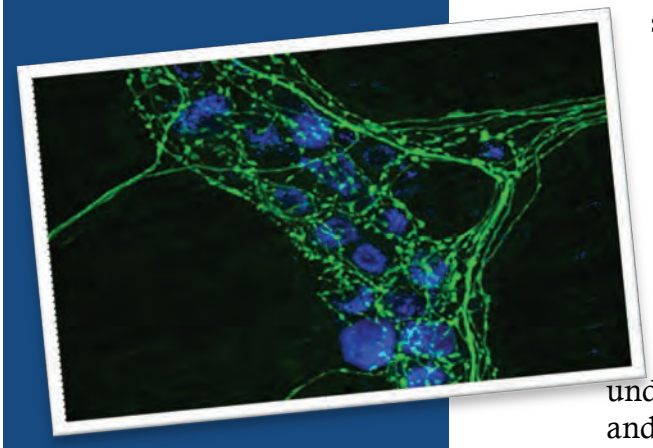
** Please note: The removable forceps (heating elements) should be viewed as consumable items and have a working life of approximately 9 months depending on usage.*

Immunohistochemistry: An Introduction

Emma Hughes – Sullivan Nicolaides Pathology



CD 10 normal kidney stained via a peroxidase



TH protein stained via a fluorophore (green) in axons of a sympathetic autonomic neuron

Immunohistochemistry or IHC refers to the process of detecting antigens (e.g., proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. IHC takes its name from the roots "immuno," in reference to antibodies used in the procedure, and "histo," meaning tissue.

Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death (apoptosis). IHC is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. Visualising an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated(joined) to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction.

Alternatively, the antibody can also be tagged to a fluorophore, such as fluorescein or rhodamine which will produce a fluorescent compound.

Sample preparation

While using the right antibodies to target the correct antigens and amplify the signal is important for visualization, complete preparation of the sample is critical to maintain cell morphology, tissue architecture and the antigenicity of target epitopes. Antigenicity is the ability of a chemical structure (referred to as an antigen) to bind specifically with a group of certain products that have adaptive immunity: T cell receptors or antibodies (a.k.a. B cell receptors). An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells.

This requires proper tissue collection, fixation and sectioning. Formalin is usually used with fixation. Depending on the purpose of the staining 2-4µm sections are taken from the tissue of interest and mounted onto positively charged slides for staining.

Continued

Because of the method of fixation and tissue preservation, the sample may require additional steps to make the epitopes available for antibody binding, including deparaffinization and antigen retrieval, these steps often make the difference between staining and no staining. Additionally, depending on the tissue type and the method of antigen detection, endogenous biotin or enzymes may need to be blocked or quenched, respectively, prior to antibody staining.

Although antibodies show preferential avidity for specific epitopes, they may partially or weakly bind to sites on nonspecific proteins (also called reactive sites) that are similar to the cognate binding sites on the target antigen.

In the context of antibody-mediated antigen detection, nonspecific binding causes high background staining that can mask the detection of the target antigen. To reduce background staining in IHC, the samples are incubated with a buffer that blocks the reactive sites to which the primary or secondary antibodies may otherwise bind.

Antibody types

The antibodies used for specific detection can be polyclonal or monoclonal. Polyclonal antibodies are made by injecting animals with peptide Ag and, after a secondary immune response is stimulated, isolating antibodies from whole serum. Thus, polyclonal antibodies are a heterogeneous mix of antibodies that recognize several epitopes. Monoclonal antibodies show specificity for a single epitope and are therefore considered more specific to the target antigen than polyclonal antibodies.

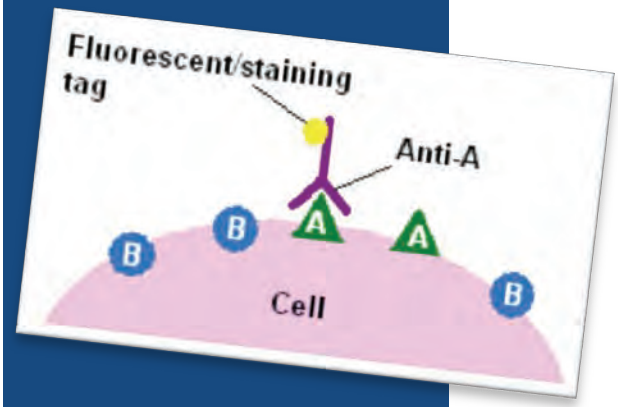
For IHC detection strategies, antibodies are classified as primary or secondary reagents. Primary antibodies are raised against an antigen of interest and are typically unconjugated (unlabelled), while secondary antibodies are raised against immunoglobulins of the primary antibody species. The secondary antibody is usually conjugated to a linker molecule, such as biotin, that then recruits reporter molecules, or the secondary antibody itself is directly bound to the reporter molecule.

IHC reporters

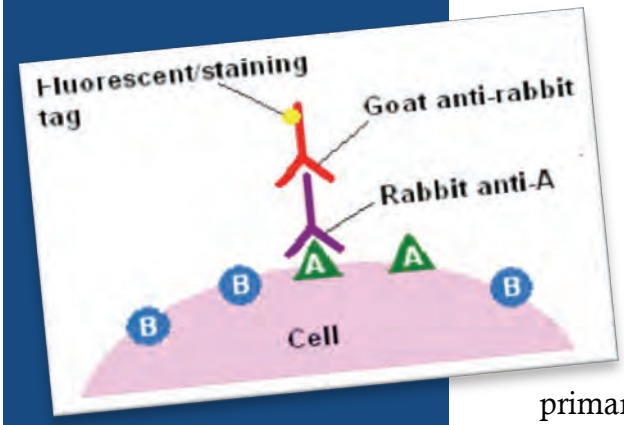
Reporter molecules vary based on the nature of the detection method, the most popular being chromogenic and fluorescence detection mediated by an enzyme or a fluorophore, respectively. With chromogenic reporters, an enzyme label is reacted with a substrate to yield an intensely colored product that can be analyzed with an ordinary light microscope. While the list of enzyme substrates is extensive, Alkaline phosphatase (AP) and horseradish peroxidase (HRP) are the two enzymes used most extensively as labels for protein detection. An array of chromogenic, fluorogenic and chemiluminescent substrates is available for use with either enzyme, including DAB or BCIP/NBT, which produce a brown or purple staining, respectively, wherever the enzymes are bound.

