Org. No. A003523F

PARAFFINALIA NEWSLETTER

VOLUME 28, NUMBER 2

HGVT

June 2024

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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The members of the Histology Group of Victoria 2024 are:

Name	Institution
Samantha Arandelovic	Mater Hospital Brisbane
Kerrie Scott-Dowell	Dorevitch Pathology/ Leica Biosystems
Mark Bromley	Sullivan Nicolaides Pathology
Kellie Vukovic	Melbourne Pathology
Alistair Townsend	Royal Hobart Hospital
Christine Gorringe	Royal Hobart Hospital
Elizabeth Baranyai	Cabrini Health
Bronwyn Christiansen	Royal Children's Hospital
Tu Anh Huynh	Royal Melbourne Hospital
Snejana Ursache	Alfred Hospital
Gulnur Orman	Box Hill Hospital
Dodie Pouniotis	RMIT University
Fatema Tajbhai	Northern Health
Kerrie Howard	Northern Health/ RMIT University
Li Shan Ong	Monash Pathology/ Melbourne Pathology
Enia Kakaflikas	Pathology Solutions
Maria Chavez	Monash Pathology

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Presidents Address

Behind the Bench with Sam Arandelovic

This year is flying so fast! Everyone is working very hard and as the winter settles in embedding shift will be the most favourable.

As I mentioned in last newsletter that Trivia Night is on Friday 26th July. I hope you got a table because it's sold out, and for those that are coming, brush up on your Trivia as we have 175 people coming. It's going to be a fun night.

DIHC Conference in Tweed Heads was well attended. Great speakers and it's also so lovely to catch up with friends and colleagues that you haven't seen for a while. I'm very much looking forward to the National Conference in Sydney. If you haven't registered there is still time.

Some people may have noticed that they haven't received any emails from HGVT. If that is the case this is because you have subscribed to HGVT with your work email many healthcare providers are ramping up their firewall's security due to all the cyber attacks. Only way around that is to update your email address to your personal email address.

See you all at our next scientific meeting!



Under the Microscope

with Kellie Vukovic

What was your first part time job?

I got my first part time job at the end of year 12. I worked in retail at Ray's Outdoors even though I had never been camping before myself. I was mainly at the register but also worked in the clothing and footwear department. During high school I also umpired junior netball on the weekend.

What is your current Job?

I am the senior scientist in charge of accessioning and dissection at Melbourne Pathology. In this role I also perform complex dissection.

How long have you been working in your role?

I transferred from SNP in Brisbane at the beginning of 2020 after completing a senior cutup scientist role there from 2016. I am now part time in this role after having 2 babies.

What skill do you want to learn and why?

My husband would love to buy a caravan and do a big trip around Australia when our boys are older. I have been putting it off for years, but I really need to learn how to drive a manual car if we are going to do it. His car is manual and the one we would take.

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

I would 100% still work which my family thinks is crazy (maybe just a couple of days a week). I would love to have an event planning business on the side to help people create their dream event. I would also love to do heaps of travel. There would always be an amazing holiday to look forward to as soon as I was back from the last one.

What's an ideal weekend for you?

I have always liked to be busy so an ideal weekend is having multiple events with my family and friends. I love a bottomless brunch with my friends and we are slowly trying as many as we can around Melbourne. My extended family is expanding rapidly with 7 kids under 3 so we now like to hire Airbnb's and get away to different places around Victoria with us all.

What's on your bucket list this year?

I don't think a bucket list exists when you have a 2 year old and a 4 month old. I would love to go for a long weekend away this year with my friends to King Valley to do a Prosecco bike riding tour.

What music/podcast is on your playlist at the moment?

I am the worst person when it comes to music. I have zero playlists on any device. I hate to admit it but I have never listened to a podcast in my life. My 2-year-old is loving Taylor Swift at the moment so 'Shake it Off' is a popular YouTube search in my house.

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

Italy – I did part of my uni placement in Edinburgh, Scotland and spent most weekends travelling to different countries. I never made it to Italy but would love to go for the food and scenery.







