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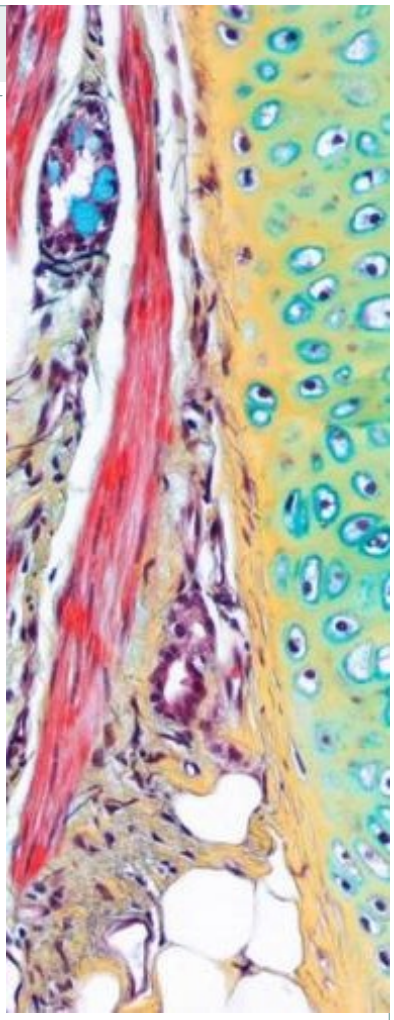
August 2020

Newsletter of The Histotechnology Society of NSW

HISTOGRAPH



Editorial

Hi and welcome to the Second issue of the Histograph for 2020. Hopefully all our member's are staying safe and well during this challenging and unprecedented times due to COVID-19.

It is with great sadness the society would like to announce the passing of a very special person, Bruce Munro. Bruce was revered by all in the field of Histotechnology in Australia and in many countries overseas.

In this issue Tony Henwood wrote a comparative review on bacterial staining. I'm sure we histotechnicians will find this article very informative and educational.

Hope you enjoy reading this issue and have some histo fun with our Test and Teach quiz. It is entertaining and highly educational.

Linda Prasad

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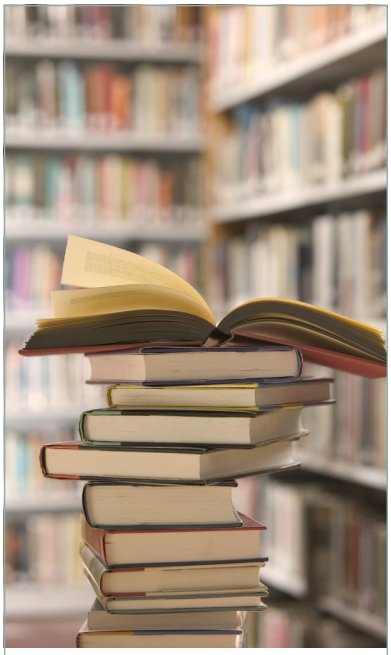

Histotechnology Society of NSW COMMITTEE MEMBERS are volunteers who work tirelessly to promote histotechnology and provide educational opportunities for continuing professional development. Thank you team for the GREAT JOB

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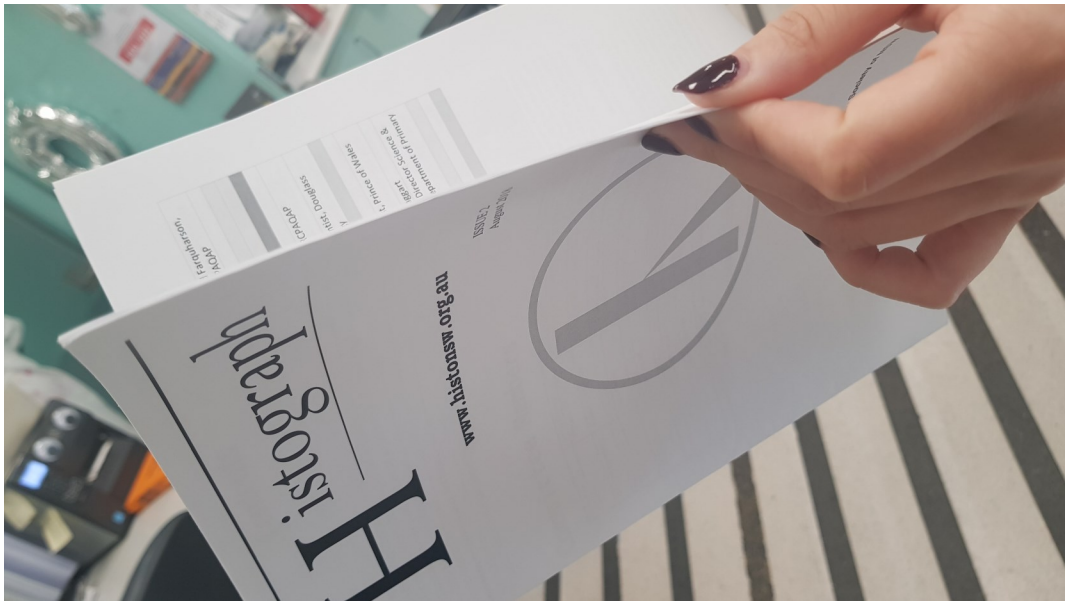


Chairman's Report

The situation with COVID-19 continues to impact on our lives and the things we do. While we still are keen to present hands on workshops it is not possible at present to do this. We are presenting webinars instead. We need to thank Dr Tamara Syztynda and UTS for their support in these challenging times.

