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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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Committee Page

The members of the Histology Group of Victoria and Tasmania 2020 are:

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Adrian Warmington	Dorevitch Pathology (Ballarat)
Mark Bromley	Sullivan Nicolaides Pathology
Elizabeth Baranyai	Cabrini Health
Alison Boyd	Northern Health
Kellie Vukovic	Melbourne Pathology
Yvette Beaber	Monash Health
Samantha Arandelovic	Mater Hospital Brisbane
Snejana Ursache	Alfred Hospital
Imogen Campbell	Alfred Hospital
Sukwinder Sohal(Romy)	University of Tasmania
Meghan Leo	Histolab
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President's Report – Behind the Bench

Welcome to 2022 Bench Buddies,
It certainly seems like we have started another year with more dark clouds hanging over our life's. We haven't had the rain like Queensland, but we still have COVID impacting our lifestyle, we have supply issues and now we have the worrying, daily news from the Ukraine.(on a side note-Apologies for the technical issues we experienced with the first educational meeting of the year – I am blaming the Kremlin) With elective surgery back in full swing, those of us in clinical pathology will be back under the pump. It is difficult to know at any given time, which crisis to spend your time worrying about.
Challenges drive innovation and strength, but sometimes distraction is the best option. With Melbourne opening back up, may I suggest The Lume exhibition, with an accompanying Flying Dutchman cocktail 🍸, to fully immerse yourself in the Van Gogh experience. A festival called Rising, incorporates many artists, and looks like fun, as does a road trip to country Victoria to see the Elvis exhibition in Bendigo.
Be kind to yourselves and find adventures to connect back to family and friends.

Kerrie Scott
(Leica/ Dorevitch Pathology)
HGVT President

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RECOGNISING DR. FRIEDA CARSON

CARSON, Dr. Frieda Born on December 2, 1926, and passed away January 11, 2022. You may not immediately recognize Dr Carson however, her name is synonymous with Histology teaching/education, and you would have probably utilized her book, "Histotechnology: A Self -Instructional Text", in your histo journey.
She graduated from Hearne High School and received her B.S. from the now, Texas Women's University. After graduation, she worked for 9 years as a chemist for the Potash Company of America in Carlsbad, NM. She moved back to Texas and became the supervisor, and later Director, of the Histopathology Laboratory at Baylor Hospital in Dallas. During her 35-year tenure at Baylor, she continued her education and received her MS and PhD degrees from Baylor University. Dr. Carson was Director of one of the first eleven Schools of Histologic Technique to receive national accreditation, and she continued to teach students in that field for all her career. She authored numerous articles in histologic technique and her book, which was first published in 1980; the book is now in its fifth edition. She was active with the American Society for Clinical Pathology, serving both on the Histotechnology Exam Committee and the

Board of Governors. Dr. Carson was among those initiating the Histology Quality Improvement Program, a joint endeavour of the College of American Pathologists and the National Society for Histotechnology. She also presented lectures and workshops both nationally and internationally and was widely respected in her field.

Dr. Carson was recognized as a Distinguished Alumnae by TWU in 1980; received three awards from the National Society for Histotechnology, the JB McCormick, MD award in 1983, the Histotechnologists of the Decade award in 1990, and the Luminary award in 2013; as well as an award by the American Society of Clinical Pathologists; and received the ASCP Mastership Award in 2010. Due to all her contributions in this field, she was honoured by Baylor University

Hospital with having the Histotechnology lab named in her honour on December 15, 2021.
Judy Brincat shared her memories of an insightful women, who taught her about heat artefact and Judy remainder me, we hosted Dr Carson, in here in Victoria, some years ago.
I had the great honour of meeting Dr Carson at the NSH symposium and found her delightful and her depth of knowledge will be sadly missed in the Histology field.

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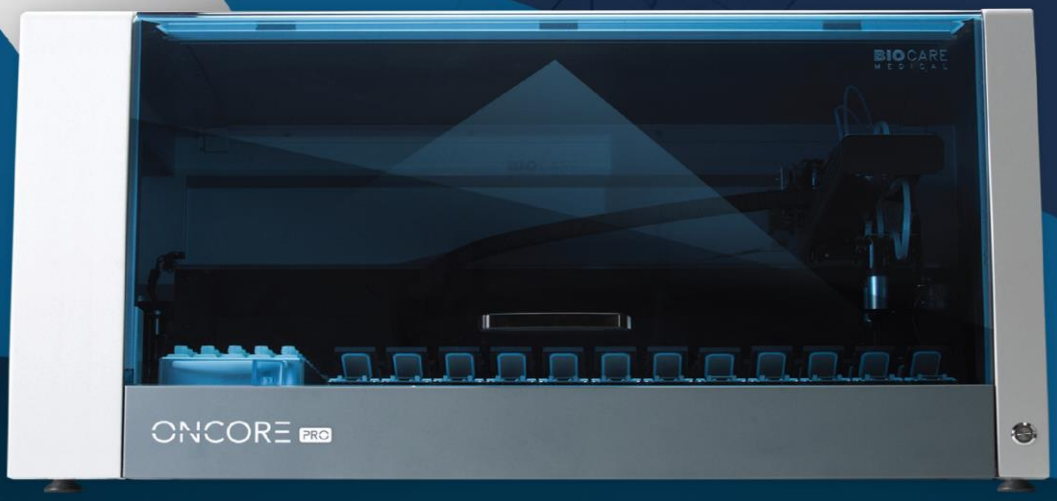