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WHITEPAPER

Sectioning: A Deeper Look

There are numerous factors that must be considered when preparing tissue for IHC staining. Due to the diversity of tissue and media that may be involved, there is significant variability in sample preparation and no single answer for best practices. However, some key contributing factors can be identified. One oft-overlooked factor is sectioning thickness.

The amount of light permitted to pass through the tissue is critical for microscopic examination.² This is partially controlled by section thickness, which affects the contrast, sharpness, and morphological details of the tissue under the microscope, thus greatly influencing staining quality.¹

Generally, thicker sections demonstrate greater staining intensity due to more protein being present and labeled in a thicker three-dimensional structure. For example, a 7zwm section will have increased staining intensity compared to a 4µm section. In addition, what is visible in a 7 μ m-thick section may be lacking in a 4 μ m-thick section since less tissue is present.

Another area of consideration in sectioning thickness is how it affects the contents and composition of the block. Cells can range in size from 1 to 100 µm. In mammals, organelles and cellular components similarly vary in size. A ribosome may be 0.2 µm in diameter, while a cell nucleus may have a diameter of 6 µm. As a result, different-sized sections will better visually represent different cellular components, such as the membrane, cytoplasm, or nucleus.^{1,2} Tissue sections >5 µm can produce more variation in staining intensity and make the assessment of cytoplasmic and membrane staining more complex than for nuclear staining.3

Tissue preservation and embedding medium should also be considered when determining section thickness.¹ Tissue is made firmer by the fixation process to preserve its structure and then may be infiltrated with a medium, such as wax or plastics, to support it, or may be fresh frozen. Section thickness typically ranges from 8-15 µm for frozen sections, 4-10 µm for wax sections, and 0.5-3 µm for plastic histological sections. For IHC staining, sections are cut between 3-5 µm.

Sectioning thickness is often overlooked as a factor in IHC staining outcomes but should always be considered in terms of tissue preservation, embedding medium, and staining quality.1 At a minimum, consistency in sectioning is critical for the quality of patient care to prevent variability and confounding results. Sectioning thickness has significant implications in medical care, both now and in the future as digital imaging pathology becomes more prominent.³

Mitochondria

1 µm

Cell Nucleus

5-10 µm

Organelles

DNA Helix

10 nm

Molecules

Sectioning a sphere with a wall of finite thickness



1. Libard, Sylwia Cerjan, Dijana , Alafuzof, Irina (2019) Characteristics of the tissue section that influence the staining outcome in immunohistochemistry. Histochemistry and Celi Biology 151, p91-96. 2. MacMillan, D.B. Harris, R.J. (2018). An Atlas of Comparative Vertebrate Histology. Diagnostic and Translational Research Guide. P 9-29

3. Shinobu Masuda, MD, PhD. Ryohei Suzuki, ME et al. (2021) Tissue Thickness Interferes with the Estimation of the immunohistochemical Intensity: Introduction of a Control System for Managing Tissue Thickness. Applied Immunohistochemistry and Molecular Morphology 2021;29: p118-126

6 um

Any news!!! We would love to hear from you! Submit a pic and a short description to "Days of our labs" to the HGVT Facebook messenger or email <u>editor@hgv.org.au</u>



The Alfred Hospital – Mark Donovan's Farewell Tea Party



Dorevitch Pathology Histology Department celebrated the Biggest Morning Tea with goodies baked by their team members.







Northern Hospital Senior Scientist of Anatomical Pathology Fatema was featured on the Northern Health's "Get to Know us"-

Read more here: https://www.nh.org.au/get-to-know-fatema-tajbhai/



MAY 31, 2024

#WeAreNorthern

Meet Fatema Tajbhai, Senior Scientist in our newest service - Anatomical Pathology.

Q: Firstly, your coffee order, Fatema?

It varies and is very mood dependent - long black or batch brew (depending on the cafe)/ soy latte/soy magic.

Q: How would you describe your role?

It is a very challenging but rewarding role. Anatomical Pathology is a new service to the Northern Hospital, only operational since 5 February 2024. Currently, I am managing the operational aspects of running the laboratory and doing all the background work to try and offer the best service we can.

Q: How did you get into this role?