Reaction with DAB can be enhanced using nickel, producing a deep purple/black staining. Fluorescent reporters are small, organic molecules used for IHC detection and traditionally include FITC, TRITC and AMCA. For chromogenic and fluorescent detection methods, densitometric analysis of the signal can provide semi and fully quantitative data, respectively, to correlate the level of reporter signal to the level of protein expression or localization. Meaning by counting the number of points of colour in a defined field you can make a statement about the amount of a particular antigen present in a tissue sample.

Continued



Direct Method



Indirect Method

Target antigen detection methods

The direct method is a one-step staining method and involves a labeled antibody (e.g. FITC-conjugated antiserum) reacting directly with the antigen in tissue sections. While this technique utilizes only one antibody and therefore is simple and rapid, the sensitivity is lower due to little signal amplification, such as with indirect methods, and is less commonly used than indirect methods.

The **Direct method** of immunohistochemical staining uses one labelled antibody, which binds directly to the antigen being stained for.

The **Indirect method** involves an unlabeled primary antibody (first layer) that binds to the target antigen in the tissue and a labeled secondary antibody (second layer) that reacts with the primary antibody.

The indirect method of immunohistochemical staining uses one antibody against the antigen being probed for, and a second, labelled, antibody against the first.

As mentioned above, the secondary antibody must be raised against the IgG of the animal species in which the primary antibody has been raised. This method is more sensitive than direct detection strategies because of signal amplification due to the binding of several secondary antibodies to each primary antibody if the secondary antibody is conjugated to the fluorescent or enzyme reporter.

Further amplification can be achieved if the secondary antibody is conjugated to several biotin molecules, which can recruit complexes of avidin-, streptavidin or NeutrAvidin proteinbound-enzyme. The difference between these three biotin-binding proteins is their individual binding affinity to endogenous tissue targets leading to nonspecific binding and high background; the ranking of these proteins based on their nonspecific binding affinities, from highest to lowest, is: 1) avidin, 2) streptavidin and 3) Neutravidin protein. The indirect method, aside from its greater sensitivity, also has the advantage that only a relatively small number of standard conjugated (labeled) secondary antibodies needs to be generated. For example, a labeled secondary antibody raised against rabbit IgG, which can be purchased "off the shelf," is useful with any

Continued

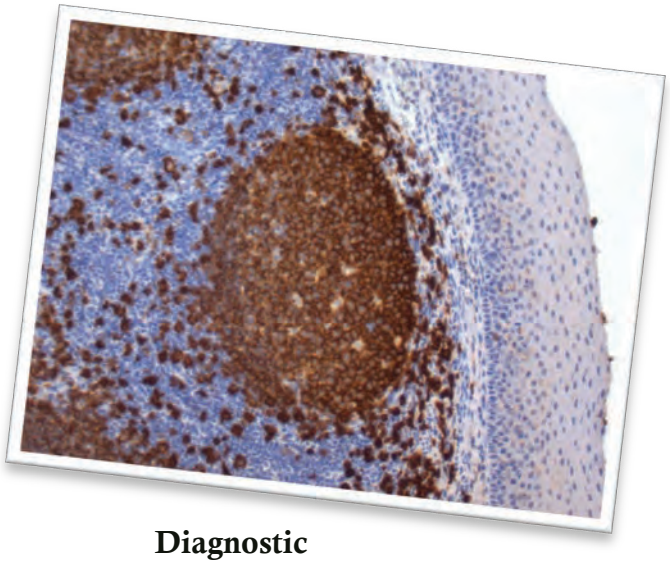
primary antibody raised in rabbit. With the direct method, it would be necessary to label each primary antibody for every antigen of interest.

Counterstains

After immunohistochemical staining of the target antigen, a second stain is often applied to provide contrast that helps the primary stain stand out. Many of these stains show specificity for discrete cellular compartments or antigens, while others will stain the whole cell. Both chromogenic and fluorescent dyes are available for IHC to provide a vast array of reagents to fit every stain, haematoxylin, Hoechst stain and DAPI are commonly used.

IHC Troubleshooting

In immunohistochemical techniques, there are several steps prior to the final staining of the tissue antigen, and many potential problems affect the outcome of the procedure. The major problem areas in IHC staining include strong background staining, weak target antigen staining and autofluorescence. Endogenous biotin or reporter enzymes or primary/secondary antibody cross-reactivity are common causes of strong background staining, while weak staining may be caused by poor enzyme activity or primary antibody potency. Furthermore, autofluorescence may be due to the nature of the tissue or the fixation method. These aspects of IHC tissue prep and antibody staining must be systematically addressed to identify and overcome staining issues.



Diagnostic IHC markers

IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues. The technique is widely used in diagnostic surgical pathology for typing tumors (e.g. immunostaining for E-cadherin to differentiate between DCIS (ductal carcinoma in situ: stains positive) and LCIS (lobular carcinoma in situ: does not stain positive).

Monoclonal antibodies

Many proteins shown to be highly upregulated in pathological states by immunohistochemistry are potential targets for therapies utilising monoclonal antibodies. Monoclonal antibodies, due to their size, are utilized against cell surface targets. Among the overexpressed targets, the members of the epidermal growth factor receptor (EGFR) family, transmembrane proteins with an extracellular receptor domain regulating an intracellular tyrosine kinase, Of these, HER2/neu (also known as Erb-B2) was the first to be developed. The molecule is highly expressed in a variety of cancer cell types, most notably breast cancer. As such, antibodies against HER2/neu have been FDA approved for clinical treatment of cancer under the drug name Herceptin. Similarly, EGFR (HER-1) is overexpressed in a variety of cancers including head and neck and colon.

Immunohistochemistry is used to determine patients who may benefit from therapeutic antibodies such as Erbitux (cetuximab).

