



# HISTOGRAPH

## Editorial

Hi and welcome to the first issue of the Histogram for 2020. Hopefully all our member's are staying safe and well during this challenging and unprecedented times due to COVID-19. Adrian Ureta has written an article based on the true experiences of Histotechs during the COVID-19 Pandemic. I'm sure a lot of us would be able to relate to it.

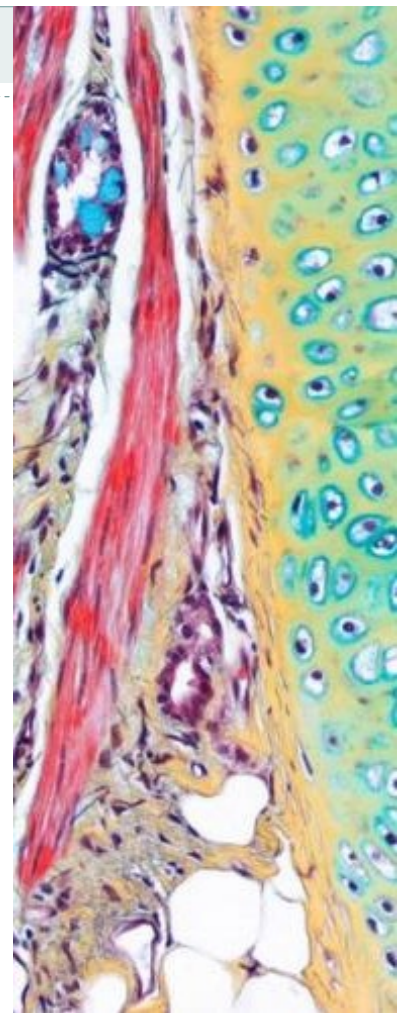
We just finished our first webinar on Tissue Processing, Pitfalls and Solutions, which was originally a workshop. Despite the changes, it was a very successful event. Please find inside a calendar for the workshops for this year.

Tony Henwood has just published an excellent Technical Note on Coronavirus disinfection in histopathology labs. This open access article was well received and read by so many across the globe. Thank you Tony Henwood for writing this timely and informative article.

To all the Histotechnology Professionals, we hope that you had a great Histotechnology Professionals Day which was celebrated on March 10th.

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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provide educational  
opportunities for  
continuing professional  
development. Thank you  
team for the GREAT  
JOB**

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Editor- Histogrpah	Linda Prasad	Children's Hospital, Westmead
Sub-committee Member	Momoko Sakaki	Children's Hospital, Westmead
Sub-committee Member	Cristina Antonio	Douglass Hanly Moir Pathology





A decision is pending on our involvement with the “Australian Council for Certification of the Medical Laboratory Scientific Workforce”. The committee’s feeling is we need to be involved as a National body.

There had been discussion on having a “Cut Up” workshop on the Sunday morning of the combined National Conference next year. We were unable to hold a wet workshop at UTS during the week. The National committee decided that Sunday morning was not a good time to do this workshop so we are looking at other options.

Adrian Ureta, Student Representative on our committee, prepared a flyer to promote our society to students enrolling this year at Universities and TAFEs. It was an excellent presentation and idea.

Our first planned activity for this year was the workshop on “Tissue Processing: Pitfalls and Solutions Workshop”, 28th of March at UTS, Sydney. Due to Coronavirus developments, we had to cancel the workshop booking with UTS and hold a webinar instead. Feedback from Dr Tamara Sztynka who organised the webinar indicates the workshop was successful with 29 attendees and around 30 minutes of question time. Di Reader (Royal North Shore Hospital) prepared and presented the presentation. Sergio Joshua, (UTS), Bill Sinai (Ex ICPMR Histology) and Ewen Sutherland (previous Histotechnician) assisted with

the question segment. There were many favourable comments from the participants.

We have several Webinars and workshops planned during the rest of this year. We will endeavour to hold these so please review our website and Facebook regularly for updates.

Stay safe,

Cheers,

Trevor Hinwood.

Chairperson.

Histotechnology Society of NSW.

# **Coronavirus disinfection in histopathology.**

Henwood AF 1,2.

