

## PARAFFINALIA NEWSLETTER

**VOLUME 25, NUMBER 4**  
**December 2020**

The HGVT aims to provide a dynamic continuing education program in which all persons with an interest in Histology and Histotechnology are freely invited to participate.

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| Adrian Warmington     | Dorevitch Pathology (Ballarat) |
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# President's Report – Behind the Bench



Greetings Resilient Mask Wearers to the last Paraffinalia for 2020  
As 2020 ends, we can say many things about the year that was. Our predictable world was shaken, and our work and home life were impacted daily by coronavirus. Suddenly everyone began talking about Pathology. Our profile in diagnostics was boosted every time Dan mentioned the number of tests performed, the positive results, turn around times of testing and the social impacts of these tests did mean that people really were interested.

I am sure many of you found yourselves working different shifts, in teams, reduced hours or reassigned to other labs. The HGVT also had to make dramatic adjustments to our program. We moved with the times and began virtual meetings. These have proven to be well attended, particularly when the state of Victoria was in lockdown. I would like to thank Sam Arandelovic for the great job she has done in co-ordinating these virtual meetings.

We hope we can get back to some face to face educational meetings in 2021, but for now I wish you all the very best for the remainder of the year and health and happiness in 2021.

Kerrie Scott  
(Leica/ Dorevitch  
Pathology)  
HGVT President



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## COVID-19 and Smoking

*Dysregulated endocytic machinery and ACE2 in small airways of smokers and COPD patients can augment their susceptibility to SARS-CoV-2 (COVID-19) infections*

Dr. Sukhwinder Singh Sohal PhD, FAPSR

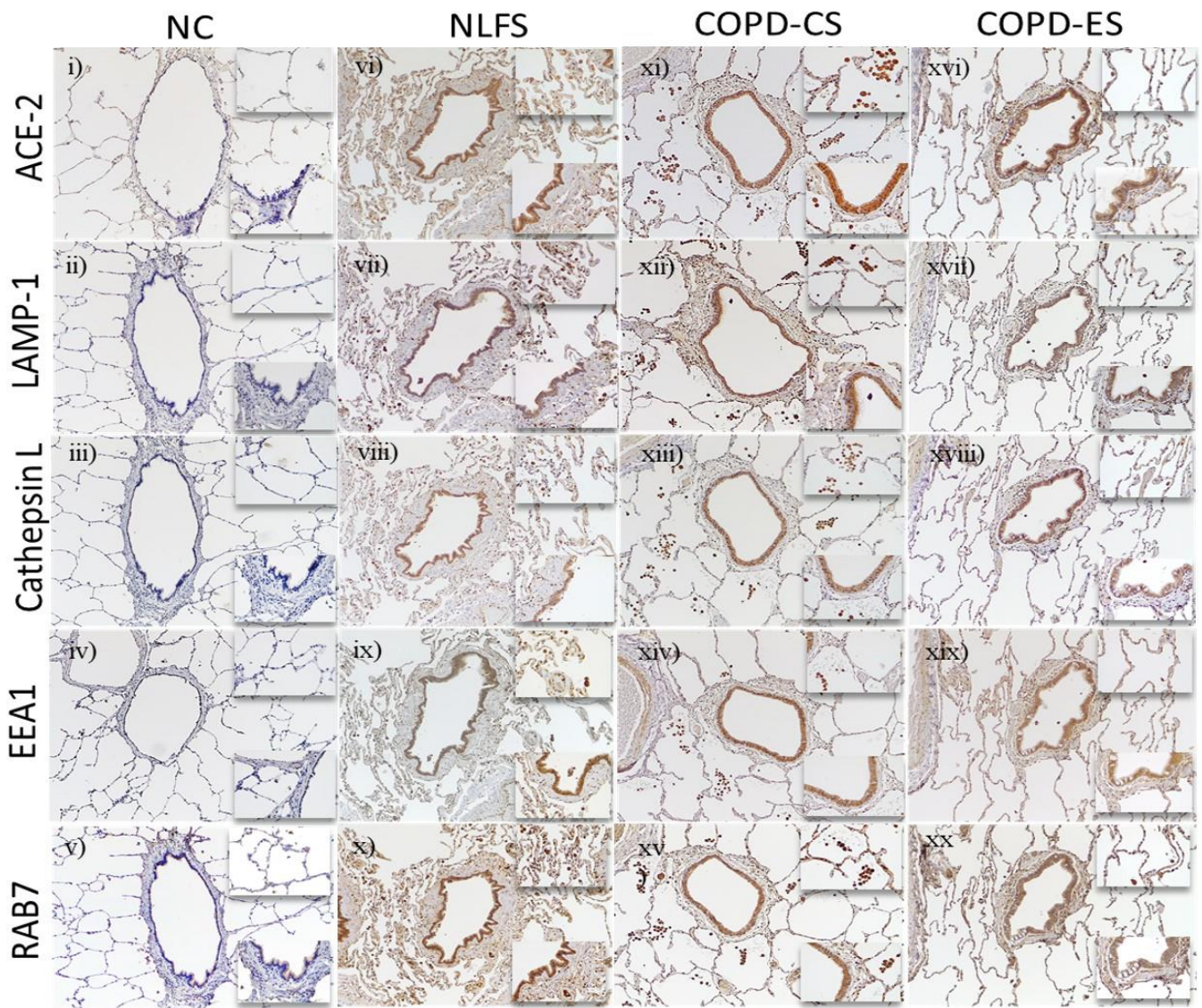
Respiratory Translational Research Group, Department of Laboratory

Little attention has been given to the role of smoking and vaping in either the transmission of novel coronavirus or mortality rate of COVID-19. Smokers contract more respiratory ailments, including colds (commonly rhinoviruses, but also coronaviruses) than non-smokers. Smokers also show double the influenza rate and increased rates of bacterial pneumonia. The damage caused to the lungs by smoking and vaping makes patients more susceptible to pulmonary infections, both bacterial and viral. Smokers are 34% more likely than non-smokers to contract the flu.

Many countries are just starting to come to terms with the fact that smokers are 14 times more likely to die from COVID-19, but few have issued warnings to quit smoking and to our knowledge, no country has ramped up its tobacco control prevention measures.

The distinguishing crown-like feature of coronaviruses is attributed to the presence of spike (S) proteins. These proteins bind to angiotensin-converting enzyme-2 (ACE2) receptor in the lungs, allowing the virus to inject its RNA and take control of the cell and make more copies. ACE2 receptor, has been the known receptor for both the severe acute respiratory syndrome (SARS)-coronavirus (SARS-CoV) and the human respiratory coronavirus NL638.

We recently published in the [American Journal of Physiology - Lung Cellular and Molecular Physiology](#) (Mathew Eapen et al, November 11, 2020), that ACE2 receptor increases in the lungs of normal lung function smokers and patients with chronic obstructive pulmonary disease (COPD), along with endocytic vacuoles, early endosome antigen-1 (EEA1), late endosome marker RAB7, cathepsin-L and lysosome-associated membrane protein-1 (LAMP-1) as lysosome markers (**Figure 1**). The histopathology suggested that there are multiple binding sites. We observed that ACE2 receptor and endocytic vacuoles were upregulated in small airway epithelium including brush borders, type-2 pneumocytes and alveolar macrophages. The expression was more in patients with COPD compared to normal lung function smokers, and little in never smokers, which shows that smoking upregulates ACE2 expression and having COPD further exaggerates it, hence more susceptibility for COVID-19 in this population. Notably, in all these cell types, COPD ex-smokers had reduced expression of both ACE2, and the endocytic proteins compared to smokers with and without COPD. These factors further elaborate on the importance of smoking cessation, considering the deleterious situation these patients could face in severe infection.



**Figure 1:** Representative images of serial sections from surgical lung resections stained for angiotensin-converting enzyme-2 (ACE2) receptor, lysosomal associated membrane protein1 (LAMP-1), cathepsin-L, early endosomal marker 1 (EEA1) and late endosomal marker (RAB7). The observation was done using brightfield microscopy at a magnification of 10x and enlarged insets at 40x magnification for the small airway wall and parenchymal spaces. Normal non-smokers (i-v) SA tissues observed to have a low expression for ACE-2, cathepsin-L and endocytic markers when compared to NLFS (vi-x), COPD-CS (xiv) and COPD-ES (xvi-xx). Compared to NLFS and COPS-CS, the expression levels of ACE-2 and endocytic makers were observed to be comparably lesser in ex-smokers across the five markers tested. A similar pattern was also observed in type 2 pneumocytes and macrophages across the patient population (figure inset). LAMP-1 expression in small airway epithelium appeared to be more diffused across the epithelium in COPD-CS (xii insets) while in both NLFS (vii inset) and COPD-ES (xvii inset) the LAMP-1 was found to accumulate close to the nucleus ([Dysregulation of endocytic machinery and ACE2 in small airways of smokers and COPD patients can augment their susceptibility to SARS-CoV-2 \(COVID-19\) infections. Eapen MS, Lu W, Hackett TL, Singhera GK, Thompson IE, McAlinden KD, Hardikar A, Weber HC, Hang G, Wark PAB, Chia C, Sohal SS. Am J Physiol Lung Cell Mol Physiol. 2020 Nov 11](#))

This is the first immunohistochemical evidence of ACE2 receptor along with endocytic vacuoles in the tissue from smokers and patients with COPD. Our observations of increased cathepsin-L expression along with lysosomes, early and late endosomal markers suggests that smoking does stimulate a highly conducive environment for SAR-CoV-2 entry and fusion in smokers and COPD patients.