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CG12432	24x32mm	$34.50/1000
CG12440	24x40mm	$38.50/1000
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	Code	Price
Plain, 90 Ground Edge	SPGE90	$2.25/50
Double Frosted, Cut Edge	SDFCE90	$2.00/50
Single Frosted, 45° Ground Edge,	SSFGE45	$3.45/50
Double Frosted, 90° Ground Edge	SDFGE90	$2.70/50
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0101222	24x50mm	$46.00/1000
0101232	24x55mm	$54.00/1000
0101242	24x60mm	$57.00/1000

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Temperature range of -70°C to +100 °C
The set consists of

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IW-2510 Staining Set **$395ea**

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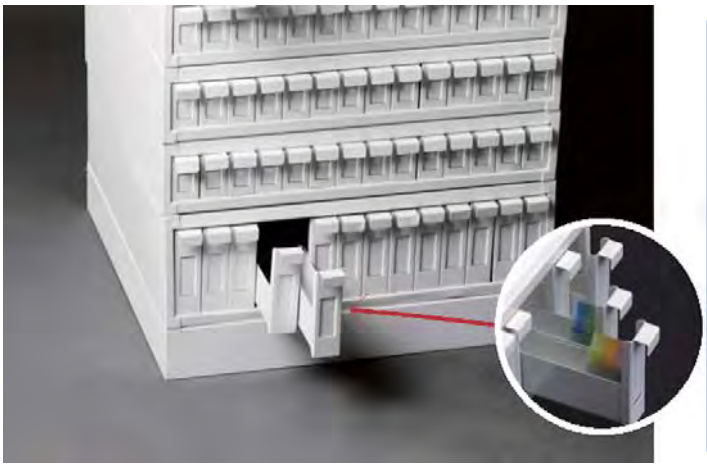

51040010	Base for Storage cabinet ext dimensions, 485x480x26mm, Metal, 485x480x26mm, Metal, White Powder Coated	**$40.00ea**
51040040	Lid for Storage cabinet ext dimensions, 485x480x26mm, Metal, White Powder Coated	**$30.00ea**
51045001	Slide Storage Cabinet, stackable, each unit contains 14 drawers and Approx. 400 slides ext dimensions, 485x480x126mm, Metal, White Powder Coated	**$295.00ea**
51040801	Paraffin Block Storage Cabinet, stackable, each unit contains 14 drawers And approx. 65 Paraffin Blocks ext dimensions, 485x480x126mm, Metal, White Powder Coated	**$245.00ea**
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HGQ AGM & Scientific Meeting 2012

"The 2012 Annual General Meeting and the final Scientific Meeting for the year was held on Friday the 7th December 2012, at Kedron-Wavell Services Club"

Emma Hughes – Sullivan Nicolaides Pathology

The 2012 Annual General Meeting and the final Scientific Meeting for the year was held on Friday the 7th December 2012, at Kedron-Wavell Services Club.

Tony Reilly then gave his President's Report. In summary the State Conference, Scientific Meetings & a Social event were held during the year with overall success. The Social event & scientific meeting schedule for 2013 is to be drafted shortly.

The 1st annual HGQ QUT Award for excellence in practical histopathology procedures was presented this year to Ashleigh Zaeza who was presented her award by Tony Riley at QUT's Graduation Ceremony.

The 2012 committee members were thanked for their contributions throughout the year especially for their work in the organization of the State Conference.

As a result of the general feelings of respect felt for the work done by Dr Brian Miller with the Overseas Specialist Surgical Association of Australia after the presentation Dr Miller and Dr Cooke gave at the 2012 State conference a motion was made to make a donation to the OSSA. Dr Miller is to be contacted to determine whether the purchase of surgical/pathological equipment of a monetary donation of $10 000 would be preferable. All attending members passed this motion unanimously.

The Treasurer's report by Stephen Riley informed the members that the HGQ had a very healthy balance after making a profit from the State Conference. As a result of this a motion was put forward that there be No membership fee from 2013 onwards with only yearly memberships registrations/renewals to continue. All attending members passed this motion unanimously.

There was then the Election of Office Bearers all positions on the committee were vacated and nominations were taken and carried unanimously for the following positions.

President – Anthony Van Zwieten (TPCH)

Treasurer – Jason Tu (PAH)

Secretary – Jerres Alcober (TPCH)

Editor – Emma Hughes (SNP)

Committee Members

Stephen Riley (PAH)

Tony Riley (PAH)

Continued

Melissa Hilas (Mater)

Lloyd Blundell (Trade)

Claire Chupin (Trade)

Mohammed Amigh (DAFF)

Helen O'Connor (QUT)

Alin Livadaru (Trade)

Vivienne Treagus (Healthscope)

Jessica Hurst (RBWH)

The final Scientific Meeting for 2012 was hosted by The Prince Charles Hospital. The Guest speakers included Claire Chupin from Abacus ALS who gave a brief talk on their New Cell Marque SOX antibodies.

Dr. Felicia Goh who works in the UQ research department @ TPCH talked about a project they were involved with which had the goal of genome sequencing Lung tumour tissue to aid in medical research. She detailed the problems and conditions involved in screening all the tissue they sent to America for inclusion in this project.

Alin Livadaru from Diagnostic Technology gave us a brief introduction to the MesoDissection System by AvanSci Bio, for automated removal of slide mounted tissue which enables the user to remove a small portion of tissue from a stained slide ready for further testing (e.g. Molecular Pathology) and the Tissue Microarray Technology which uses both manual and automated methods for the production of Microarray blocks to be used in the IHC, H&E and Special Stains areas of Histology.

Finally there was a wonderful Trivia competition held after the buffet dinner, which was organized by the fantastic Jerres Alcober. We had prizes donated by the Trades in attendance to be given away and ended the night with the fun game heads and tails, which was won by yours truly.

I hope to see you all at the next Scientific Meeting which is tentatively scheduled for Thursday the 2nd of May at 6:30pm.

PST

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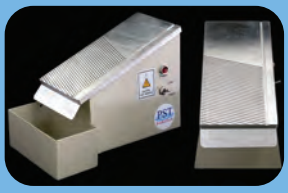


ProSciTech supplies a full range of histology instruments and most supplies. Instruments include tissue processors, paraffin wax dispensers, embedding centres, wax block trimmers, microtomes and cryostats, tissue flotation workstations, slide warmers, and slide stainers.