Since our last Histograph we have held two successful Webinars:

Processing Machines on the 23rd of May. We need to thank the companies who contributed to this presentation as well as Ewen Sutherland who prepared the power point. The Webinar covered: What is Tissue Processing, History, processing process, changes, types of processors, microwave processing and xylene free processing. 38 people participated.

Stratified Squamous Epithelia on the 25th of July. Special thankyou to Ewen Sutherland and Askar from Thermofisher for preparing the slide scans. Presentation covered a revision of Epithelium, the Skin Epidermis, accessory glands & hair follicles, Dermis and Stratified squamous epithelia. 66 people participated.

Our next Webinar is on the 29th of August on Digital Histology.

Our committee meetings are focussing more on video conferencing. To enable us to meet current requirements for Microsoft Teams, Zoom, Skype etc., we are replacing our main laptop computer which is now quite old.

The planned Joint National Conference next June has been cancelled. The International Academy of Pathology (IAP) have decided to cancel their Conference booking with the International Convention Centre (ICC) in Sydney and hold a virtual Conference instead.

Doing a survey through the Histology Group of Australia (HGA) and discussions with our NSW Committee we have decided to reschedule and proceed with a separate National Conference at a time to be decided. The preference was for a personal Conference and not a virtual one.

The NSW Conference organising committee recently met with ICC and Daltone House (Conference Dinner) and have arranged for the bookings to be rescheduled to a date to be confirmed. Our organising committee will work on a new plan with these facilities and develop a new conference program.

Stay Safe,

Cheers,

Trevor Hinwood

Chairperson

Histology Society of NSW

Bruce Munro - Vale

I regarded Bruce as a mentor in my early years (70'/80's) as a budding product specialist in Histology products working for Selby's. Products that came from Reichert (Vienna), Jung (Heidelberg) and American Optical (Buffalo) factories.

As the Laboratory Manager for the Histology Department in the Blackburn Building at Sydney University, he had a good knowledge of Histology and was always approachable to discuss Histology and encouraged interested people to become involved in this field.

Over the years I spent many hours discussing Histology with him in his small office at the rear of the Histology Department's office in the Blackburn Building. His encouragement and support for me to become more involved in this area helped me further develop my interests in this field.

Bruce was heavily involved in the formation of the Histotechnology Group of NSW (now Society) along with others such as Bill Sinai (separate Vale) and Grant Taggert (now deceased).

I can recall being asked by Bruce to join the committee as a general committee member. Some committee meetings being held in the Blackburn building and Veterinary School at Sydney University. They were good times expanding and developing the Histology discipline in Pathology.

One of my roles as a developing product specialist was to work with the manufacturers on new developments and provide technical feedback on customer requirements. At one point my involvement related to the development of motorized rotary microtomes and the sectioning of resin tissue blocks. Bruce suggested I should write an article for the "Tissue Tek" newsletter which had world wide distribution. The article was prepared, given to him and he forwarded it to the editor for publication, which it duly was.

I could provide other examples of Bruce's involvement in my life, suffice to say Bruce holds a special place in my memory as he would in others. A great man and I would not be where I am today with my Histology involvement, if it was not for people such as Bruce.

Trevor Hinwood,

Chairman

Histology Group of Australia.

Vale: Bruce MUNRO

Bruce was revered by all in the field of Histotechnology in Australia and in many countries overseas. He was a very private person away from his beloved Histotechnology.

I think there are many people who will remember Bruce fondly for his enthusiasm to teach the subject and to encourage students to become the best they could in this field.

Bruce was the father of Histotechnology in NSW and possibly Australia and a great individual, he began teaching Histotechnology at Sydney TAFE on behalf of what was then AIMLS (AIMS) in the late 1950's until 1970's. His insight into how we could progress Histotechnology in NSW, at first by the formation of the Histotechnology Study group followed by the formation of the Histotechnology Group of NSW (HTG) in 1981.The group met at Sydney University in the Anderson Stewart building, when he took on the role of the President for the first 8 years.

I was more than happy, although nervous, when he suggested I could become the President of the group in 1989 as I thought they were very big shoes to fill.

He organised some of the best Histotechnologists to attend the HTG weekend conferences from the first conference in Albury Wodonga (1982), individuals such as Lee Luna the editor of the AFIP Histotechnology Staining manual and other overseas luminaries in Histotechnology from USA, Europe and England. He was keen to have weekend seminars and encouraged the initial joint meeting between the Victorian (HGV) Albury Wodonga and several years later the Queensland (HGQ) in Armidale NSW. As we now know this liaison has become the norm for National meetings of Histotechnologists in Australia.

As one of teachers I most admired, his thoroughness and knowledge of Histotechnology, and the way in which he managed to get experts in the field as lecturers is the main reason it was a privilege to be taught by him and then to have him as one of my mentors along with Dr Ken Taylor.