The increases seen in smokers for ACE2, endocytic vacuoles and associated risk for COVID-19, further raises the question of whether this is also true for people engaged in waterpipe smoking and those switching over to the more recent alternatives such as electronic cigarettes and “heat-not-burn” IQOS devices. It is essential to recognize that these devices are not “safer”, they are still a tobacco product that produces vapor or smoke and similarly could cause and exacerbate infectious lung damage as we see with traditional cigarettes. Governments should promote smoking cessation to further avoid this calamitous respiratory viral pandemic and should reject proposals to legalise any such hazardous products.

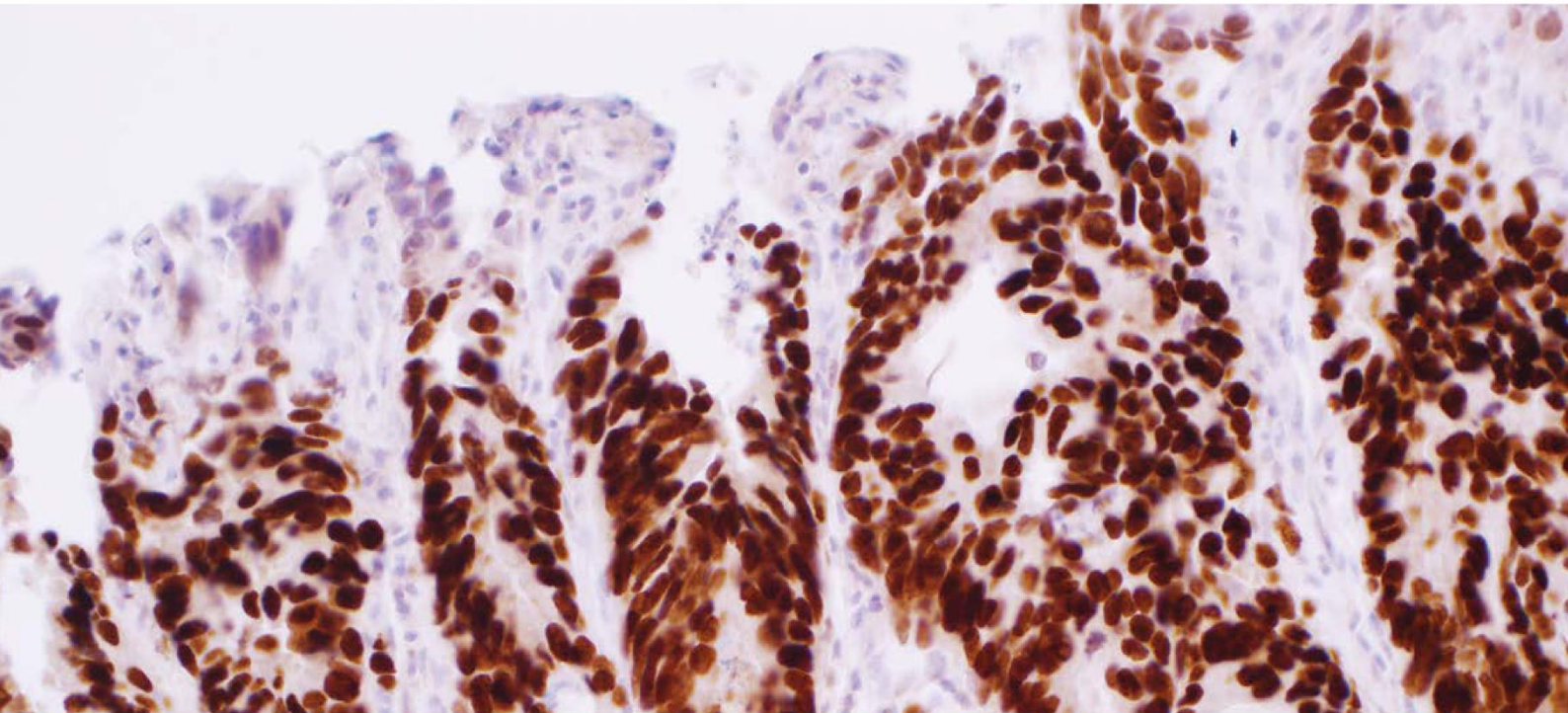
Since this deadly virus attacks the lung it is vital people should not be inhaling any tobacco products and avoid any other insult. This could exacerbate the viral load due to higher ACE2 expression and also recipe for increased spread. It would be a “double whammy” on your lung and may lead to fatal consequences.

There can't be a better motivation than coronavirus to quit smoking.

# SATB2 (EP281)

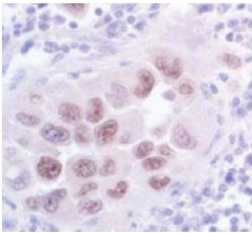
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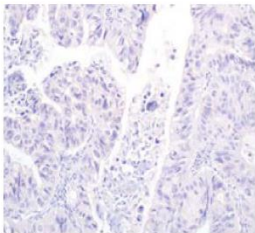


| Product Name  | Ordering Code | Comments   |
|---|---------------|------------|
| <b>SATB2 (EP281) Rabbit Monoclonal Primary Antibody</b> | 08313415001   | Colorectal |

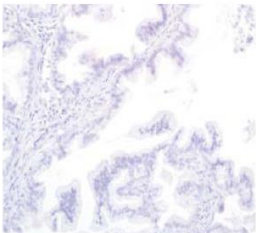
Special AT-rich sequence-binding protein 2 (SATB2) is a transcription factor involved in gene regulation. Among epithelial cells, it is only expressed in the glandular epithelium of the lower GI tract.<sup>1</sup> SATB2 has been reported to have 93% specificity and 100% sensitivity for colorectal carcinomas when utilised in the immunohistochemical panel with cytokeratin 7 and cytokeratin 20.<sup>2</sup> The sensitivity and specificity of SATB2 for colorectal cancers make it a valuable antibody for differentiating carcinomas of unknown primaries.<sup>3</sup>



Tumour cells of medullary carcinoma of the colon are positive for SATB2.



Tumour cells of oesophageal adenocarcinoma are negative for SATB2.



Rabbit monoclonal anti-SATB2 stains negatively for invasive mucinous adenocarcinoma of the lung.

References

1. Brocato J, et al. Carcinogenesis. 2015; 36:186-91.
2. Dragomir A, et al. Am J Clin Pathol. 2014; 141:630-8.
3. Ordonez NG. Adv Anat Pathol. 2014; 21:63-7.

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# UNDER THE MICROSCOPE WITH IMOGEN CAMPBELL

## **What was your first part-time job?**

I worked in hospitality as a waitress at Hogs Breath Cafe in Darwin..

## **How long have you worked in histology?**

I have worked in histology for approximately 3 years.

## **When people ask, “So, what do you do?” How do you explain Histology?**

“ When people have tissue/organs removed it is my job to help process these for analysis by a pathologist.. it’s different but cool”



## **What is a skill you’d like to learn and why?**

I would really like to learn surgical/complex cut up. I find cut up one of the most interesting parts of histology and expanding my knowledge in this field would be amazing.... Also I would like to learn how to play rugby (lol).

## **If money was no object, what would you do all day?**

I would travel around the world doing two things. The first would be as part of an archeology expedition exploring ancient cultures and civilisations. While the other would be exploring the animal kingdom to take part in animal conservation.

## **What’s an ideal weekend for you?**

My ideal weekend is a trip away with friends to go camping/bush walking/swimming and exploring.

## **If you could take only THREE items with you to a deserted island, what would they be?**

- A. Camera
- B. Water
- C. A fully stocked boat so I could leave the island

## **What’s on your bucket list this year?**

As the year is nearly over and it has been a super weird one, I haven't created a bucket list. However, if there was one thing I could do it would be to go explore the Great Barrier Reef.