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J Histotechnol. 2020 Mar 1:1-3. doi: 10.1080/01478885.2020.1734718. [Epub ahead of print]

## **Abstract**

The 2019 Coronavirus epidemic, provisionally called 2019-nCoV, was first identified in Wuhan, China, in persons exposed to a seafood or wet market. There is an international push to contain the virus and prevent its spread. It is feasible that potentially infectious samples may be received in histopathology laboratories for diagnosis. This technical note presents disinfection procedures and histotechnology processes that should alleviate the risk of infection to laboratory staff. Using data obtained from similar coronaviruses, e.g. severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), experts are confident that 70% ethanol and 0.1% sodium hypochlorite should inactivate the virus. Formalin fixation and heating samples to 56°C, as used in routine tissue processing, were found to inactivate several coronaviruses and it is believed that 2019-nCoV would be similarly affected.

## **KEYWORDS:**

Coronavirus; SARS-CoV-2; biosafety; disinfection; histopathology laboratory; histotechnology

Open access download link:

<https://www.tandfonline.com/doi/full/10.1080/01478885.2020.1734718>

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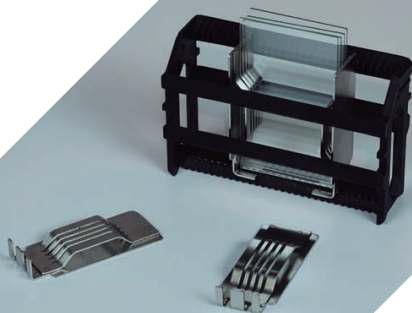
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## Happy Histotechnology Professionals Day!

Linda Prasad – Children's Hospital at Westmead

Histotechnology Professionals Day (HPD) was launched in 2010 by National Society of Histotechnology (NSH) in the United States to raise awareness about the histology profession and educate other health professionals. Celebrated every year on the 10<sup>th</sup> of March, it is a day dedicated to recognise the work of our unseen heroes of the lab which are our Scientists, Lab Aides, Technologists, Registrars, Pathologists and Administration Officers who work constantly to save lives one slide at a time.

Most of us would agree that majority of people are not aware of this field of pathology. The Histotechnology Society of NSW is trying to bring awareness about this important day.

A big thank you to Carlie Dawn Wiersma from Royal Prince Alfred Hospital for sending in a picture of her Histo Day celebrations (see below).

Carlie ordered these amazing custom cookies from The Cookie Crafter AU a dessert shop in Melbourne, to give to her colleagues in celebration of Histotechnology Professionals Day. How lovely and thoughtful! The placenta and severed leg are delightfully horrifying!

Thank you again Carlie for sharing!





Some fun and very creative pictures of HPD celebration from the Children's Hospital at Westmead.