Histotechnology Group of Queensland Award

QUT Awards Ceremony – 2012 Academic Year

Photos: Jerres Alcober

When: 13 March 2013

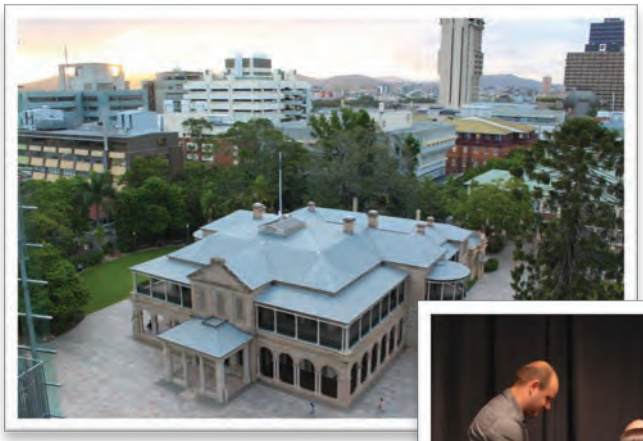
Where: Room Three Sixty, Level 10 - Y Block, Gardens Point Campus, QUT

Award: Student with the highest aggregate mark for the practical component of Histology course topics

Prize: $300 cash from the HGQ

Presented by: Anthony van Zwieten – HGQ President

CONGRATULATIONS to Pauline Lim



Letter of appreciation from OSSAA to the HGQ - $10,000 donation

OSSAA

28th January 2013

Mr Stephen Riley
Anatomical Pathology
Princess Alexandra Hospital
Ipswich Road
WOOLOONGABBA QLD 4102

The Histology Group of Queensland's donation
to the
Overseas Specialist Surgical Association of Australia

Dear Stephen

Please accept the grateful acknowledgement and thanks, expressed on behalf of OSSAA, for the Histology Group of Queensland's most generous donation to our organisation.

The donation will support OSSAA's efforts to assist poor people suffering disability and deformity in Timor Leste and Eastern Indonesia. It will also make a most significant contribution to OSSAA's ongoing training and rehabilitation work, which we conduct with local nursing, medical and other staff in the two countries.

OSSA looks forward to keeping you informed of this work, noting the contribution that the Histology Group of Queensland has made. We consider ourselves very fortunate to have your support.

Yours sincerely

Patrick Markwick-Smith

Patrick Markwick-Smith
President, Overseas Specialist Surgical Association of Australia

Surgical Teams Volunteering Abroad

NATIONAL HISTOLOGY CONFERENCE 2013

Conference Program

National Histology Conference Program final as of 18th March 2013. Program may be subject to change and the Conference Organisers and/or organising Committee will endeavour to let you know prior to the Conference.

Friday 26th April 2013 - Workshops

8.00		**Registration Desk Opens** (will close at 6.00pm)
10.00	Dr Guy Orchard / Mohammed Shams	**Workshop #1:** MOHS Technique (Advanced)
10.00	Dr Thomas Haas	**Workshop #2:** Tissue identification for the Histologist (Basic)
14.00	Dr Thomas Haas	**Workshop #3:** Stalking the big Four: New developments in the diagnosis of breast, prostate, colon and lung carcinomas (Advanced)
14.00	Jason Kelly / Alex Laslowski / Mark Bromley / Rita Au	**Workshop #4:** Basic Immunohistochemistry Stain Identification: Focus on stain identification & Recognition of some popular antibody markers (Basic)
18.00	Social Function	**Welcome Reception** | Exhibition Hall: Crown Conference Centre

Saturday 27Th April 2013

7.30		**Registration Desk Opens**
9.00	Adrian Warmington	Welcome
9.10	Dr Rowan Story	The Role of Health Reserves in the Defence of Australia
9.50	Keith Byron	Introduction to Molecular Techniques
10.35		**Morning Tea**
11.05	Tony Van Galen	Laboratory practices for the diagnosis and management of prostate cancer
11.35	Mark Koina	Electron Microscopy diagnosis and infectious diseases – the ACT Pathology Experience
12.05	Dr Guy Orchard	IHC Diagnosis of Malignant Melanoma
12.50	Anne Prins	"Animal, Vegetable, mineral or….?"
13.20		**Lunch**
14.10	Piero Nelva / Dr Beena Kumar	Technical aspects related to Breast cancer diagnosis
14.55	Natalie Kvalheim	Abalone virus ISH
15.25	Soeun Mom	Male infertility : Testicular biopsies
15.45		**Afternoon Tea**
16.15	MyHoa Huynh	GVHD: An Introduction
16.35	Nina Fotinatos	Career paths/ research opportunities post MLS
19.00	Social Function	**Conference Gala Dinner** | River Room: Crown Entertainment

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NATIONAL HISTOLOGY CONFERENCE 2013

Complex

Sunday 28th April 2013

8.30		**Registration Desk Opens**
9.30	Naomi McCallum	EM diagnosis : Neuropathology case studies
10.00	Dr Thomas Haas / Heather Renko	SLN: A look at the significance from a Histotech's perspective
11.00		**Morning Tea**
11.30	Suzanne Svobodova	Companion diagnostic molecular testing for BRAF, KRAS and EGFR
12.00	David Gan	Making the most of your specimen in IHC
12.30	Andrew Griffin	NATA: Aspects of the standard in reference to laboratory accreditation
13.00		**Lunch**
14.00	Professor Peter Choong	Targeted therapies in sarcoma
14.30	Sarah Morabito	Leishmaniasis
15.00	Greg Jenkins	Histology disasters

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p40 IHC at The Prince Charles Hospital