## **Where do you most want to travel, but have never been?**

EGYPT! To be able to see the pyramids, museums, and to be able to partake in their culture would be the most fantastic experience! Also Rome is a very close second, to be able to stand in the Colosseum.



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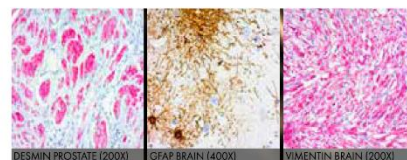
## Cytokeratins (CK, Keratins)

Cytokeratins are intermediate filament keratin-containing proteins that can be found in epithelial tissue. Cytokeratins are further categorised into Type I (acidic) and Type II (basic or neutral) in addition to subdivisions based on molecular weight. ScyTek offers a wide variety of both specific and broad spectrum antibodies.



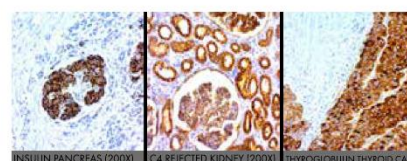
## Intermediate filaments (Type III)

Type III intermediate filaments are proteins that include Desmin, Vimentin and GFAP. ScyTek offers multiple clones to specifically label and differentiate these proteins.



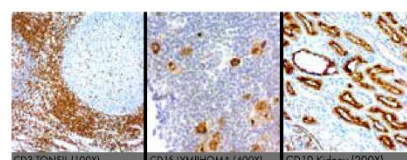
## Autoimmune markers

Autoimmune disease affects an increasing number of people throughout the world. These diseases generally arise from the cell-mediated attacks against one of more constituents of the body's own tissue. ScyTek offers an expanding list of antibodies targeted at this diverse group of disorders.



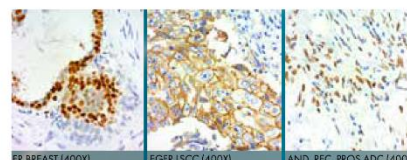
## Immune regulation (CD markers)

CD markers are well established in the scientific literature for their importance in a wide array of immune response systems. ScyTek continues to expand this family of products as relevant new markers are developed.



## Receptors

Numerous receptor sites are important for initiation of signaling pathways that lead to changes in the behaviour of the target cells. High quality, well characterised markers for receptors have become increasingly important in a variety of disease states.



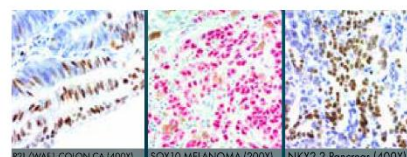
## Tumor markers

In recent years, tumor markers have become more useful as leading-edge research has identified numerous markers with ever increasing specificity for individual tumor types.



## Research antibodies

This category includes many new antibodies that are focused primarily on basic research. In an effort to provide research scientists with new and unique tools, ScyTek constantly monitors current research publications to find valuable new markers.



## VIRTUAL SCIENTIFIC MEETING REVIEW

### *Interesting Cases*

By Meaghan Leo and Kerrie Scott-Dowell

26<sup>th</sup> of November, 2020

#### Ujenia Renganathan: *The Curious Case of Cutaneous Cryptococcus*

*by Meaghan Leo*

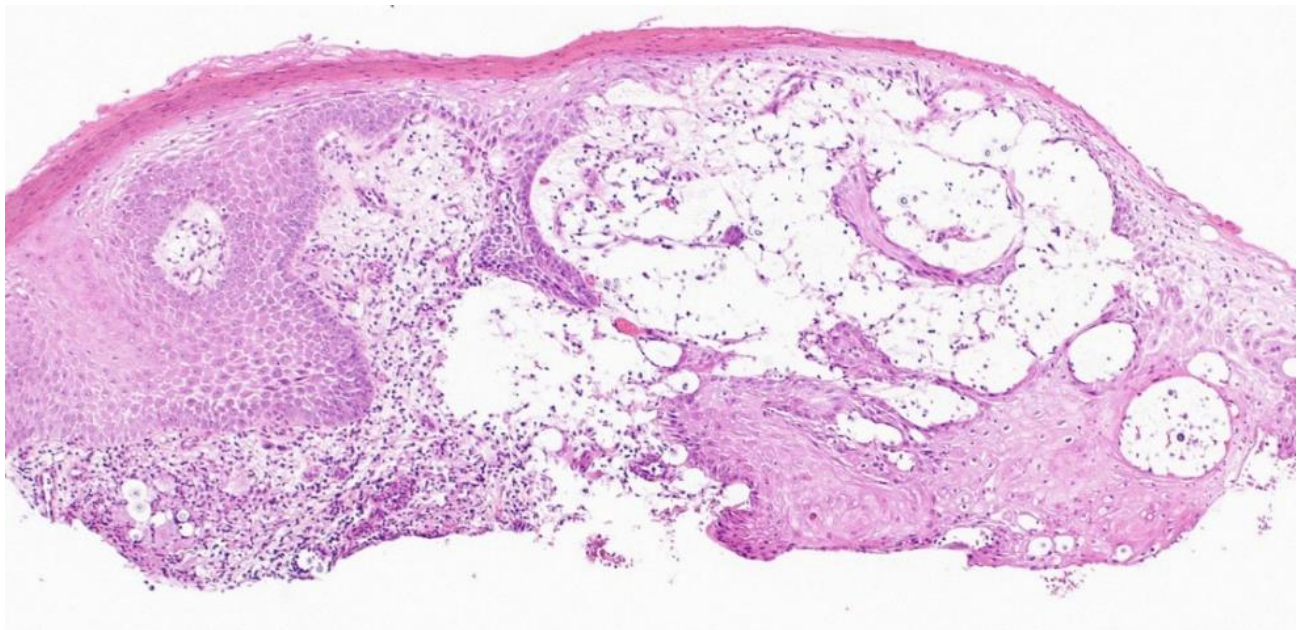
Ujenia Renganathan from Histolab started off the November meeting with a great presentation of a cryptococcus case study.

First Ujenia explained that cryptococcus is a fungus known as *Cryptococcus neoformans*, and cutaneous cryptococcus is when it's found in the skin. Cryptococcus is primarily found in the lungs and to a lesser degree in the kidneys, prostate and bone. It is rarely found in skin.

Cryptococcus is spread via an infected individual, pigeon droppings or consuming unwashed fruit.

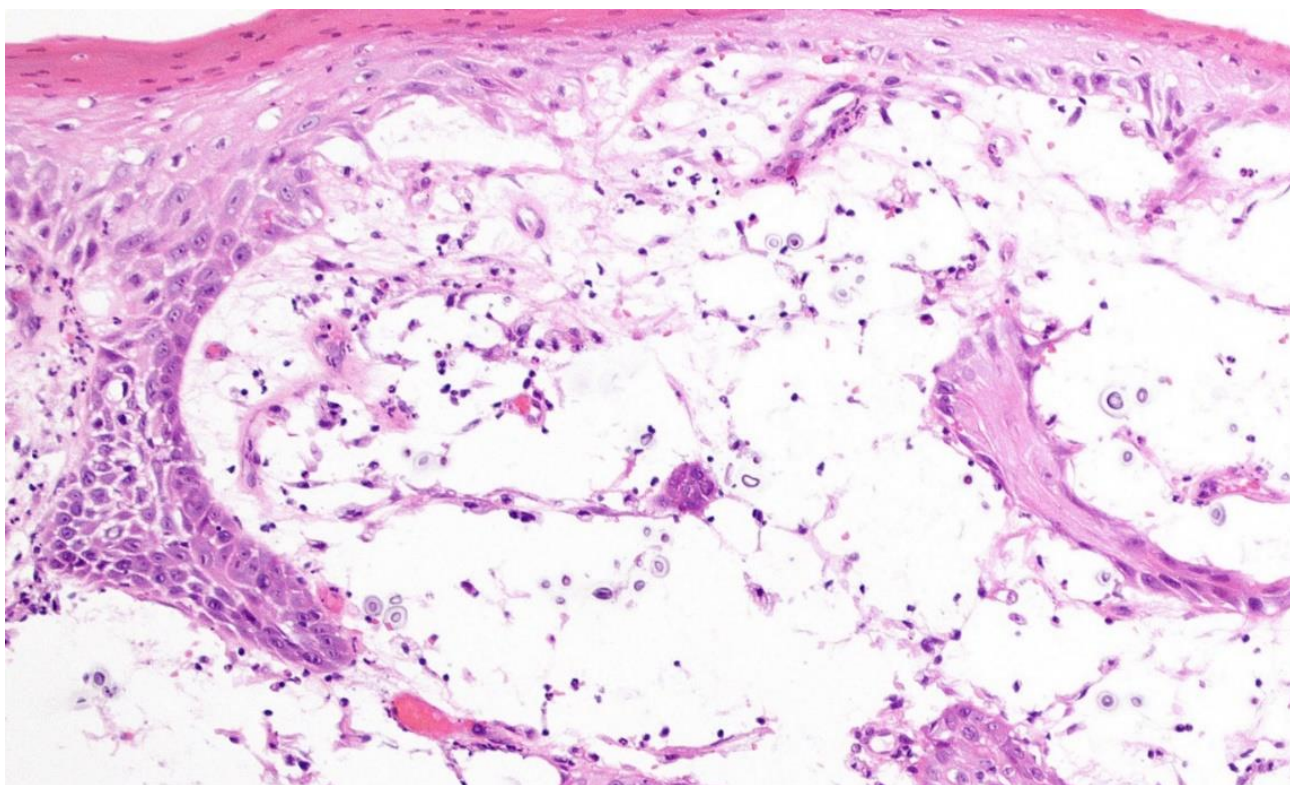
The main symptoms of a cryptococcus infection are fever, fatigue and headaches. It can also cause meningitis.