I was fortunate in 1979 to be encouraged by Bruce and Ken Taylor to take up a role as tutor in my favourite subject then lecture at UTS (St Leonard's Campus) for 7 years. I continued to appreciate his advice once he retired. I followed his guidance on how to be the best teacher of Histotechnology, enough to be asked to with one of my ex-students and Bruce's assistance we design the Histotechnology Course at Granville TAFE and we taught this subject for 5 years followed by a few years teaching part time at Sydney TAFE.

Sadly missed.

Bill Sinai
0407077572

A Review of Bacteria Staining

Tony Henwood, Principal Scientist, Histopathology, the Children's Hospital at Westmead, Sydney. NSW.

In 1676, Antoni Van Leeuwenhoek discovered bacteria using his home-built microscope. He described bacilli, cocci and spirillum from the human mouth (Porter 1976, Gest 2004). Two hundred years later (1875), Karl Weigert developed the alcoholic methylene blue stain for bacteria (Cambau & Drancourt 2014).

With a Haematoxylin and Eosin stain (H&E), bacteria are haematoxylinophilic or weakly eosinophilic. If they are very small, they can be very difficult to see (Winn 1995). A good general bacterial stain is the routine Giemsa and Methylene blue stains. Most bacteria are stained blue. But these stains will not tell you whether they are Gram positive or negative.

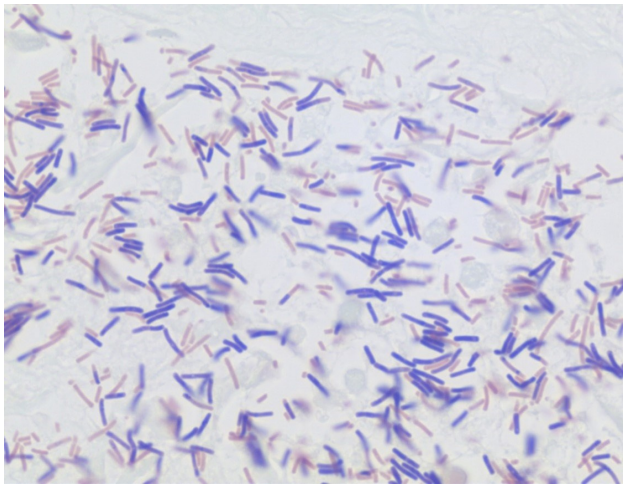
In 1884, Hans Gram, a histochemist, discovered that gentian violet plus iodine stained bacteria in paraffin sections and that some bacteria resisted alcohol differentiation. He later found that this same technique could be applied to smears. He noted that "Hopefully, the method will prove useful in the hands of other investigators." Well it became the bread and butter stain for microbiologists (Cantey & Doern 2015). The Gram is a simple stain: apply the blue crystal violet (or similar), mordant with iodine, decolourise with alcohol, rinse in water and apply the red counterstain. So why are some bacteria Gram positive or negative? Gram negative bacteria have more lipid in their cell walls, so the lipid is extracted by acetone/alcohol causing the crystal violet-iodine complex to fall out. Gram positive bacteria have thick peptideoglycan cell walls that retain the crystal violet-iodine complex.

While differentiating bacteria into either Gram positive or negative is fundamental to most bacterial identification systems, researchers have argued the Gram staining method is prone to error and "is poorly controlled and lacks standardization" – something Gram himself warned of when his work was published in 1884 (Cantey & Doern 2015).

In the real world nothing is perfect. Legionella, Bordetella, Bacteroides, and Brucella are Gram negative but often stain poorly (Winn 1995). What can we do? Some options are to use acid fuchsin instead of neutral red or safranine as the counterstain, use Loeffler's Methylene Blue, which was mentioned earlier or use the Warthin Starry Silver Stain.

The issue often facing histochemists is that some bacteria should be Gram positive but aren't.

Let's look at the Gram stain of our anthrax bacilli in tissue (figure 1). It should be Gram positive but many of the rods are Gram negative. In this instance, there is a significant population of dead bacteria that stain red. Gram positive bacteria are notorious for becoming gram negative when they die.



There can also be technical reasons for false Gram negative staining. Remember that tissues are fixed in formalin and processed through alcohol and xylene to wax. This could be an issue. We may be overenthusiastic with our differentiation. Another less known fact is that iodine solutions lose their iodine activity, even though the solution still looks dark brown (Magee et al 1975). Have you ever noticed the brown discolouration of plastic or glass containers and especially of the parafilm that is often used to seal flasks? What are some tips for a reproducible Gram? Consider partly drying sections after Crystal Violet – Iodine treatment prior to differentiation, short rinses in water and rapid alcohol dehydration prior to coverslipping.

What internal Quality control can we use when doing a Gram stain? Fibrin, keratohyaline, elastic fibres and Paneth cell granules should be Gram positive, whereas neutrophils and epithelial cells should be Gram negative.

The demonstration of mycobacteria, such as TB and leprosy is important and atypical mycobacteria are an issue with immune deficient patients and also little children who like to eat mud pies. Since many mycobacteria are difficult to culture, the histopathological features are important. Histopathologically, recognition of the Langhans giant cell is important. The classic cell is a result of the fusion of many macrophages and their nuclei are arranged in a horseshoe pattern. Giant cells are often seen in biopsies and are either big cells with lots of nuclei or cancer cells. Mycobacteria are considered Gram positive, but most are Gram neutral (ie Gram ghosts). For this reason, in the latter 19th century, Ziehl and Neelsen developed an acid-fast stain known as the ZN stain. Simply sections are treated with a red carbol-fuschin, heated (which mordants the stain), decolourised with acid-alcohol and then counterstained with methylene blue.