I studied and worked as a medical scientist back in the UK and when I moved to Melbourne, I continued in the same line of work. When the Anatomical Pathology Service was being set up at Northern Health, I was excited to get involved and be a part of this new venture. I applied for a position and was fortunate enough to get it!



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Review of Scientific Meeting

HGVT Scientific Meeting Review

2nd May 2024 By Ker

By Kerrie Scott-Dowell

We were lucky enough to have Agilent sponsor our meeting with 2 Tasmanian Speakers presenting some rare and interesting cases. What else would you expect from Tassie.

Presenter- Christine Gorringe

CASE 1 Ewing's Sarcoma

A 25yo male who had a BMT and a BM aspirate. The aspirate showed aggregates of malignant cells. In the trephine H&E, 40% of the bone marrow showed contiguously spreading, tumour cells. IHC showed very strong membrane staining for CD99 and 60-70% nuclear positivity for Ki67. Scans showed disseminated malignancy in the spinal cord and lung. LDH (Lactate Dehydrogenase) levels were very high.



Fig 1 Bone Marrow Aspirate

Fig 2 CD99

The diagnosis was metastatic Ewing's Sarcoma, which has a very poor prognosis.

Ewing's is a highly metastatic form of sarcoma that affects predominantly young adults. The high LDH level has a poor prognostic significance. The differential diagnosis was Ewings vs other small round cell tumours. 80% of Ewing's cases are CD99 positive. 70% of cases have recurrence. This patient unfortunately died 5 months after diagnosis.

CASE 2 Amyloidosis

A 68yo male presented with renal impairment. The EGFR (estimated glomerular filtration rate) was 26 and the normal range is greater than 90, so it indicates a very low renal function. A renal biopsy was performed, and all glomeruli showed disruption of the tissue architecture by amyloid deposits.



Fig 3 Renal H&E

The amorphous infiltrate was visible on the H&E, PAS and PASMMT stains and the Congo Red showed the classic birefringence. A BMT was also performed and also showed Congo Red staining amorphous infiltrate. Amyloidosis occurs when beta sheet fibrillar proteins aggregate in various tissues. There are different types and different treatments. The current treatment for this patient is initial drug treatment followed by a stem cell transplant.



Fig 4 Renal Congo Red in Polarized light



Fig 5 Renal Congo Red

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MILESTONE H E L P I N G P A T I E N T S

Presenter Alistair Townsend Case3 Tuberculosis

Right testicle 75yo – No clinic notes. Macroscopically the testis is identified, but no normal epididymis identified. An area of necrosis and cystic area containing fibrin and haemorrhage. The H&E shows necrosis, with a lymphatic infiltrate, giant cells and granulomatous change. ZN and Wade Fite showed positive staining bacteria. Diagnosis of an isolated tuberculous epididymitis would be very rare (Only 4% of TB are genitourinary), and most masses of testes are tumours, so without any patient history a large number of IHC stains were performed to eliminate.





Fig 1 Bisected gross specimen of the Testis

Fig 2 H&E showing necrosis with an area of surrounding activated macrophages and a lymphocytic infiltrate with occasional multinucleated giant cells

When more details were obtained about the patient, it was seen he had previous respiratory TB, spends a lot of time in Asia, resulting in large gaps in his medical history, but probably most significantly has been frequently recatheterised in Vietnam, making this the most probable source of infection.

Diagnosis -Tuberculous Epididymitis. Treatment 6-9 month drug therapy.

Case 4 Schwannoma

36 male Pleural Mass (no clinical notes).

Pathology had to call to get information such as chest tightness for some time, all blood test normal and a history of mine-work.

H&E showed spindle cells. A large range of IHC antibodies were used . S100 and SOX10 were strongly positive, as was TTF1, BCL2 and CD99 was weak patchy staining. CD34 was negative for the spindle cells and Ki67 positivity was low. All other IHC were negative. The diagnosis was a Schwannoma, which is a benign, slow growing, peripheral nerve sheath tumour. It is rarely seen in the thoracic area (2% only).

Alistair lamented the waste of resources and time in both of his cases presentations, due to a lack of clinical notes accompanying the specimens.