The patient in Ujenia's case study was a male in his 60's presenting with a lesion over his knee joint that had been present for three months. A biopsy querying pyogenic granuloma was taken.



*Figure 1 H&E x4*

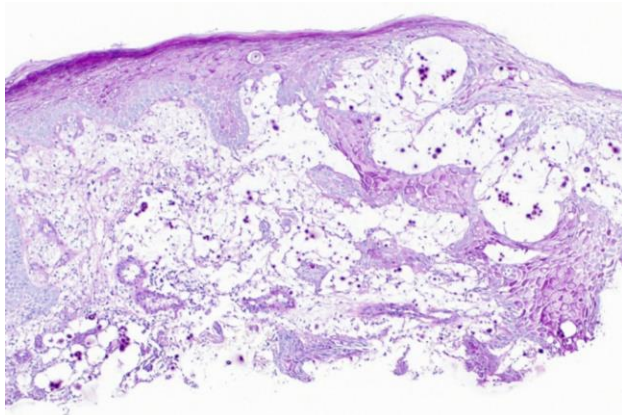
Curious Case of Cutaneous Cryptococcus



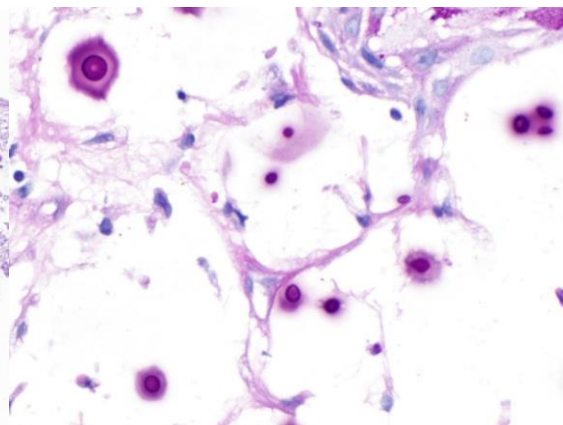
*Figure 2 H&E x20*

The H&E of the biopsy shows reactive epidermal hyperplasia overlying cleared spaces in the upper dermis which contain rounded yeast-like organisms with mucinous capsules.

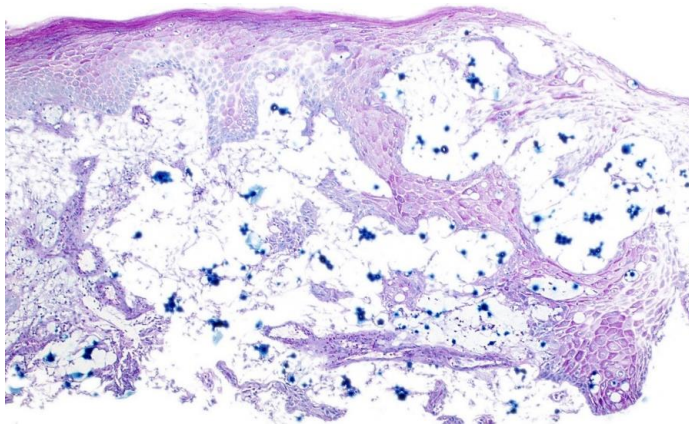
PAS, ABPAS and Toluidine Blue special stains were also performed. They all showed positive staining of the acidic mucins in the cryptococcus capsules. Confirming a diagnosis of cutaneous cryptococcus.



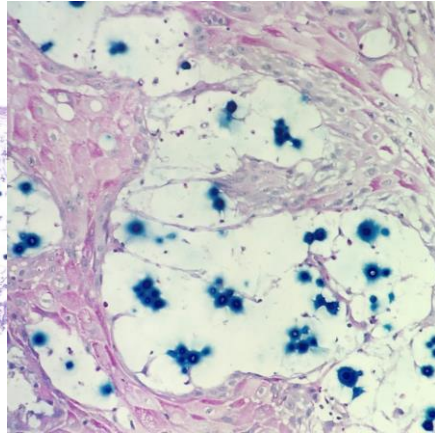
*Figure 3 PAS x4*



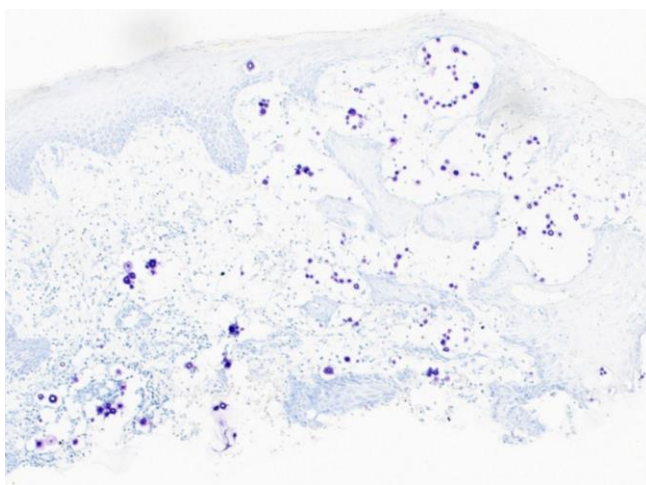
*Figure 4 PAS x40*



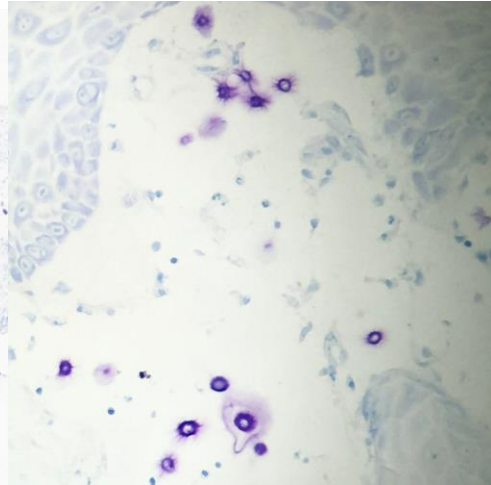
*Figure 5 ABPAS x4*  
*ABPAS x20*



*Figure 6*



*Figure 7 Tol Blue x4*



*Figure 8 Tol blue x20*

Grocott, mucicarmine, methenamine silver and methylene blue are all other special stains that can be performed for a cryptococcus diagnosis.

Cryptococcus can be treated with antifungal medicines but some cases may also require surgical debridement.