Syringe filled with tomato sauce



Sterile jars with jelly





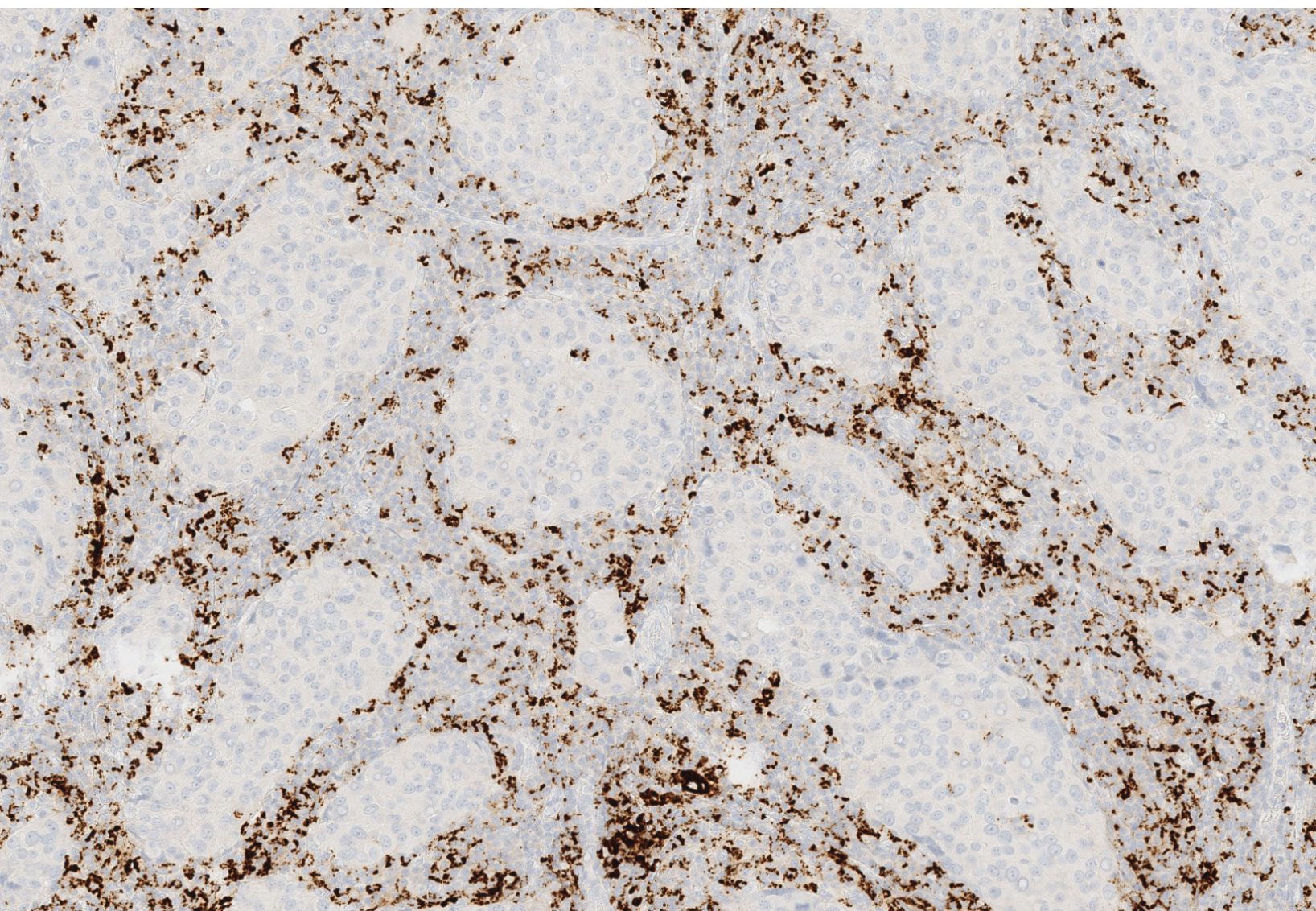
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# PROCESSING WORKSHOP

EXPERIENCE OF DEALING WITH SUBOPTIMALLY PREPARED  
TISSUE SAMPLES

**PRESENTED BY MRS. DIANNE READER (RNSH)**

**Hosted by Dr. Tamara Sztynnda & Zooming expertise of Mr. Sergio Joshua (UTS)**

The workshop - which was going to be a group gathering - eventuated being a Zoom meeting just before UTS restricted their staff and students to working/learning from home. The seminar was held at 10am on Saturday, 28<sup>th</sup> of March being broadcast from the University of Technology Sydney for Histotechnology Society of NSW. We were grateful for - the cumulative greater than 80 years of expertise - added to those of Dianne Reader provided by other attending contributors Bill Sinai, Ewen Sutherland and Kristopher Avery (RPAH) who gave examples from their experience re-enforcing Di's comprehensive presentation.

There were 23 delegates making a total of 29 attendees. Di's presentation lead to vibrant and valuable discussion leading to the use of more than the two hours which were set aside for the meeting.

As social distancing for COVID 19 pandemic prevented us enjoying the networking opportunity morning tea or lunch could bestow we all enjoyed a cuppa at home.

The seminar was recorded and the video edited by Sergio has now been put onto the society website, as has a PDF of the Powerpoint of the presentation. We were also grateful to have recorded the chat and written questions in a .txt document so we have for future reference to ensure we can address specific issues we could not elaborate on in detail during the two hour meeting.

Thank you for the kind words of appreciation from the participants. We believe everyone took away something from this workshop and will make their contribution to their work of patient care or research better.