Anthony van Zwieten – Pathology Queensland

What is it? - Actually an isoform of p63 - Ab recognises ΔN domain of protein

- Described as a sensitive marker of pulmonary SCC

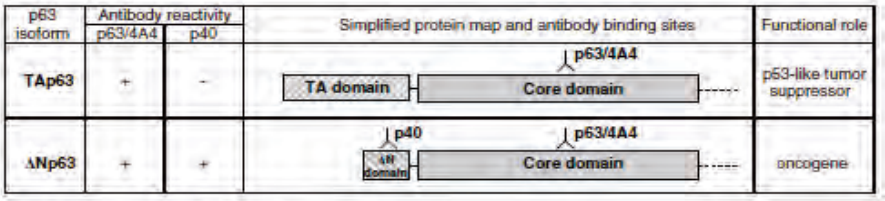
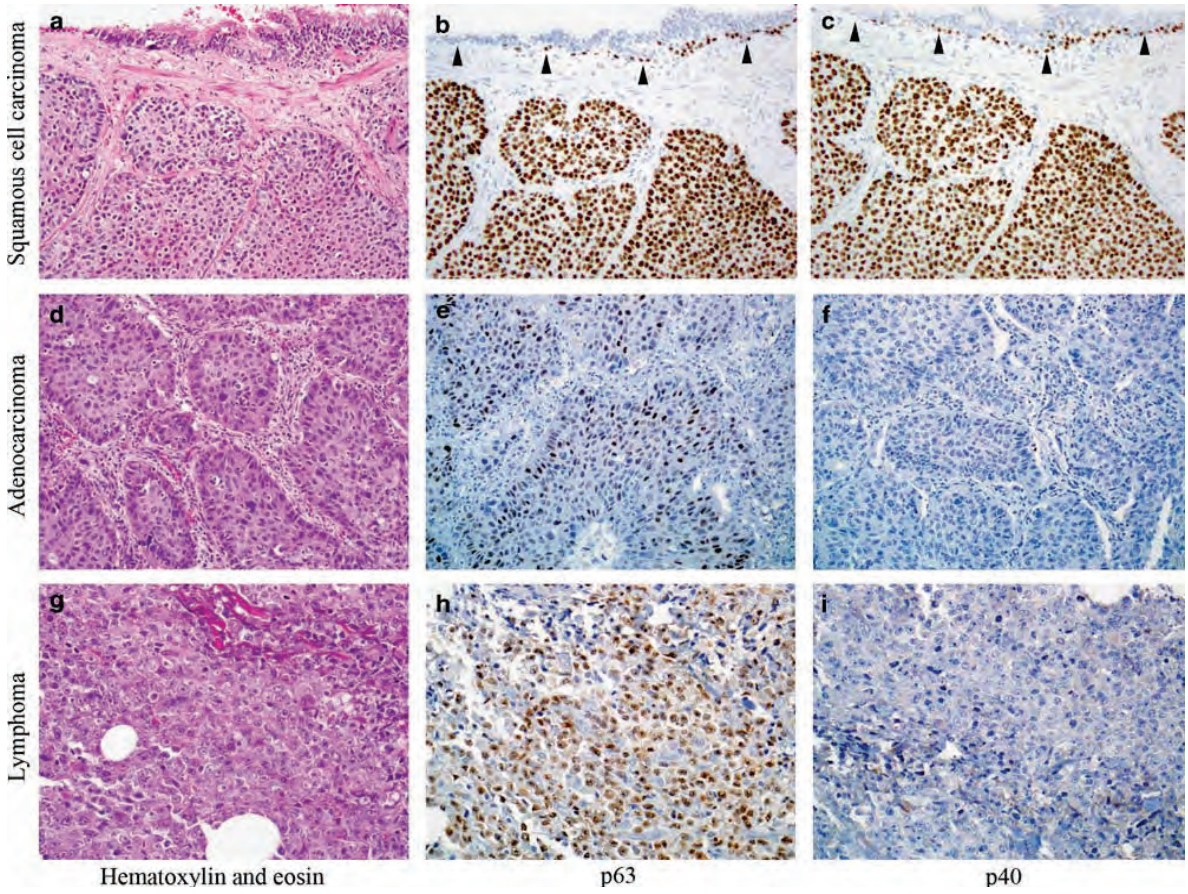


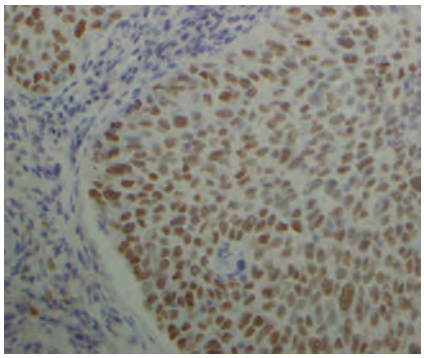
Figure 1 Diagram of p63 isoforms and antibody binding sites.