Reviewed by Meghan Leo



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Joel Dowsett: *Bowel Carcinoid*  
*by Kerrie Scott-Dowell*

Joel Dowsett of Dorevitch Pathology presented a case at the November meeting. Here are some images from the presentation:

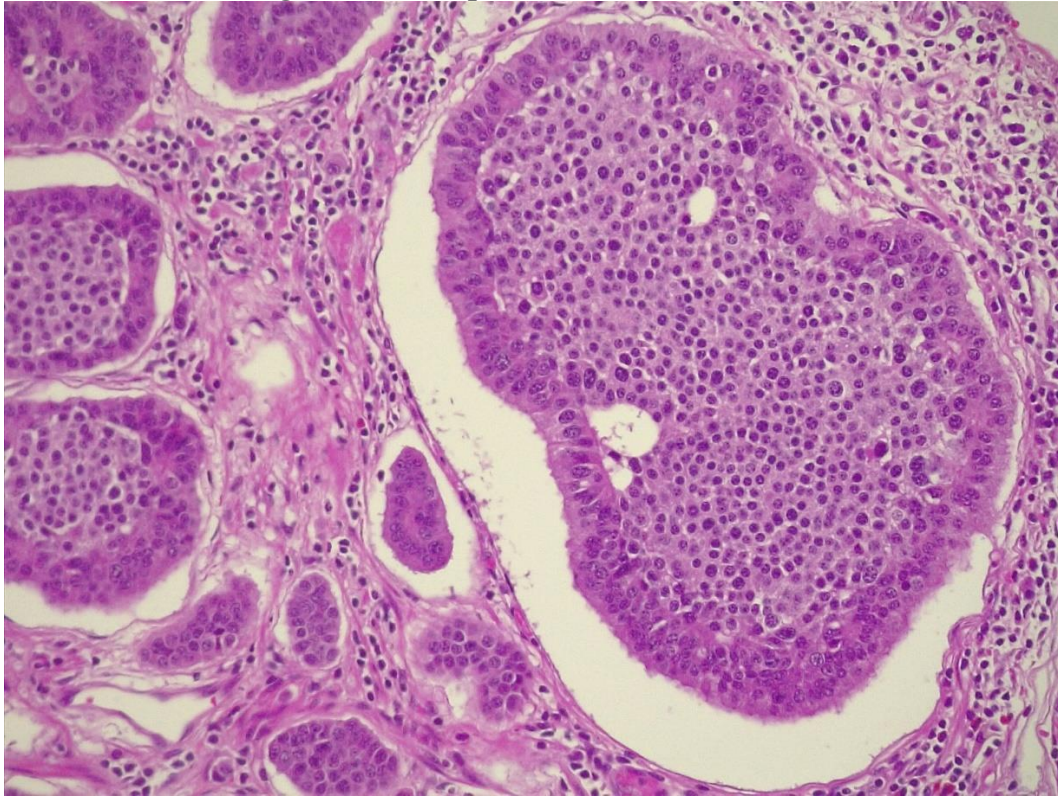


Figure 1: Carcinoid of the bowel H&E staining showing nests of tumour

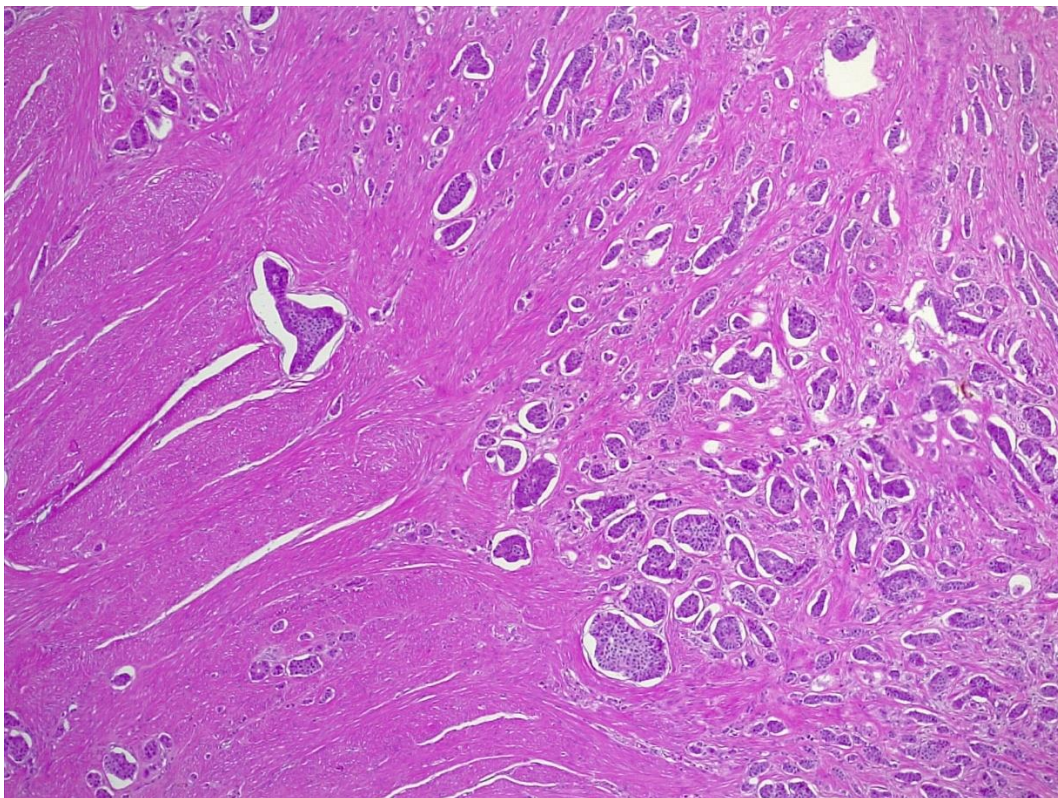


Figure 2: Carcinoid tumour extending through muscle

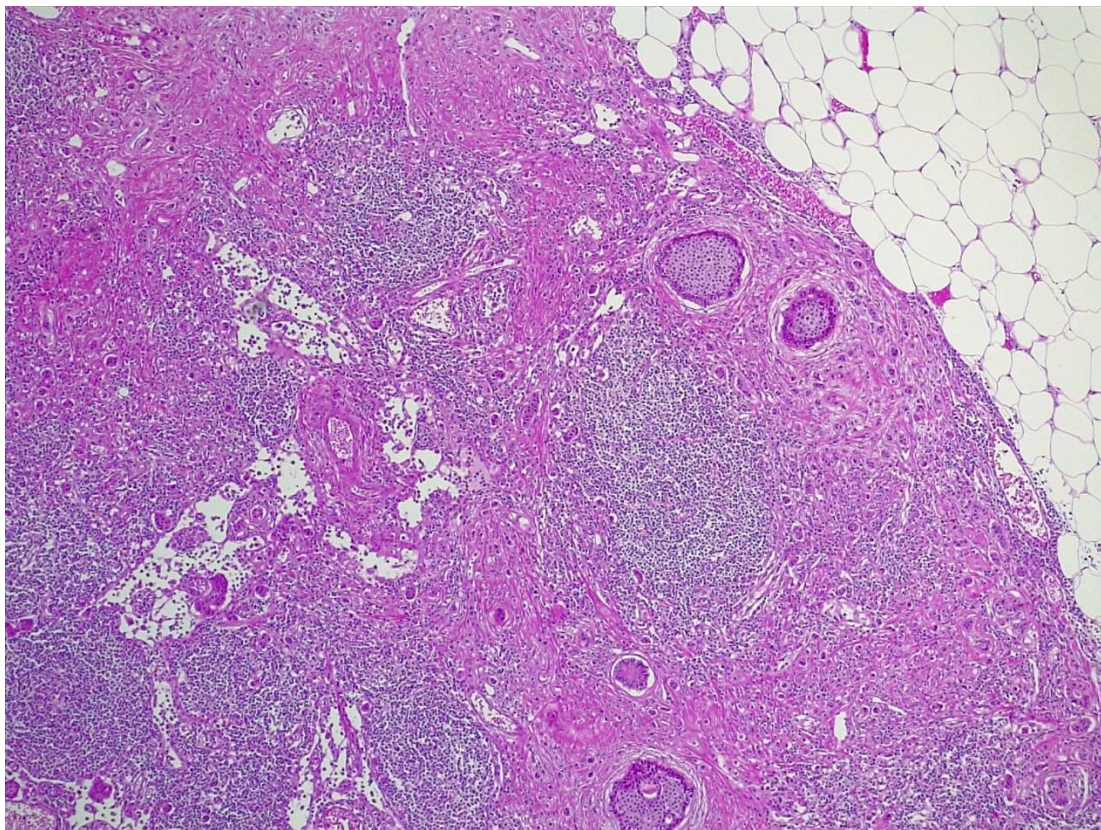


Figure 3: Carcinoid tumour extended into lymph nodes

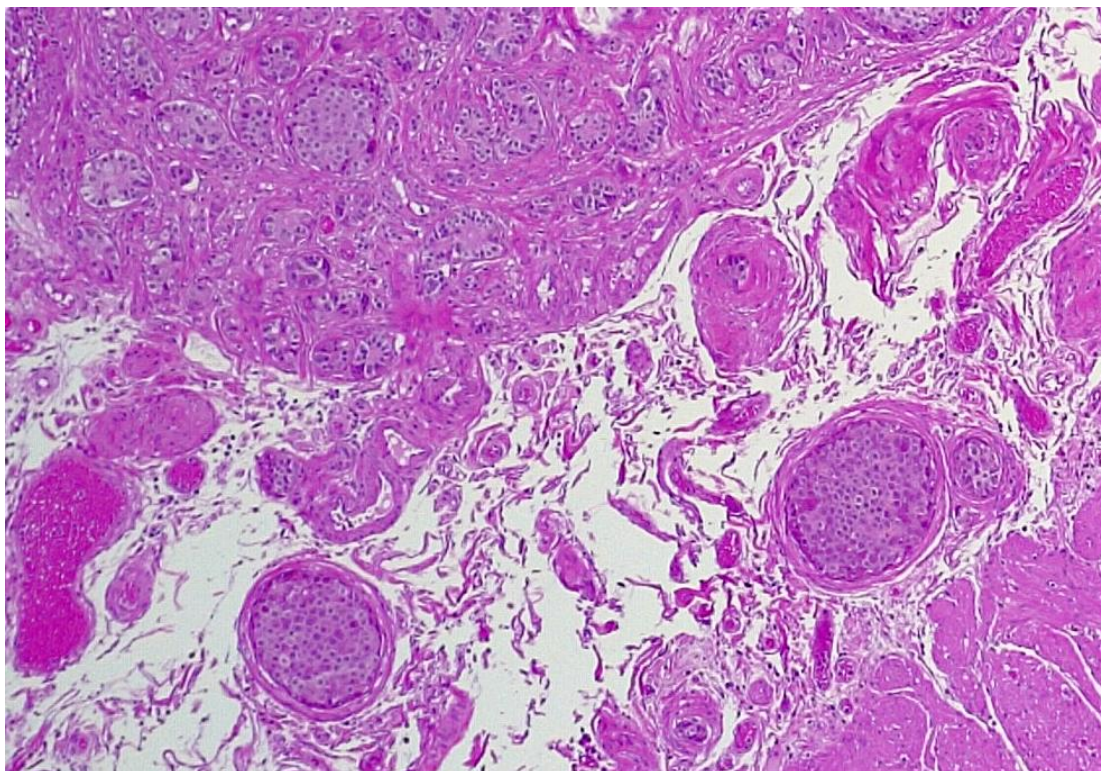