Fig 3 H&E x 400 showing Spindle Cells

Fig 4 S100 IHC staining demonstrating Spindle Cells

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Article Review By May Chung

Bridging bytes and biopsies: A comparative analysis of ChatGPT and histopathologist in pathology diagnosis and collaborative potential.

M L Oon et al. (Histopathology 2024, 84, 601-603.)

ChatGPT (an AI chatbot, using <u>Generative Pretrained Transformer</u>) has shown capabilities in analysing human input and generating responses to mimic humans. It can be useful in performing clerical tasks, refining dialogue and written text and as a clinical decision support system (CDSS). It has shown it can perform just as well as humans in providing factual and relevant answers in several knowledge domains, including one instance where it was reported ChatGPT had outscored humans in a virtual specialist Obstetrics and Gynaecology Objective Structured Clinical Examination (OSCE). Its usefulness in histopathology, where visual interpretation is vital, has not been determined. The authors set out a study to analyse this.

The study was completed in two parts, the first hoping to establish a benchmark for ChatGPT against pathologists. The second explores the potential of pathologists using ChatGPT as an aid to achieve more accurate diagnoses. A group of junior pathologists and trainees with less than 10 years' experience was recruited.

In Part 1, Chat GPT was primed to answer a series of questions pertaining to diagnostic histopathology. The pathologists and ChatGPT then had a set of standardised histopathology questions to answer. These questions covered different disciplines of pathology and focused on common diagnostic challenges. It also evaluated specific knowledge domains. The outcome showed on average, pathologists scored 8 out of 10, compared to ChatGPT which scored 6 out of 10.

In Part 2, the pathologists reviewed a set of 10 challenging virtual slides and provided their answers, with no assistance in any form. Afterwards, they were instructed to use ChatGPT to aid them. The interaction with ChatGPT could take any form, i.e. asking ChatGPT to provide a list of differential diagnoses based on descriptions of microscopic features or prompt ChatGPT to pose questions to refine the diagnosis. The pathologists were then given the opportunity to revise their diagnosis after using ChatGPT, if they wished. The outcome showed pathologists scored 8 out of 10, both before and after consulting ChatGPT. A deeper look at the results showed some pathologists improved their score after using ChatGPT, whereas some performed worse after using ChatGPT. Some diagnoses were incorrect and remained incorrect after ChatGPT.



In their discussion, the authors conclude that the current version of ChatGPT was less useful in reaching an accurate diagnosis. This has to do with its understand of language and linguistics versus its inadequate mathematical knowledge, vital for the pathologist when including and excluding differential diagnoses. It also showed inconsistency, sometimes outputting different answers to the same prompts.

By contrast, using ChatGPT as an aid was more promising, with the pathologists praising its ability to function as an advanced search engine. A pitfall to be aware of in this scenario however, is the quality of ChatGPT output is dependent on accurate descriptions being entered in the prompts. If the pathologist does not identify or accurately describe a microscopic feature in their prompts, the output may not be useful. Another pitfall is the risk of confirmation bias, where the prompts entered in to ChatGPT based on the pathologists first impressions, confirms the diagnosis.

An upcoming version of ChatGPT will have a feature to accept imagebased prompts. This may work around the limitations of pathologist's interpretation, as well as going some way to resolve the issue of confirmation bias. Current sentiments towards ChatGPT show that pathologists are willing to embrace it as a diagnostic aid, with every confidence it will not be replacing the important role pathologists play any time soon.





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CD3- Ki67 Dual Immunohistochemistry Stain | By Maria Charvez

CD3 is a multimeric protein complex composed of four distinct polypetide chains; gamma, delta, epsilon and zeta. This rabbit monoclonal antibody (Roche -Ventana) detects the epsilon chain of CD3 which is expressed on T cells and natural killer cells. ¹

In T cells, CD3 is initially expressed in the cytoplasm of early thymocytes and then later, on the cell membrane of mature T cells. The CD3 rabbit monoclonal antibody is highly specific and an ideal pan T cell marker, useful for the detection of normal and neoplastic T cells. Approximately 80% of T cell lymphomas produce a positive staining pattern with CD3.²

Ki67 is a nuclear protein expressed in proliferating cells through all active stages of the cell cycle and plays a critical role in cell division. It is not expressed in resting cells. The absence of



Normal Tonsil: CD3 DAB Brown, Ki67 Alkaline Phosphatase Red.