We hope to continue with more meetings later this year - as we can - though we may need to modify our program somewhat depending on the ever-evolving measures during this pandemic.

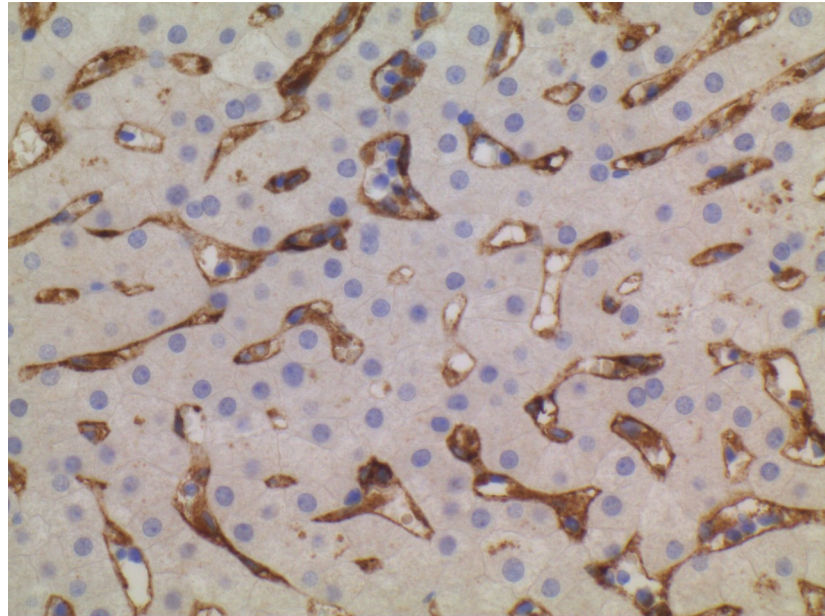
We all hope everyone reading this are well and dealing with this pandemic with fortitude and resilience and counting their blessings rather than tribulations.



## QUESTIONS

THIS IS A CD4  
IMMUNOSTAIN

1. What is the tissue?
2. What is it staining?
3. Why is this?



**2020 Workshop Webinars Series at**  
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- Mar Sat 28 Tissue Processing, Pitfalls and Solutions Workshop **FREE**
- 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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200148 Rev A - IMC-3115-REV-A 03/2020

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# Test and Teach – From the Literature

**Tony Henwood, Histopathology, the Children's Hospital at Westmead, Sydney**

One of the important aspects of individual Continuing Education is the reading and analysis of published journal articles. This will also be an important feature of the upcoming National Registration Scheme for laboratory professionals. It is sometimes perplexing trying to understand the “Methods” section of some of these articles and I have collected a few and presented them as snips for your comments. Answers below:

1. This from a table in a paper concerning immunohistochemical tests on a cohort of tumours. Can you pick the typo, well at least we hope it is a typo?

Biological marker	Functions	Clone	Species	Dilution	Antigen retrieval	Source
Fragile histidine triad (FHIT)	Tumor suppression	RB-9232	Rabbit	1:300	HIER pH 6	Lab Vision/Neo Markers
p53	Tumor suppression, apoptosis	M7001	Mouse	1:1000	HIER pH 6	DakoCytomation
p16	Tumor suppression	NCL-p16-432	Mouse	1:100	HIER	Novocastra
Retinoblastoma protein (Rb)	Tumor suppression	554136	Mouse	1:300	HIER pH 9	Pharmingen
CD4 <sup>+</sup>	Immune defense	NCL-CD4 <sup>+</sup>	Mouse	1:25	HIER pH 9	Novocastra
IL-10	Immune suppressor	RHCIL1000	Rat	1:100	HIER pH 6	Caltag
EGFR	Proliferation	28-0003	Mouse	1:40	Proteinase K	Zymed
Ki-67 (MIB-1)	Proliferation	M7240	Mouse	1:200	HIER pH 9	DakoCytomation
CK 10	Cytoskeleton	M5-611	Mouse	1:600	HIER pH 9	Lab Vision/Neo Markers
E-cadherin	Cell-cell adhesion	13-1700	Mouse	1:2500	HIER pH 9	Zymed
Cox-2	Inflammation, angiogenesis	18-7379	Mouse	1:2000	HIER pH 9	Zymed
c-myc	Cell cycle progression, malignant transformation	M3570	Mouse	1/600	HIER pH 19	DakoCytomation

Answer: According to the table, c-myc requires antigen retrieval at pH 19. The pH range is from 1 to 14. pH 19 does not exist.