Figure 4: Vascular involvement of the carcinoid tumour

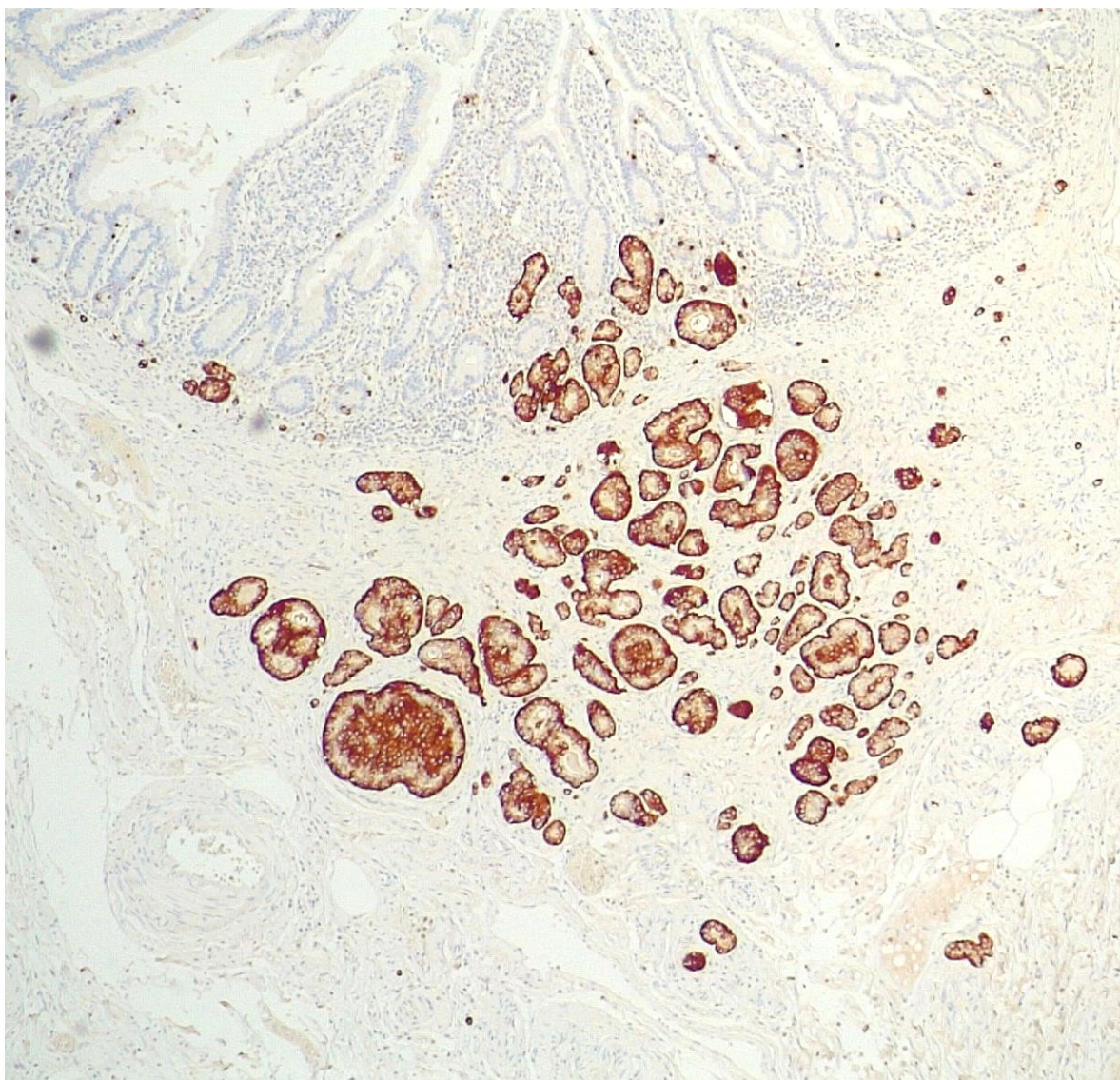
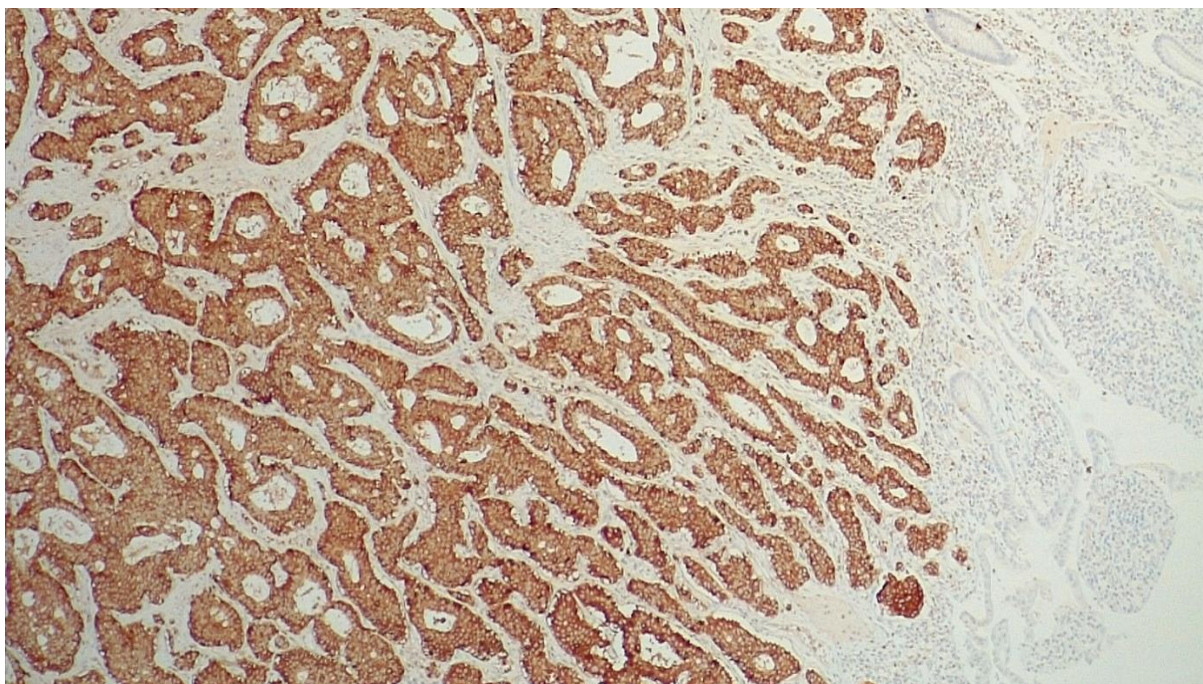


Figure 5 & 6: Chromogranin staining carcinoid tumour nests and normal bowel mucosal neuroendocrine cells



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## VIRTUAL SCIENTIFIC MEETING REVIEW

### *Latest from the Trade*

By Alex Johnston

15<sup>th</sup> of October, 2020

The HGV were fortunate to have several trade partners to come in and offer their insights into the exciting innovations and perspectives currently within their respective companies.