In our laboratory, the test is performed on the Roche Benchmark Ultra instrument. The combined CD3 -ki67 dual stain utilises alkaline phosphatase red detection kit to demonstrate Ki67 (nuclear stain) and DAB brown detection (membranous stain) for the demonstration of CD3. This test has been extremely beneficial when determining the proliferation index of cases of lymphoma, in particular B cell, marginal and follicular lymphomas. A rich reactive (benign) T cell population is often associated with low grade B cell lymphomas, a single Ki67 IHC test does not differentiate between proliferating neoplastic B cells and Ki-67 in resting cells and its expression in all proliferating cells, whether normal or neoplastic, makes the Ki-67 antibody useful for determining the growth fraction of any given human cell population. Ki67 is often used to differentiate between benign versus malignant disease, assist in grading of tumours and determine the prognosis of breast, bladder and prostate cancers and chordoma. Cancers with a high Ki67 proliferation index have a worse prognosis.



Marginal Zone Lymphoma: CD3 DAB Brown, Ki67 Alkaline phosphatase red. Cells which are positive for both are excluded from the Ki67 proliferation index.

proliferating reactive (benign) T cell lymphocytes. To the reporting pathologist, these types of cases may initially appear as high-grade disease, when it is actually a low-grade lymphoma. The CD3 – Ki67 dual stain allows for an accurate evaluation and enumeration of ki67 proliferation index of cases of lymphoma as it provides a clear distinction between the neoplastic B lymphocytes and reactive benign T cell lymphocytes. Furthermore, this has a direct impact on the prognosis and patient management.

² de Boysson H, Geffray L. Granulomatose lymphomatoïde [Lymphomatoid granulomatosis]. Rev Med Interne. 2013 Jun;34(6):349-57. French. doi: 10.1016/j.revmed.2012.08.017. Epub 2012 Oct 1. PMID: 23036780.

¹ https://elabdoc-prod.roche.com/eLD/api/downloads/49729da6-7333-ea11-fa90-005056a772fd?countryIsoCode=us

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Under the Microscope

with Alistair Townsend

What was your first part time job?

Working in the Automotive section of the local K-Mart store

What is your current Job?

Medical Scientist in Charge – Anatomical Pathology, Royal Hobart Hospital

How long have you been working in your role?

16 years

What skill do you want to learn and why?

I'm currently learning Spanish and I'd like to get to a level of fluency where I can have basic conversations when travelling in Spanish speaking countries.

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



Retire and travel. I'd also want to do more volunteering at major sporting events and any sort of voluntary work in developing countries.

What's an ideal weekend for you?

Catching up with friends at a sporting match and then grabbing a great meal out.

What's on your bucket list this year?

Travel to Greece

What music/podcast is on your playlist at the moment?

I just listen to whatever is on the radio at the time.

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

Antarctica.



Australasian Association of Histology and Histotechnology



Doltone House, Darling Island, Sydney, Australia

HISTOTECHNOLOGY

10th NATIONAL CONFERENCE August 2024



Scan for more information





TRIVIA NIGHT 2024

- Date: Friday 26th July
- Time:

6.30pm-10.30pm



Burnley Brewing 6-B/650 Bridge Road actimond 3121

Price: \$30 per person (Tables of 6, 8 and 10)

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Next Meeting

27th June 2024

HGVT Scientific meeting **Topic:** Introduction to Cut Up Kellie Vukovic and Kerrie Howard

Date: 26th July 2024 HGVT Trivia Venue: Burnley Brewing 650 Bridge Rd, Richmond.

Date: 9th-11th August 2024 National Histology Conference Venue: Sydney, New South Wales **Presenters:** Various

Date: 5th Sep 2024 HGVT Scientific meeting **Topic:** Student Presentation **Presenters:** TBA