“pH” is an abbreviation for pondus hydrogenii and means the weight of hydrogen. This term was introduced in 1909 by the Danish biochemist S. P. L. Sørensen who created the original logarithmic definition of pH:

$$\text{pH} = -\log[\text{H}^+]$$

The pH scale is a log scale and was defined as such to replace the less convenient molar concentration scale. The pH scale simply ranges from 0 to 14, and each pH value corresponds to the power of 10 in the molar concentration. A change of one pH unit changes  $[\text{H}^+]$  by a factor of ten. For example,  $[\text{H}^+]$  for a solution with a pH of 1 is 10 times larger than a solution having a pH of 2, 100 times larger than a solution having a pH of 3, and so forth.

2. Sometimes we need to reproduce the staining conditions presented in an article for possible introduction in our laboratory. Would the following snip allow this?

Needle biopsy tissues were fixed in 10% neutral buffered formalin. Multiple 3-um paraffin sections were stained using haematoxylin and eosin. All tissues were also stained using a monoclonal antibody to SV40 large T antigen (Oncogen Research Products, Cambridge, MA, USA), using standard immunoalkaline phosphatase methods preceded by pressure-cook antigen retrieval for 5 min in Ventana retrieval buffer (pH 10.0; Ventana Medical Systems, Tucson, AZ, USA). Diaminobenzidine tetrahydrochloride was used as a chromogen. The monoclonal antibody detects epitopes unique to the PV large T antigen, shared by SV40, BK and JC viruses.

Answer:

NO, this could not be duplicated since DAB is not a chromogen that can be used in a “standard immunoalkaline phosphatase” method. DAB is the most common chromogen used in immunoperoxidase methods.

3. A mathematics problem for you. The following snip was from an article on urine cytology. Would you be able to use the parameters presented to duplicate the centrifugation conditions in your laboratory?

*Urinary cytopathology protocol*

The urine sample collected was the midstream of the first voided urine in the morning. Next, 10 ml of urine was centrifuged at a radius of 155mm for 10 min. The supernatant was removed, and the pellet was cytocentrifuged (cytospin) at a radius of 100.6mm for 6 min. Two smears were subjected to Papanicolaou staining. The whole circular area of

Answer: No

When a suspension is rotated at a certain speed (given as revolutions per minute (rpm)), centrifugal force causes the particles to move radially away from the axis of rotation. The force on the particles (compared to gravity) is called Relative Centrifugal Force (RCF). For example, an RCF of 500G indicates that the centrifugal force applied is 500 times greater than Earth’s gravitational force.

The RCF generated by a rotor depends on the speed of the rotor in revolutions per minute (rpm) and the radius of rotation (i.e. the distance from the axis of rotation). The equations that permit calculation of the RCF from a known rpm and radius of rotation and calculation of the rpm from a known RCF and radius are:

$$\text{RCF} = 11.18 \times r (\text{rpm}/1000)^2$$

$$\text{rpm} = 299.07 \sqrt{(\text{RCF}/r)}$$

where  $r$  = radius in cm.

As you can deduce, the RCF is the important factor in duplicating centrifugation conditions between different centrifuges. If you know the RCF needed and the radius of rotation (the length of the arm)

of your centrifuge, then you can calculate the speed needed. The longer the radius, the higher the G forces applied to a sample. Note that the time of centrifugation is an independent factor.

So, to duplicate the centrifuge conditions you will also need the RCF as well as the period of centrifugation.

## Reference

Henwood A. A survival guide for laboratory professionals. Scotts Valley, CA, USA: Amazon Create Space Independent Publishing Platform; 2019. Chapters 6 and 16.

<https://www.amazon.com.au/Survival-Guide-Laboratory-Professionals/dp/1074504933>



# LABBY AND THE CORONAVIRUS

Due to the nature of our work, most Histology professionals are not able to work from home. So while we are still going to work, we have to keep ourselves, our colleagues, our family and our community safe.