p63 V p40 in pulmonary tumours

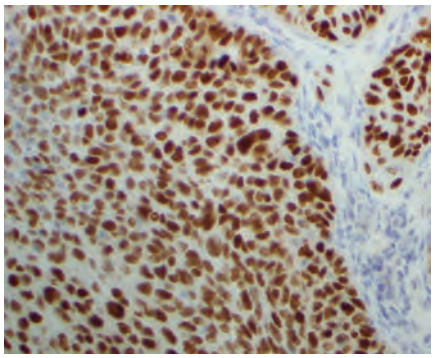


Antibody Comparison

p40
- Biocare
- Rabbit polyclonal
- Dilution 1/100



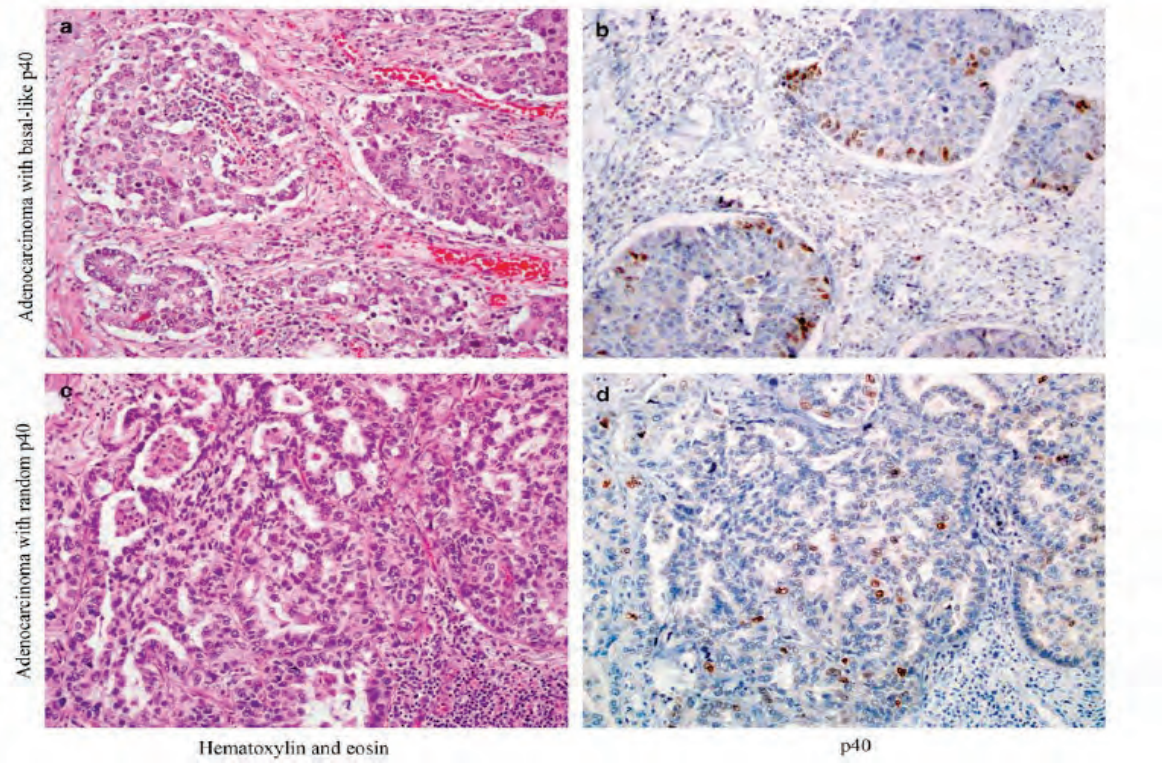
p63
- Dako
- Mouse monoclonal (4A4)
- Dilution 1/500



Reference Findings

Diagnosis	p63	p40
Lung SCC	100%	100%
Lung ADC	31%	3% (<5% of cells)
Large Cell Lymphoma	54%	0%

p40 detection in Lung Adenocarcinoma



P63 and pulmonary lymphoma

Frequent (>50%)

?Labelling germinal centre lymhocytes

Lymphomas in Lung

Can clinically mimic carcinoma

Anaplastic large cell lymphomas

Negative CD45; Positive EMA

Potential pitfall with IHC interpretation

p40 not recognised in lymphoma?

Other Abs for consideration

- CK 5/6, CK 34BE12, SOX-2, Glypican 3
- Desmocollin-3 (<100% specificity of SCC, cytoplasmic marker)
- None as sensitive as p40
- p40 is a nuclear marker

Further information

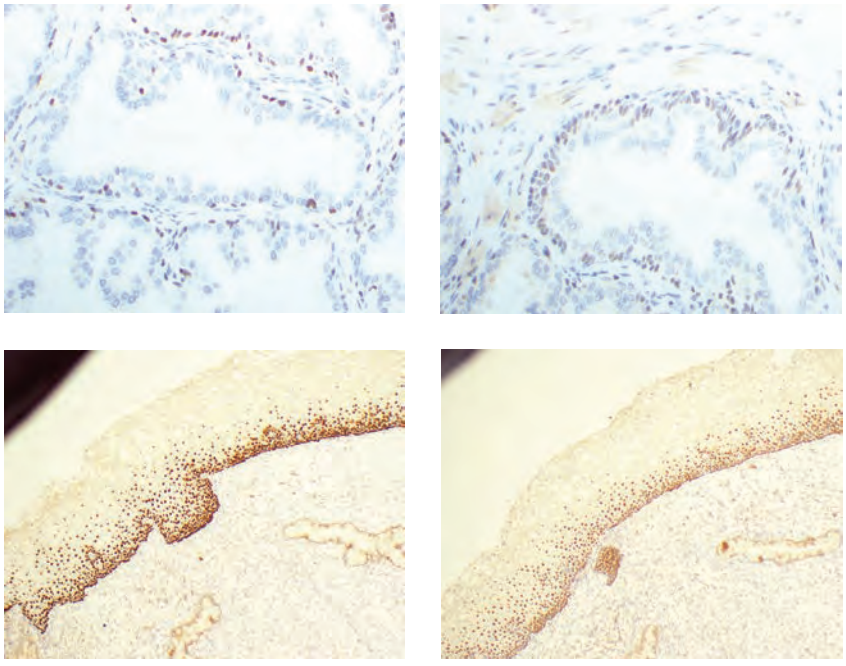
- Minimises reliance on other markers
  - Ordering of CK 5/6
  - Result of TTF-1 not as critical
    - p63 positivity when TTF-1 also positive regarded as non-specific
    - TTF-1 cross-reactive with

Recommendations

- p40 sensitive and specific marker for pulmonary squamous cell carcinoma
- Almost no immunoreactivity in lung adenocarcinomas
- No staining of Lymphomas
- No need for CK5/6 & not reliant on TTF-1