It was a fascinating thought throughout that while Histology is an accomplished discipline, it is still being refined and evolving to be even more optimized while embracing new modern technology with these approaches.

A brief overview of each is provided below however or those seeking more detailed accounts from the speakers themselves, the presentation, along with other Virtual Meetings throughout the year, have been uploaded to the HGV's Google Drive by clicking [here](#).

#### ***Abacus:***

**Topic: Microwave Tissue Processing**

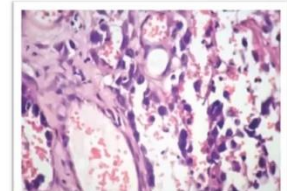
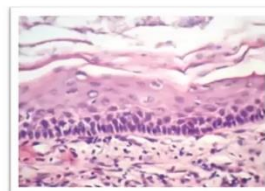
**Speaker: Christie Chee**

Christie gave a brief but interesting account of history on the emergence of microwaves and their application within Histology in the past 20-30 years. The company Christie represented, Milestone, specialises in microwave technology and were the first to build and design tissue processors with several machine solutions incorporating the concept.

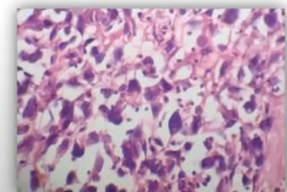
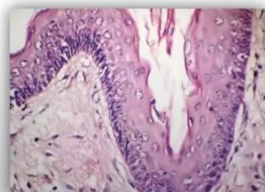
Given early speedbumps in using household microwaves to develop a procedure, eventually machines were tailor made for laboratory settings that included many more safety measures as well as the ability to monitor and control variables such as temperature and time. Exploiting the effects of microwaves on molecules to produce thermal and kinetic energy, the tissue processing procedure was able to be performed within smaller time periods while not compromising histological detail in the final

slides produced when compared to traditional processing methods. A trial performed in New Zealand supported this statement where they found comparable results in stained sections between traditional and microwave methods however were able to produce them in shorter time frames. Some papers supported the improvement of HER2 staining in immunohistochemistry from tissues processed via the microwave method.

Microwave  
Tissue  
Processing



Conventional  
Tissue  
Processing



Odontogenic Keratocyte  
40x

Angiosarcoma 10x

Comparison images of conventional and microwave processed stained sections from Christie's presentation.

## Agilent:

### Topic: Fishing and its application in oncology

Speaker: Scott Reed

Scott discussed the application of fluorescent in situ hybridisation (FISH) in oncology cases.

After giving an overview of the technique Scott delved into how there are four different probe types available for use depending on the desired application. They include:

LSI (Locus specific identifier) which uses include:

- Copy number changes including gene amplification and deletion
- Gene rearrangements including translocations and inversions.

The other main type discussed were CEP/CEN (Chromosome Enumeration Probes) which are typically used for investigating chromosomal gain or loss but are also used together with LSI's to ensure only cells with whole nuclei are counted.

ALK (anaplastic lymphoma kinase) and KRAS mutations were highlighted as some of the most common genetic markers tested for however, Scott iterated that many more existed.

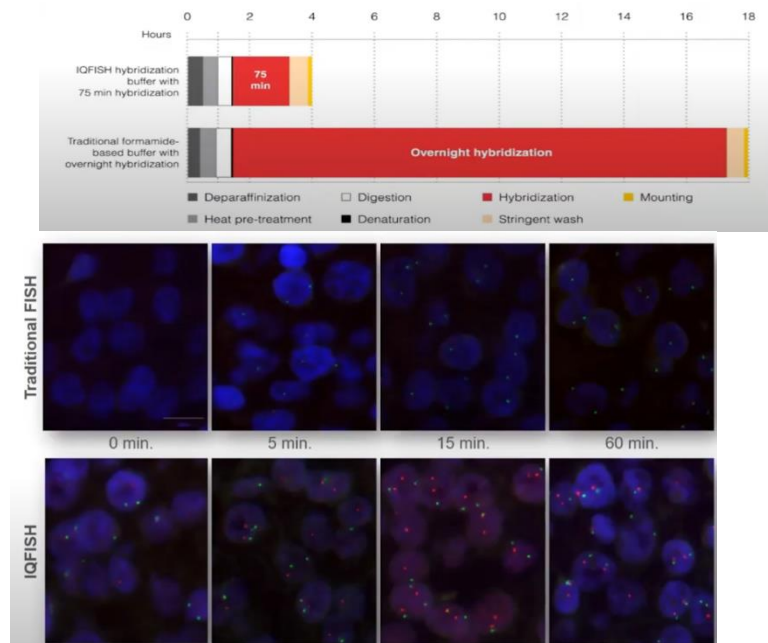
In terms of innovations to next-gen FISH Scott discussed several options. Two included the Fast IQFISH buffers which aims to provide almost instant binding of targets compared to current methods that employ overnight hybridisation. Another innovation was the use of micro-gap technology which allows for more distinct signals, making it easier to determine positive and negative results.

## Next-generation ISH

### 1. Fast IQFISH buffers

Traditional ISH uses formamide, which requires overnight hybridisation for **genomic targets**

IQFISH uses **ethylene carbonate**, (almost) **instant binding** possible



Slides from Scott's presentation with an example of FastIQFish buffers vs. traditional FISH methods.



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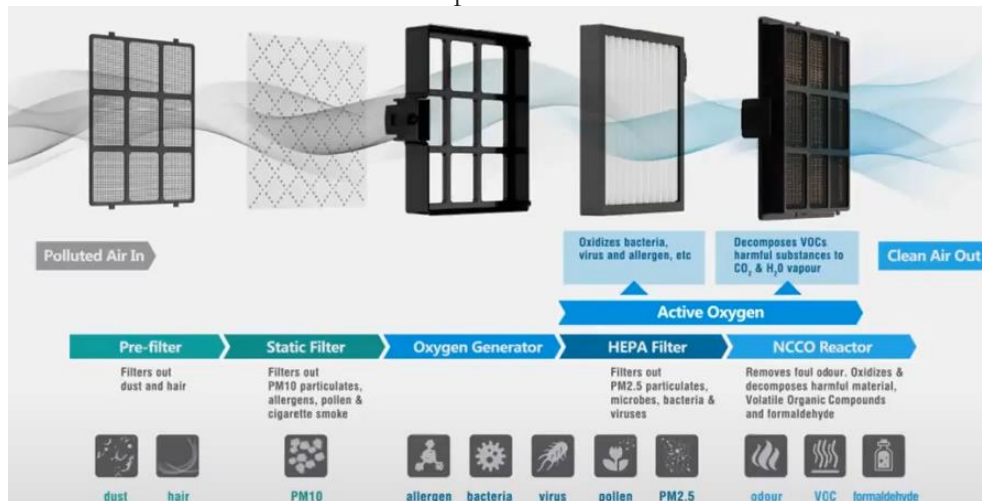
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## Veto Medical Technology

### Topic: Reducing Risk in Pathology

### Speaker: Jarrad Webber

Jarrad discussed air purification as a point of interest for improving the safety in laboratory, an apt point for discussion at all times but especially in 2020. Using a NCCO (nano confined catalytic oxidation) process, it aims to trap small pathogens and immerse them in a layer of ozone. The end result of this is trapping process produces carbon dioxide and water vapour via a catalytic reaction. For formalin fumes it has been shown to remove 99.9% of fumes from the environment and 99.7% of other vapours from the environment.



Slide from Jarrad's presentation visually breaking down the NCCO filtration system.

The other technology discussed was the ability to live share digital slide images for online

collaboration. One of these technologies was iMEDHD2 which delivers full HD1080p images and videos for sharing these images. The program aims to be as user friendly as possible in regard to ease of use, sharing and annotation of images to outline some key features offered with ongoing updates to ensure the program remains current and optimised.

**Roche:**  
**Topic: Digital Pathology**  
**Speaker: Warren Gouin**

Warren outlined the advantages of digital pathology including the ability to work of long distances with different collaborators and ultimately optimise the diagnostic process.

One of the big considerations raised was the storage of all the digital data produced from the process as well as the requirements for secure networks.