Here are some things Labby has been doing to minimise the potential spread of the SARS-CoV-2 virus in the lab.

## Disinfect surfaces regularly



## Regular hand washing or sanitising



## Wear appropriate PPE for the task



## Stay home when sick



## Practice Social Distancing



# Cutting through the Curve:

## A Histotech's Journey through the COVID-19 Pandemic

By Adrian Ureta & Momoko Sakaki

***This story is based on the collective experiences of Histotechs during the COVID-19 Pandemic***

Free coffee from Macca's! Free coffee for health professionals! Those were my first thoughts before this Covid-19 pandemic started. Just before work, I quickly claimed my flat white with two sugars from the nearest Mcdonald's and headed straight to work.

I parked my car and walked towards the lift. Took a big sip of coffee. The fine aroma and taste of the arabica beans, the soft texture of the micro-foam and a hint of sweetness formed a smooth blend that rushed through my body, giving me a buzz of slight euphoria; I knew this would be a good day.

As I approached the lift, I couldn't help but read the sign that said, "A maximum of 4 people per lift." Baffled by the bizarre sign that was just recently placed, I brushed it off and got in the lift with two others as we all headed straight to work.

Walking into the lab, the change of setting was instant – the beeping sound of auto-stainers endlessly coverslipping slides, the subtle scent of xylene lingering in the air, and colleagues pushing through routine work as the swift clunks of their microtome handles rotated like machine guns while a voice yelled, "is anyone staining?!" You'd think that this was a particularly busy and stressful day but it's just a normal day in the Histology lab.

Before I had a chance to get stuck into my work, we were all called for an impromptu meeting. Gathered in our huge lab, we were told to spread apart from each other as management updated us on the current situation. Silence permeated the room as we listened to them speak with slight hesitation as if they were forced to make these decisions. We were now required to practice social distancing from each other. 1.5 metres apart they said; and going on breaks together were limited to 3 people max. In our minds we thought this was ridiculous. How are we supposed to work together with such distance? Especially when it was almost Easter when doctors push all the work out so they can go on their holiday. But regardless of how difficult the new rules would be to implement, we all knew it made sense. Being the biggest department in the whole building with over a 150 staff, it was our responsibility to be healthy and avoid the spread of the virus in the lab at all costs. Cancer didn't get the message that we were in lockdown. Our work was still essential.

The next day, a black mark in the lift sign was noticeably scribbled with it being written, "A maximum of 2 people per lift". Surprised by this new rule, I entered the lift with another person. As we go up, the lift doors open again on the next floor and another worker attempts to walk in before realising we were there. He steps out and glares at us with annoyance as the doors slowly close. I avert my eyes to the floor, feeling a twinge of guilt for not being able to let him in.

As I walk into the lab, the pace and atmosphere is noticeably slower. The sound of microtome clunks and auto-stainers beeping has dropped. I sit down at my microtome station and glance around at my colleagues who seem so far away. Our stations have been moved to adhere to the 1.5 metre rule. My microtome buddy smiles and dramatically reaches out, "I'll never forget you!" she declares. We laugh, trying to relieve the feelings of general uncertainty and anxiety. The workflow has significantly changed; resulting in us having to take different measures for simple tasks like walking around the lab just to avoid getting close to one another. Training new staff wasn't easy either. We were required to wear extra PPE if we were training just so we can interact in close proximity. These were difficult times.

A scratchy sound coming from the radio and a mumbling voice. The short chrome antenna made an irritable static noise that I had to fix. The sound started to clear up and a gentle voice of a news anchor could be heard speaking with sincerity. Silence in the room as we listened to the announcement: "Elective surgeries will be banned until further notice". Shock and horror spread across the room. By this time, many people in different industries had lost their jobs because of this pandemic. Suddenly, our work which mostly relies on elective surgery started to seem less essential. Frightened from what we just heard, we started to wonder what will happen next?

Still with disbelief, I quickly sent a message to the Mohs Surgeon I work with asking if this ban will affect Mohs surgery. Working in a skin histology lab and a Mohs technician on the side, most of my work relied on elective surgery. It was terrifying as this might severely affect my employment. Anxiously waiting for his reply, a loud buzz sound came from my phone. My heart pounded as I read the text message. "It is still on for now", it said. . I was slightly relieved but still fearful in the back of my mind.