Continue reading on page 13

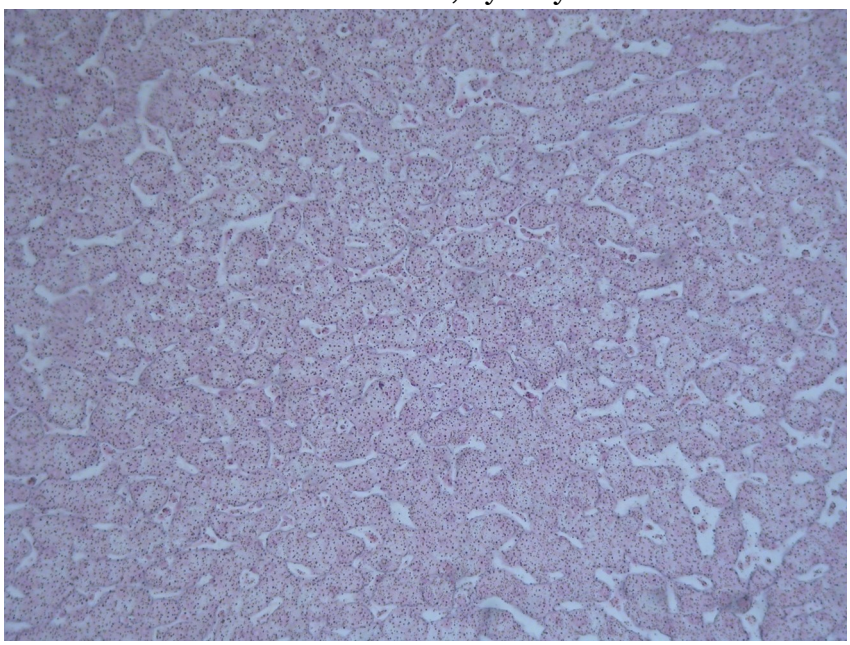
TEST AND TEACH

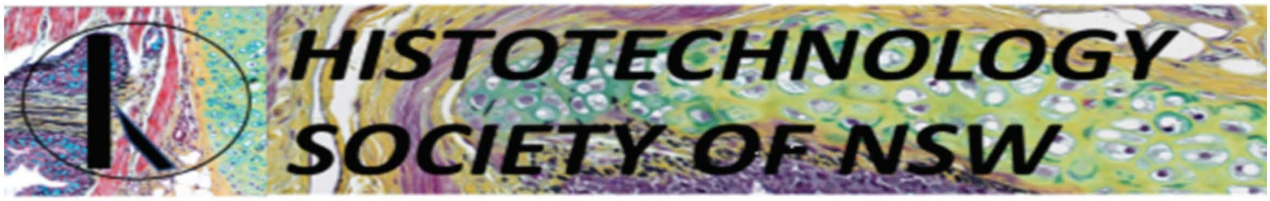
Linda Prasad Histopathology, the Children's Hospital at Westmead, Sydney.

QUESTIONS

1. **What is the tissue?**
2. **What is the stain?**
3. **Why is this?**

Answers will be published in the next issue of the histograph





2020 Workshop Webinars Series at

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- *May Thurs 21* ***Technical Update Webinar - Processing machines. FREE***
- *Jul Sat 25 Skin - Histology & regional variation - Microscopy Workshop****
- *Aug Thurs 27* ***Technical Update Webinar – Digital Histology. FREE***
- *Sep Sat 12 Skin Pathology Microscopy Workshop**** *(inflammation, healed wounds, keloids, moles, warts and malignancy)*
- *Nov Sat 14 Skin special staining Workshop**** *(to highlight different pathologies - including immunohistochemistry)*

***Lab workshops are at cost for rental of the university premises.

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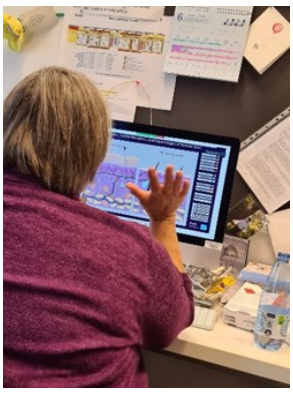
HISTOLOGY MASTERCLASS – STRATIFIED SQUAMOUS EPITHELIA

Held on the 25th July at UTS via ZOOM.

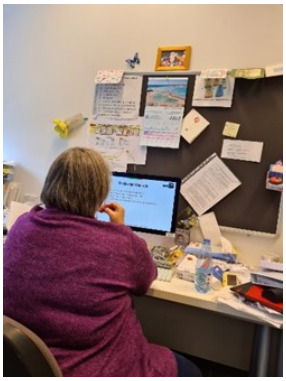
Due to circumstances beyond our control and 5 computers and two buildings later we started (20 seconds early).



Thank god for Sergio Joshua, who looked after the waiting room and played music to entertain the growing number of attendees, while Tamara and I ran around trying to get a working computer.

Eventually, we ended up in Tamara's office, socially distancing, her at the "MAC" and me at the door.