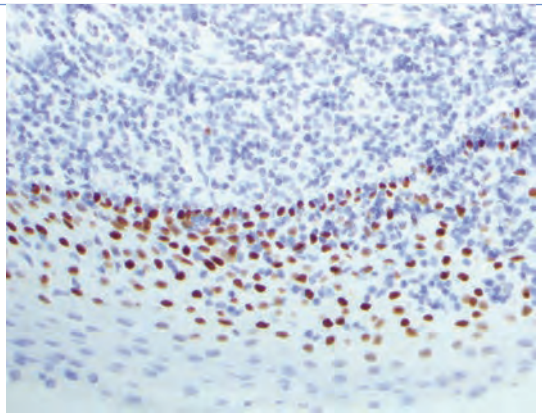
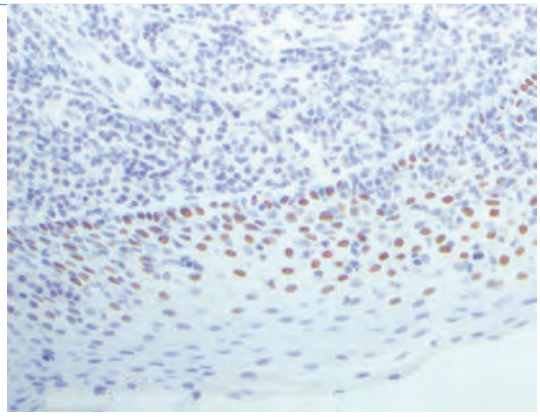
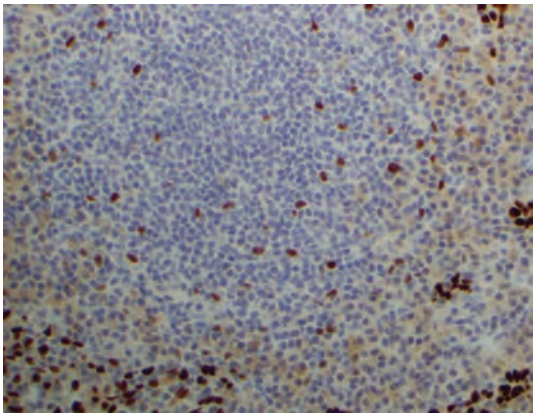
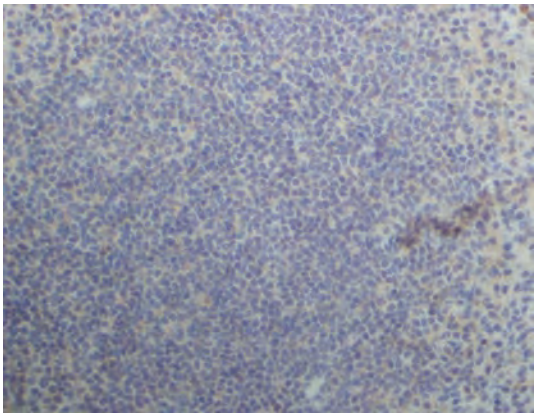
Antibody may become more accessible in AUS in near future i.e. Napsin A

p63 v p40 in normal tissue



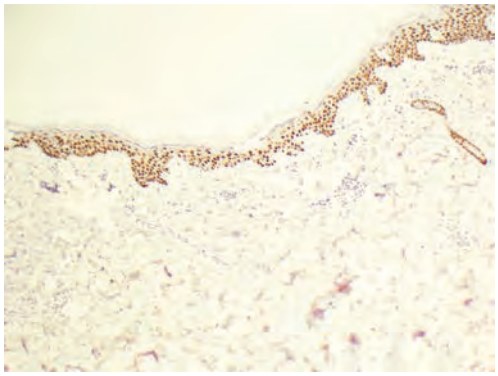
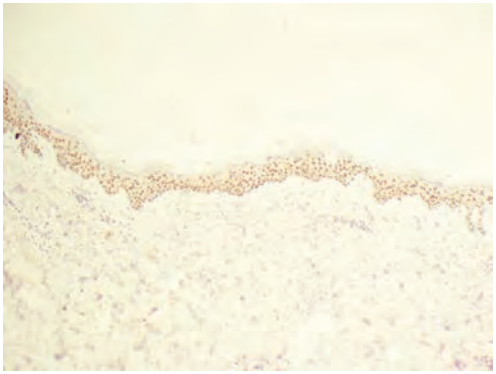
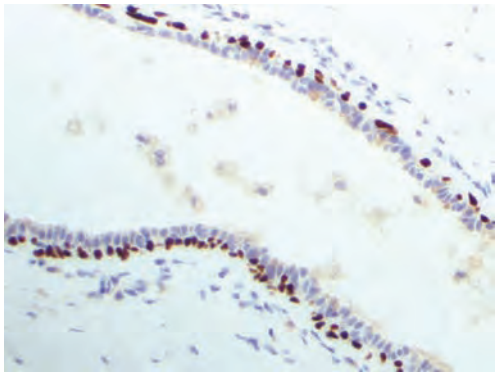
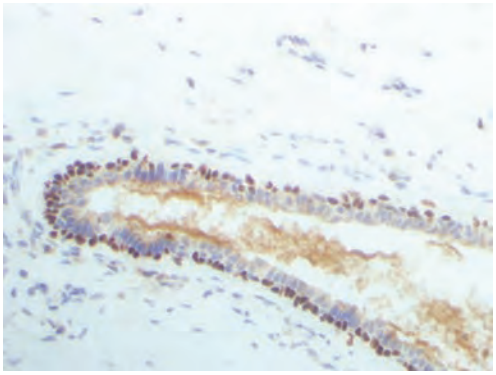
p63 **p40**