A few days later, I was doing Mohs in a day surgery. The feel of stress and anxiety surrounded the hospital as I walked to the theatres. These were the last few days of elective surgery. Nurses and doctors were panicking and rushing to finish but limited with the number of patients they could bring in because of the social distancing restrictions. It was hell. Worked for 15 hours with only 6 patients to clear. I knew I shouldn't be complaining as many people have lost their jobs. But I was stressed as from next week, we will be the only surgical procedure happening in the hospital. Putting more pressure on me as everyone will be waiting for me to hand in results so we can finish in a timely manner. I also knew the main lab I also work will be flooded with work as surgeons try to push as much work as they can before the ban.

Days after the elective surgery ban, the mood had become a curious mix of calm overlaying a simmering unease. While the flow of work had slowed, a different sort of madness developed – racing to keep up with the daily changes to procedures, the obsessive wiping of surfaces with disinfectant, the anxiety over rumours of job cuts and the sharp looks at anyone who coughed. There were whispers of a potential alcohol shortage. My mind reeled at the thought, mentally checking all the things that needed alcohol: tissue processing, H&E and special stains, reagent preparation, fixation, immunohistochemistry, cryostat decontamination, cleaning of surfaces... The list went on.

One lazy Sunday evening, I was laying on the couch, idly watching a movie when my housemate came bounding out of his room. “The gyms are closing!” he shouted. I sat up and switched the channel to the news. Our faces sunk as we listened to the announcement: gyms, pubs, restaurants and places of worship were being closed.

Driving to work the next day felt like being in an apocalyptic movie. Normally I would wake up early to battle the traffic, but the roads were now eerily quiet. The cafes that would usually be bustling with the morning crowd were almost deserted. A few people hovered around the places serving take away coffee. As I walked into work, I passed the staff cafeteria. Hastily strung black and yellow tape roped off the tables and chairs, almost like a crime scene. I stood for a moment reminiscing about the celebrations, the collective lunches and the hilarious but ill-advised chilli eating dare that happened in this cafeteria. I wondered if things would ever go back to normal.

As the cases of COVID-19 went up, the number of people in the lab dwindled. Some administrative staff and managers began to work from home. Quite a few pathologists also took up the opportunity, lugging home a microscope and only stopping in to collect slides. The lab staff were split into teams and rotated on different shifts in case anyone caught the virus.

We began to settle into a new normal. Every workday we lined up at the entrance, waiting to get our temperature taken. Meetings were either cancelled or moved online. Breaks became a generally solitary affair. We religiously washed or sanitised our hands and meticulously disinfected our stations after each shift.

This morning I went to McDonald’s again for a free coffee, but this time my mood was a bit more subdued. As I pulled into the parking lot at work and took a sip of the coffee, I reflected on the pandemic. I was grateful to the businesses who were supporting healthcare workers. I was grateful that I still had a job. I was grateful for my amazing colleagues who still focused on patient care and giving out a quality section despite the challenges we have all faced. I was grateful I lived in Australia, where the virus was still controlled, and the number of new cases were getting down to single digits. It gave me hope.

As I stepped out of the car, my phone buzzed. It was the Moh’s surgeon. “Elective surgeries are back on next week!”

I smiled and headed into work.

*Adrian Ureta*  
*Technical Officer, Douglass Hanly Moir Pathology*  
*Mohs Technician, Ramsay Healthcare*

*Momoko Sakaki*  
*Technical Officer, Histopathology, The Children’s Hospital at Westmead*



## The Histotechnology Society of NSW is on Facebook!

<https://www.facebook.com/Histotechnology-Society-of-NSW-466949980048716/>

Like the page to keep up-to-date with the latest HSNSW events and to follow the adventures of our mascot, Labby the Histo Lab as he learns and has fun in the world of Histology.

### **What's new?**

The Histotechnology Society of NSW has a **Discussion Group**. Join the group today to ask questions, share your knowledge and connect with your fellow Histotechs all around NSW and other states.



Find us on 



**Stay Connected**