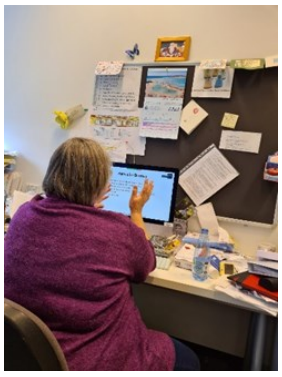
When we started the "MAC" could not run the hard drive containing Ewen Sutherland's beautiful scanned images, as the software was not compatible for the "MAC". But the ZOOM was soon in full swing with an enlarged mouse pointer.



Tamara presented the ZOOM session with professionalism and knowledge.

Unfortunately, the animated Tamara could not be seen by the attendees as it was not possible to have Tamara and her presentation on-line at the same time (but I enjoyed it).

Thank you to the "MAC" and UTS lockdown.



Sergio Joshua kept his eye on the chat from his home and answered some of the questions and relating them to Tamara, so we had his technical expertise exuding from his home again, social distancing. I had emails and text messages from attendees who could not speak more highly of the way Tamara conducted the webinar and most were surprised at her breath of knowledge (that comes with years of experience and answering silly questions).

While Tamara's computer (the "MAC") kept us amused with the telling of the time every 15 minutes, my conclusion to this extremely challenging day is that as Histotechs we learn to be very adaptable in every environment and circumstances.

This Webinar could not have been possible if it were not for the wonderful team of:

Ewen and Askar for the scanned slides, Tamara for her patience, resilience and knowledge on the Masterclass and Sergio for his smarts and ability to take charge when all else fails.

That is what I call TEAMWORK.

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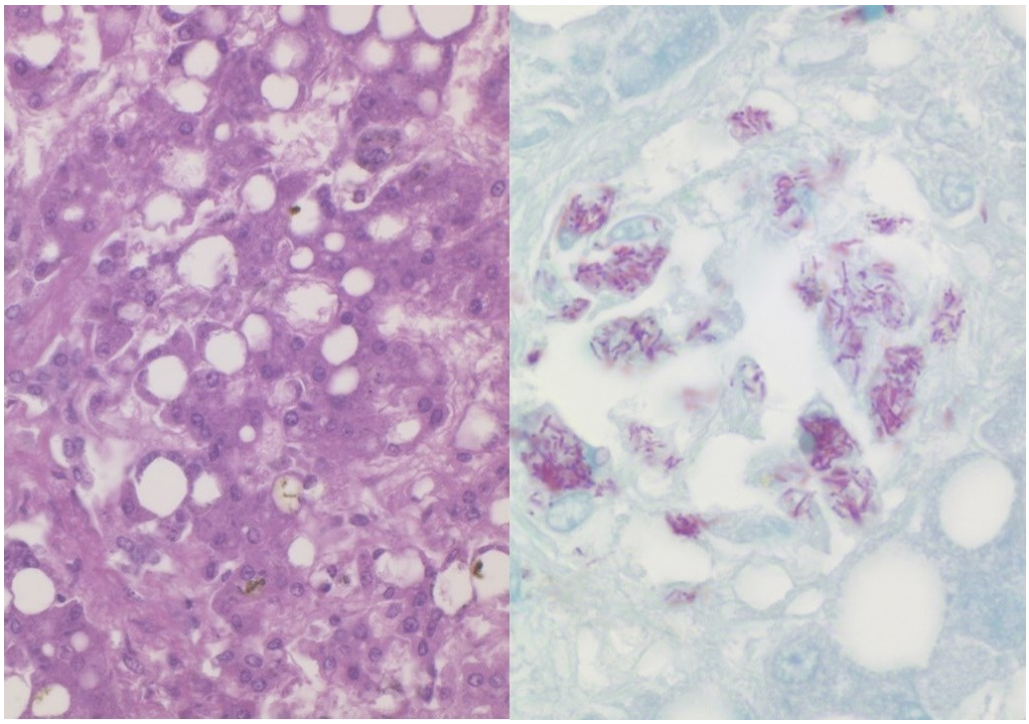
Figure 2, a case of tuberculosis in the liver. You cannot discern the bacteria on the H&E but they stain quite well with a ZN stain.