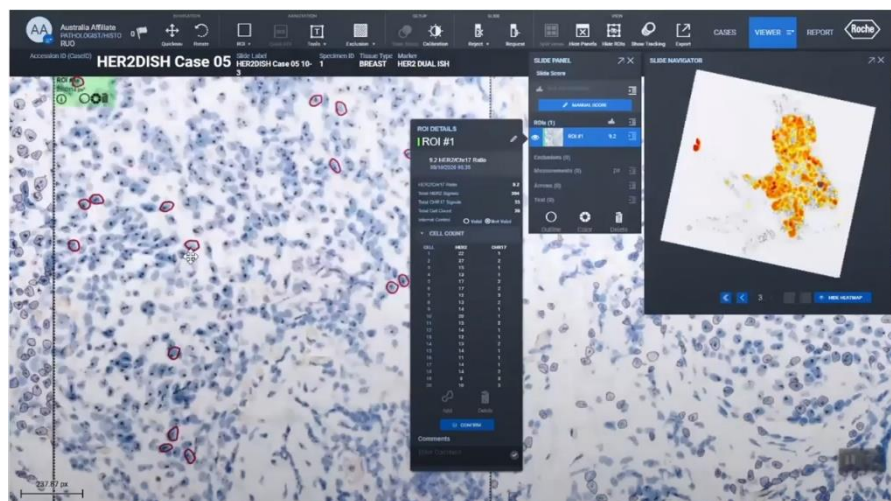
From the application side of things, Warren highlighted the potential for both teaching and also training remotely. The concept of telepathology was introduced to describe the ability to have remote access to cases and collaborate from anywhere, which could also be extended to frozen sections.

The medium also lends itself to easing the organisation of multidisciplinary meetings being able to coordinate slides ahead of time.

A point of excitement for Warren was the utilisation of algorithms to assist pathologists in reassuring their diagnoses with their ability to learn to recognise areas of interest. This concept was presented via an example of a 'heat map' of suspicious cell clusters being provided on the image of the slide to attempt to identify area of interest sooner.

Given the advantages Warren pointed out that the degree of change requires support from several levels both financially and with infrastructure in order to be implemented within a laboratory which may be a limiting factor currently to its uptake.

However, he was optimistic in saying that a partial move into digital can still provide several benefits to a laboratory.



Slide from Warren's presentation showcasing an example of 'heat map' algorithm assistance available to pathologists.



**We would like to thank our customers  
for their ongoing support through  
what has been a very challenging year for all.  
We look forward to continuing to work  
with you in 2021.**

***We wish you and your  
family a safe and healthy  
Christmas and New Year.***



## **Leica:**

### **Topic: Digital Pathology and Aperio GT450DX**

**Speakers: Laura D'Angelo and Marne Prinsloo**

The two speakers from Leica were excited to present the company's latest digital solution offering, the Aperio GT 450 DX. Marne highlighted the importance and utility of having digital pathology as a tool in healthcare, with its ability to function during this pandemic.

The Aperio GT 450 DX is able to scan a slide per 32 seconds or 81 per hour.

The speed and usability were regarded as some of the biggest umbrella advantages for histotechnicians in their day-to-day duties with the ability to batch slides and even prioritise more urgently required images. More specifically, part of the usability also comes from the compatibility of the slide racks across different Leica machines i.e. the ability to remove a rack of slides from the coverslipper and place them onto the scanner while in the same rack.



Slide from Marne's presentation of the Aperio Gt450DX

Aperio GT 450 DX images were reviewed by Pathologists in the development of the machine in order to ensure quality was of a standard conducive to an accurate and efficient means of diagnosis for pathologists and patient's alike.

An intriguing feature of the software was the 'Tissue Finder' which aims to include all the tissue on the slide including harder staining components like fat cells.

Marne explained that digital pathology is popular with hospitals and that for research purposes, other options existed that could be better suited to their purposes. The proposition of artificial intelligence and machine learning for replacing pathologists was posed as a question however, Marne explained that machine learning already existed in the Aperio GT 450 DX as a means of optimising and maintaining quality of scanned images.

## **Metagene:**

### **Topic: New rapid methods & tips and tricks for improving diagnoses using IHC**

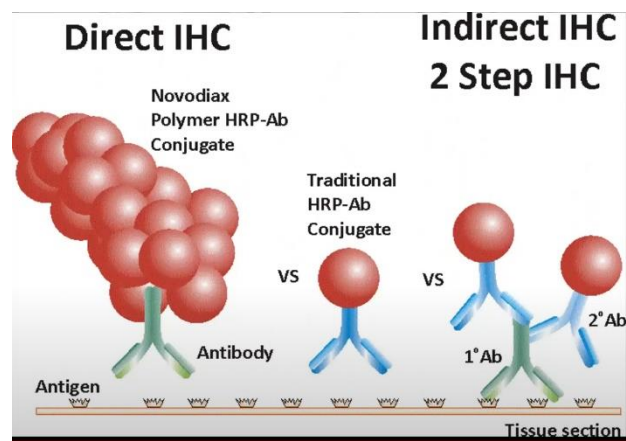
**Speaker: Sarah Dower**

Sarah was excited to discuss ways to optimise immunohistochemistry diagnoses to improve patient outcomes and laboratory workflow as well as reduce costs.

The way she proposed Metagene was going to provide this solution was by offering Novodiox, a faster direct IHC method to rival indirect IHC methods.

With reagents that are compatible with current machines, no further technology is required to implement this solution for both frozen and FFPE tissue sections.

The advantages of this method meant a simpler protocol with less steps that could be as short as 18-20 minutes leading to quicker diagnoses and achieve the aims of the innovation.



Slide from Sarah's presentation displaying Novodiox's direct IHC method vs. traditional IHC method's employed currently.



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## 'Slice of Life'



It's sad when people leave but amazing to see them follow their passions!

A special handmade going away card from a former member of Peter MacCallum Cancer Institute aiming to pursue her art career. All the best!

Artist credit Emma Davis

Had a recent exciting life event? While Histology is a fascinating science, it's no less fascinating than the members that make it all work!

We're welcoming photos to highlight your awesome team, lab social events, marriages, babies, pets like the beautiful examples in this issue or anything you've achieved that you'd like to shine a light on. We'd be delighted to celebrate you!

Send your contributions to ([editor@hgvt.edu.au](mailto:editor@hgvt.edu.au)) with a caption and look out for yourself in future newsletters!

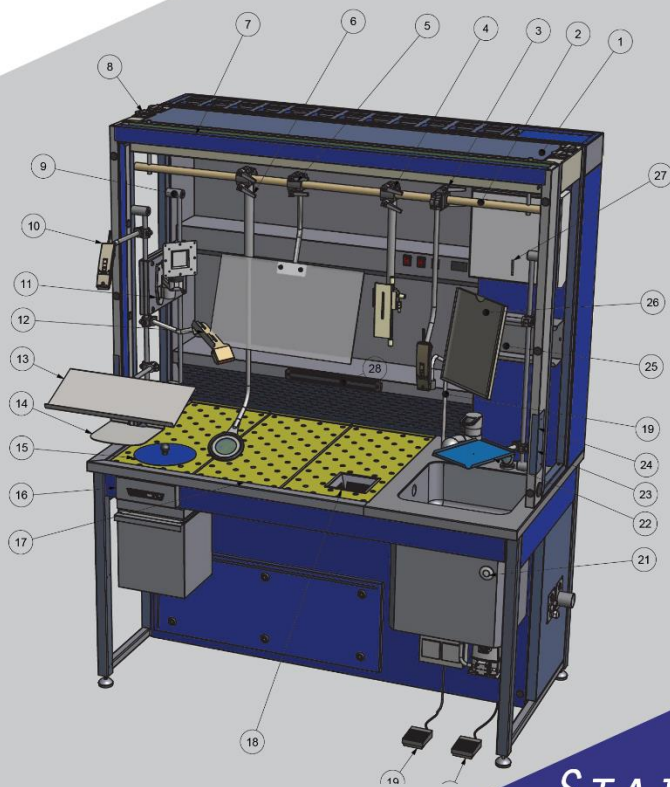


Eva the Maltese-Shitzu  
(Belongs to Alex Johnston)

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## Future Events: 2021

Org. No. A0035235F

### *Date: February 25<sup>th</sup>*

Scientific Meeting

**Topic:** MOHS

**Venue:** Streamed live and recorded using G-suite

### *Date: April 22<sup>nd</sup>*

Scientific Meeting

**Topic:** TBA

**Venue:** Streamed live and recorded using G-suite

### *Date: June 24<sup>th</sup>*

Scientific Meeting

**Topic:** TBA

**Venue:** Streamed live and recorded using G-suite

### *Date: August 6<sup>th</sup>*

Trivia Night

**Venue:** TBA

### *Date: September 9<sup>th</sup>*

Scientific Meeting

**Topic:** TBA

**Venue:** Streamed live and recorded using G-suite

### *Date: October 14<sup>th</sup>*

Scientific Meeting

**Topic:** TBA

**Venue:** Streamed live and recorded using G-suite