p63 v p40 in tonsil

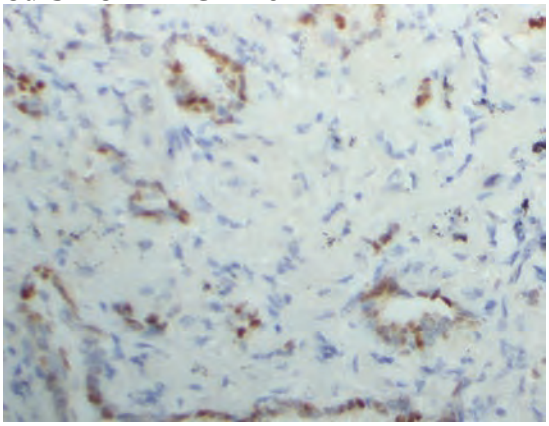
p63 **p40**

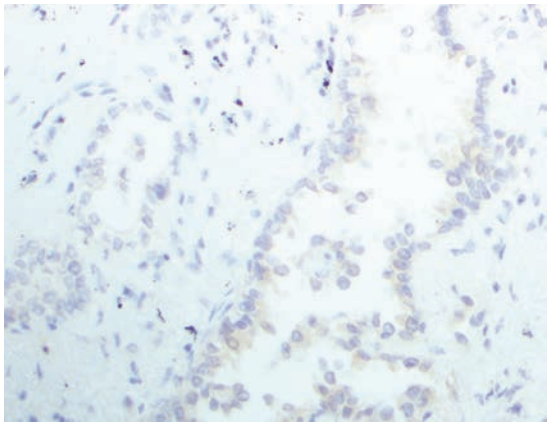
p63 v p40 in skin and breast

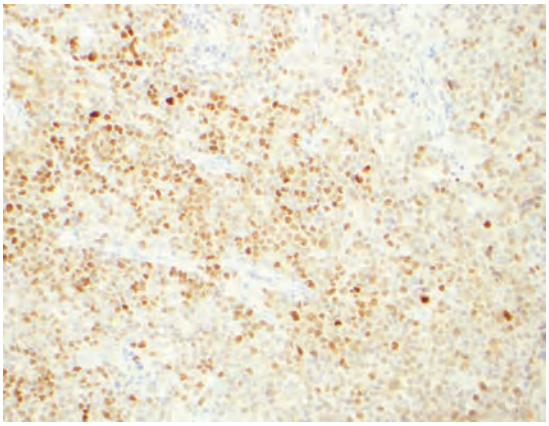





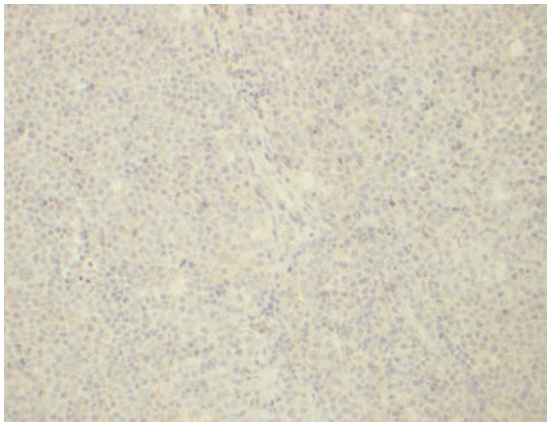
p63 **p40**

p63 v p40 in lung tumours from TPCH 2012



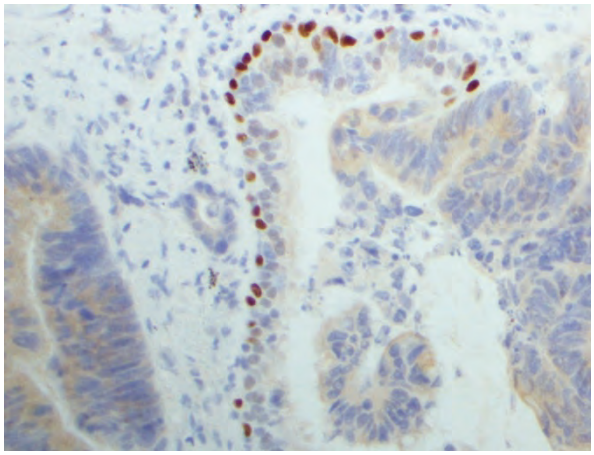


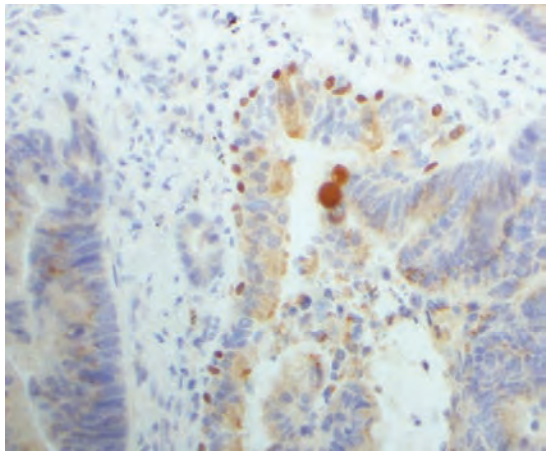




p63 **p40**

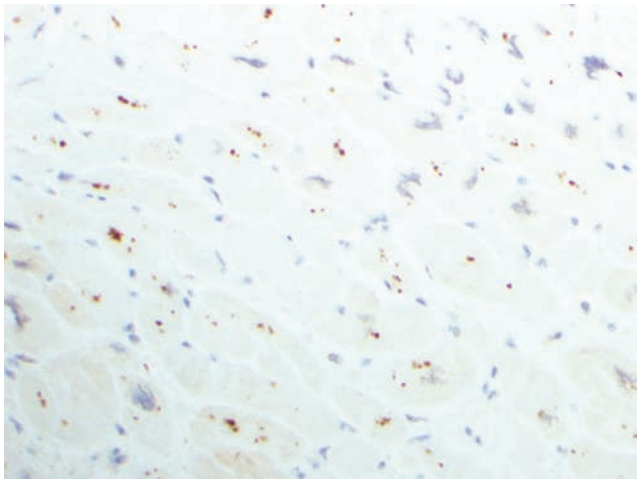
Metastatic colorectal carcinoma in Lung *(Note internal control positive staining of bronchial epithelium)*



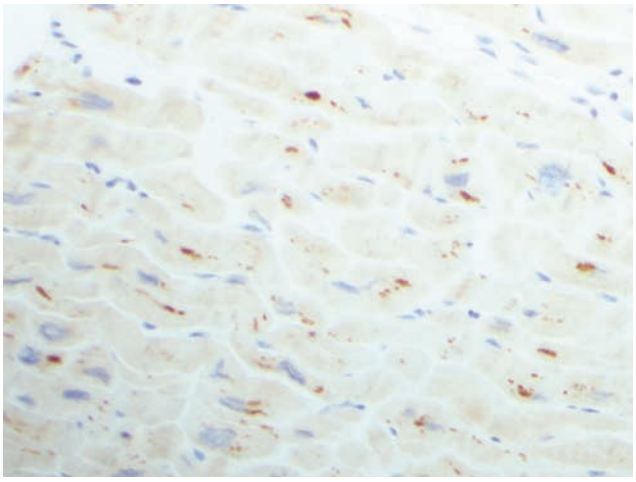


p63 **p40**

p63 v p40 in heart



p63



p40

References

- Bishop JA *etal.* **p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma.** Department of Surgical Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA. Mod Pathol. 2012 Mar;25(3):405-15
- Nylander **Differential expression of p63 isoforms in normal tissues and neoplastic cells.** J Pathol. 2002 Dec;198(4):417-27.

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

Full Name: _____

Employer & Site: _____

Job Title / Position: _____

Mailing Address: (Work Preferred)

_____

_____

_____

Email: (Work Preferred) _____

Contact Number: _____

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: Mr 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