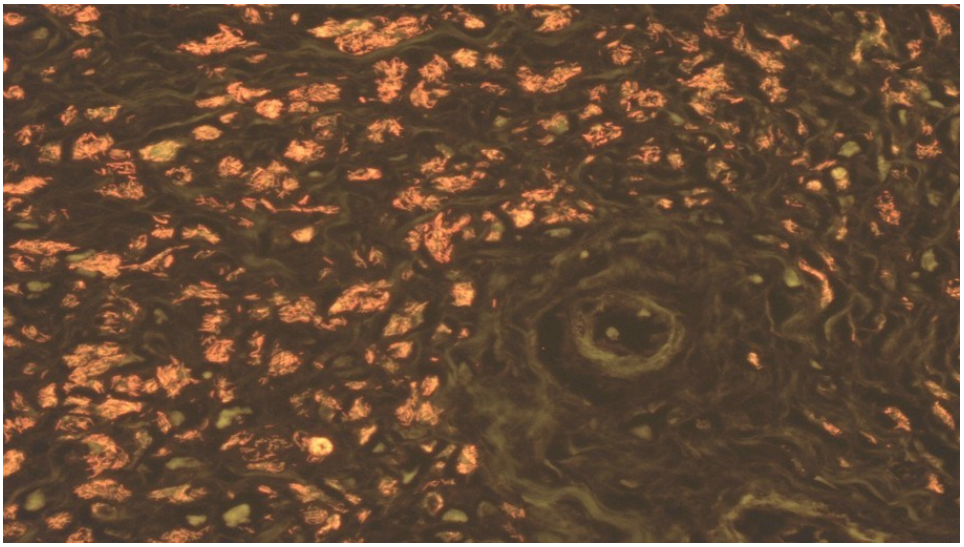


Why do certain bacteria resist the acid-alcohol removal of carbol-fuschin? Both mycobacterium and Nocardia have unusual cell walls that are waxy and nearly impermeable due to the presence of the molecule mycolic acid. This retains the red dye. But not all mycobacterium react the same, though TB is resistant to acid alcohol Leprosy mycobacteria are not, but Leprae are resistant to acid alone (called a Fite stain which is a modified ZN).

Nocardia and Actinomycetes are similar morphologically and it is important to differentiate them. Though both actinomycetes and nocardia are Gram positive, only nocardia is Fite positive. There are several structures that may be present in sections that are ZN positive such as hair shafts, red blood cells and Russell bodies amongst others.

There are other techniques that can be used to aid in the detection of mycobacterium, especially atypical mycobacteria which are usually present in few numbers. One of these uses fluorescent microscopy. To reiterate, Fluorescent microscopy relies on the ability of certain substance to absorb high energy light (for example ultraviolet), convert a small amount to vibrational energy (causing an electron to jump to a higher orbit), finally releasing the remaining energy as visible light (of a lower energy) as the unstable electron returns to its original orbit. The modern fluorescent microscope has an excitation filter which absorbs low energy light, allowing the passage of ultraviolet light, a dichroic mirror that reflects the UV light to the sample and allows transmittance of the reflected visible light to the observer. An emission filter selects the appropriate emitted light and acts as another filter to block harmful UV light from damaging the microscopist eyes.

Figure 3 is mycobacteria stained with Auramine-Rhodamine. The bacteria fluoresce orange red on a green background (Winn 1995).



Silver impregnation methods such as the Dieterle or Warthin Starry are more sensitive and may be used to assist with the identification of mycobacteria in those cases where carbol fuchsin methods have failed. They show beaded bacilli, nocardia-like filamentous organisms, and granular debris probably representing degenerate mycobacteria. Their specificity is limited as morphological similarities are shared with cat scratch disease and nocardiosis (Hale 2000).

The Warthin-Starry is a silver stain that when it works well will demonstrate most if not all bacteria. The bacteria, usually pre-treated with uranium nitrate, will bind to bacteria, induce a silver nucleation reaction whereby more silver ions are deposited and then using an external reducer, such as hydroquinone, resulting in a black deposit. The Warthin-Starry stain demonstrates syphilis as well as Legionella bacteria. Both are virtually unstained with Gram and Giemsa based methods (Winn 1995).

In 1984, Marshall and Warren at Royal Perth Hospital, made an amazing discovery (Marshall & Warren 1984). They ascertained that stomach ulcers were caused by Helicobacter pylori. This had a big effect on the treatment of ulcers and a decrease in stomach ulcers that they were later awarded a Nobel Prize in 2005.

Helicobacter affects up to half the world's population. It causes gastritis, ulcers, gastric carcinomas and lymphomas. At the time, mainstream gastroenterologists did not believe this since the belief was that no bacteria could survive in the acidic environment of the stomach. So, what changed? Helicobacter were found to produce ammonia that neutralises hydrochloric acid (Goodwin et al 1986). Helicobacter also produce lipase and phospholipases that dissolved mucins (Ruiz et al 2007). This allowed them to survive in the stomach by using the stomach's own protective layer.

One of the major issues that they faced was satisfying Koch's Postulates.

How do we know that a pathogen causes a specific disease? According to Koch, the pathogen must be present in every case of the disease, the pathogen must be isolated from the diseased host and grown in pure culture, the disease must be reproduced when a pure culture of the pathogen is inoculated into a healthy susceptible host and finally the pathogen must be recoverable from the experimentally infected host.

Now, unable to make his case in studies with lab mice (because H. pylori affects only primates) and prohibited from experimenting on people, Marshall grew desperate. Finally, he ran an experiment on the only human patient he could ethically recruit, himself. He took some H. pylori from the gut of an ailing patient, stirred it into a broth, and drank it. As the days passed, he developed gastritis, the precursor to an ulcer: He started vomiting, his breath began to stink, and he felt sick and exhausted. Back in the lab, he biopsied his own gut, culturing H. pylori and proving unequivocally that bacteria were the underlying cause of ulcers. He did make sure that he had an effective antibiotic, bismuth plus metronidazole, before he started this madness – a very brave man!

Helicobacter are very small, curved like bacilli, that can sometimes be seen on a H&E and they are weakly Gram negative. At the time, I was working in Adelaide, and it was a challenge to convince physicians that helicobacter existed. For this reason, we started doing Warthin Starry stains on all gastric biopsies and they stand out quite well. Unfortunately, Warthin-Starry stains are time consuming and difficult to do. Finding a stain that is easier to do than the Warthin -Starry, has resulted in a large number of stains being proposed including Giemsa and Methylene blue. Helicobacter are also quite well demonstrated using acridine orange and a Fluorescent microscope (Walters et al 1986). Antibodies to helicobacter are also available and have been used in immunoperoxidase techniques.

Fich et al (1989) found that helicobacter could not be detected using H&E and Giemsa stains in gastric tissues that had been stored in 10% formalin for longer than two years, even though helicobacter were present in the initial tissue blocks (with less than 24 hours fixation).

It is important to remember that some histochemical stains not usually associated with detection of bacteria may reveal their presence in histologic sections. For instance, the periodic acid-Schiff and Gomori methenamine silver techniques may both stain conventional bacteria, especially if the incubation time in the silver stain is prolonged. The methenamine silver stain is, in fact, an excellent method for demonstrating the aerobic actinomycetes, such as Nocardia asteroids (Winn 1995).

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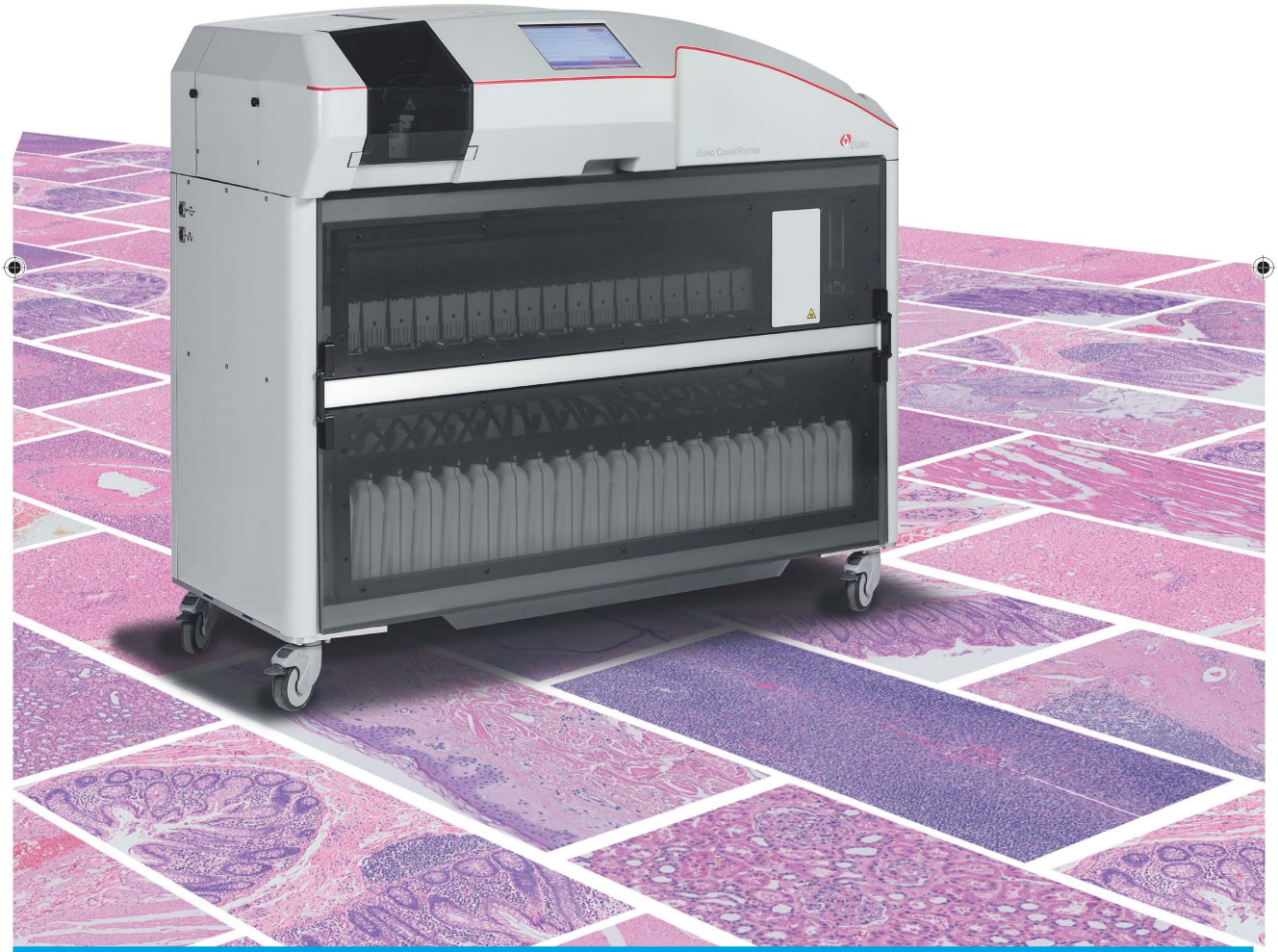

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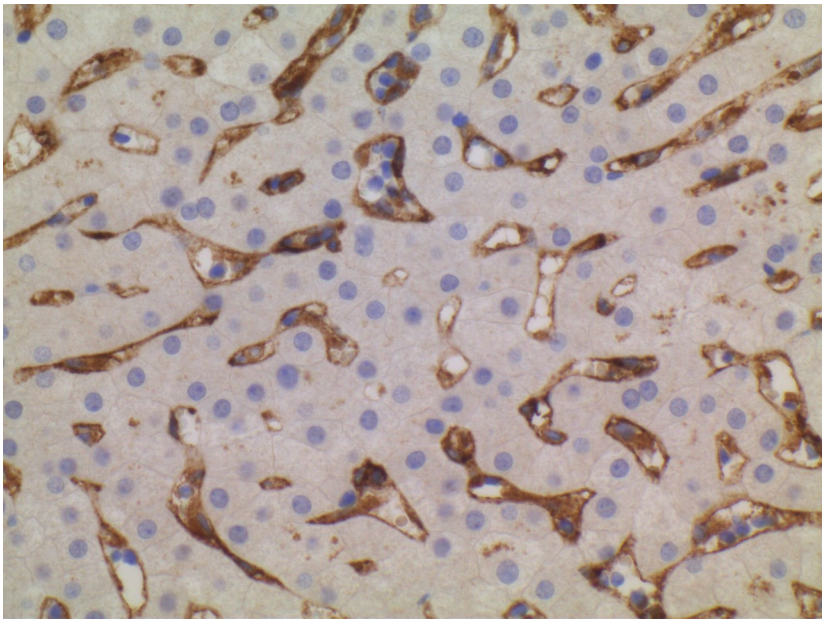
ANSWERS

THIS IS A CD4 IMMUNOSTAIN

1. **What is the tissue? Liver**
2. **What is it staining? Liver Sinusoids**
3. **Why is this? Read below for answer**

TEST AND TEACH FROM LAST ISSUE

Linda Prasad, Histopathology, the Children's Hospital at Westmead, Sydney.



CD4 (cluster of differentiation 4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells (Antigen-presenting cells). CD4+ T helper cells are white blood cells that are an essential part of the human immune system. They are often referred to as CD4 cells, T-helper cells or T4 cells. It is a marker of helper T-cell population.

In Paediatrics, CD4 staining is mainly used to look at the T-cell population. It is used for the classification of lymphocytes, e.g. in inflammatory lesions and classification of malignant lymphomas. CD4 is also expressed in Langerhans cell histiocytosis and is also a receptor for the immunodeficiency virus.

Unlike other organs, which are supplied by arterial blood through arterioles, the liver receives venous blood at low pressures through the portal vein as well as arterial blood via the hepatic artery. The intrahepatic portal venous system consists of conducting and distributing systems that ensure blood is carried throughout the parenchyma and evenly delivered to individual hepatocytes via the sinusoidal network.

The liver is home to a large repertoire of immune cells, and its unique architecture permits direct contact of circulating T cells with liver-resident cells . Liver sinusoidal endothelial cells are unique liver-resident Antigen Presenting Cells that are strategically located in the liver sinusoids and interact with passenger leukocytes, cross-present Antigen, and subsequently mediate naive CD4+ and CD8+ T cell tolerance. CD4 is not only found in the hepatic sinusoidal endothelial cells but also found on Kupffer cells which are the liver's largest population of tissue resident-macrophages. CD4 is an adhesion molecule related to antigen presentation and is continuously found in the sinusoidal walls. This is why liver sinusoidal endothelial cells stain positive with CD4 Antibody.

NordiQC suggest using liver tissue as a control for CD4. Tonsil is always recommended as a positive and negative tissue control. As a supplement to tonsil, it is recommended to verify the protocol on liver tissue as well. The Kupffer cells and endothelial cells in the liver sinusoids must at least display a moderate, distinct staining reaction.

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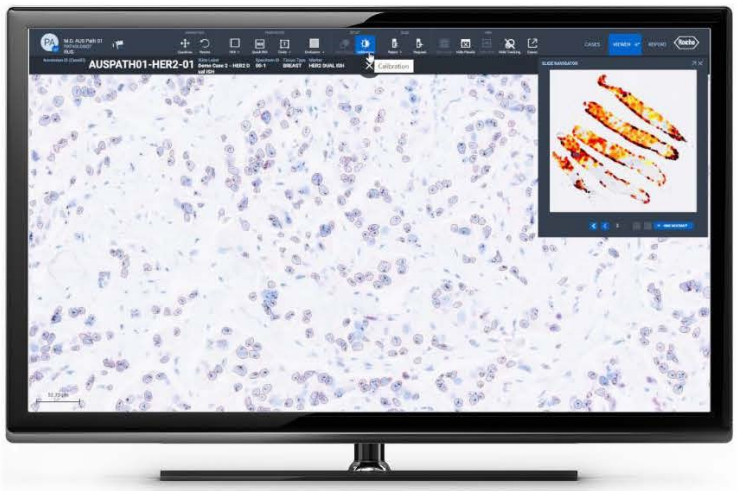

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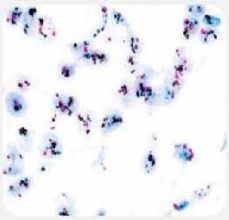

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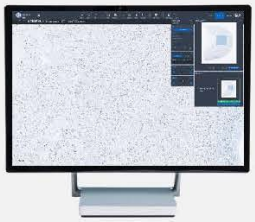

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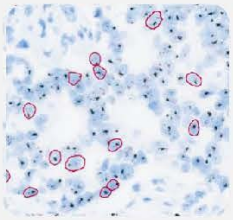

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