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AUSTRALIA DAY HONOUR 2022

Professor Prithi Bhathal (MBBS, PhD, FRCPA)

Congratulations to Professor Prithi Bhathal from Melbourne Pathology, who was awarded an AM (Member in the General Division of the Order of Australia) on Australia Day 2022 for his outstanding contribution to pathology, education, mentoring and medical research.
Professor Bhathal is one of the leaders in Gastrointestinal (GI) pathology in Australasia. He has a passion for research and has contributed significantly to this field including the teaching of medical students. He achieved his medical degree in 1958, his PhD in 1966 and became a Fellow of the Royal Australasian College of Pathologists in 1970. His name is a familiar one in our industry with over 9500 citations and 176 publications.

Biography

A graduate of the University of Adelaide, Professor Bhathal trained in histopathology at the Royal Melbourne, Royal Children's and Alfred Hospitals. He completed his PhD at the University of Melbourne's Department of Pathology and followed this with appointments as senior lecturer in Pathology at the University of Queensland and Reader in Pathology at the University of Melbourne.
From 1978 to 1999 he was Professor/Director of Anatomical Pathology at the Royal Melbourne Hospital. In addition to his role as Histopathologist at Melbourne Pathology he continues to teach and research part-time in the Department of Pathology, University of Melbourne, where he is an Honorary Professorial Fellow.

Professor Bhathal has an international reputation in hepatic and gastrointestinal pathology, and was presented the Distinguished Pathologist Award of the International Academy of Pathology in June 2007, and the Distinguished Research Prize of the Gastroenterological Society of Australia in October 2007. He was awarded the Distinguished Pathologist Award of the Australasian Gastrointestinal Pathology Society in October 2019 and was inducted as a Fellow of the American Gastroenterological Association (AGAF) in February 2021.

Academic commitments

Honorary Professorial Fellow at Department of Pathology, University of Melbourne

Awards

Distinguished Pathologist Award of the International Academy of Pathology - June 2007
Distinguished Research Prize of the Gastroenterological Society of Australia - October 2007
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Under the Microscope with Linda Lu

1. What was your first part-time job?

I worked at Sumo salad as a cashier and making salads.

2. How long have you worked in histology?

Between Histology and Cytology, it'll be 10 years this year, how time flies.

3. When people ask, "So, what do you do?" How do you explain Histology?

I generally say something like "When people have surgeries to remove tumours or say their appendix, it comes to us! We describe it, we sample it, put it onto a slide and give it colour so it can be seen under the microscope."

Japan, Nara Prefecture – Nara Park

4. What is a skill you'd like to learn and why?

At the moment its crocheting, actually kind of started. It's pretty relaxing.

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

I think travelling the world doesn't seem like a bad idea. I would probably also be buying more plants. Maybe hire a team of designers to create a cover-slipping machine that will NEVER crash.

6. What's an ideal weekend for you?

A clear schedule; then doing whatever comes to mind.

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

Volley ball, pair of ice skates, a FedEx parcel – he survived.

8. What is the best conference you have ever attended?

Pathology Horizons New Zealand – great content, all whilst with a backdrop of crystal-clear lakes and mountains dusted with snow.

9. What's on your bucket list this year?

Haven't really thought about, probably another year of "play it by ear". Managed it make it to Harry Potter (finally) and Moulin Rouge last year; which by the way I highly recommend.

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

To the Northern hemisphere, where I could see the aurora borealis.

IHC ANTIBODY SPOTLIGHT:

Calponin (Basic)

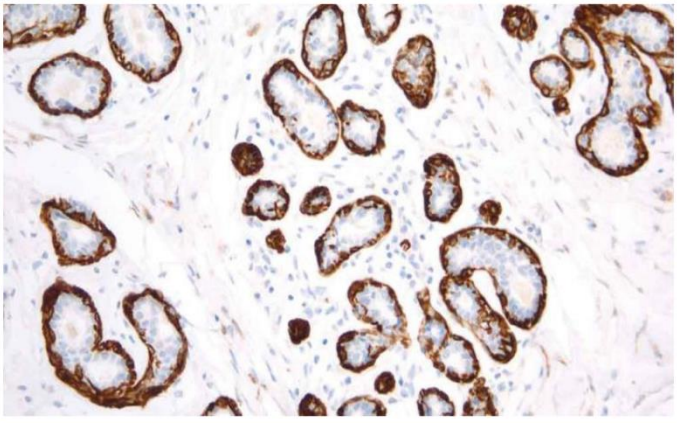
Basic calponin (calponin-h1) is an actin, tropomyosin and calmodulin binding protein thought to be involved in the regulation of smooth muscle contraction.

There are three calponin isoforms: the basic (CNN1), neutral (CNN), and acidic (CNN3) isoforms, all separate gene products. Basic calponin is a selective biomarker for differentiated smooth muscle whereas the neutral and acidic isoforms are more widely expressed. (Vetterkind and Morgan, 2012)

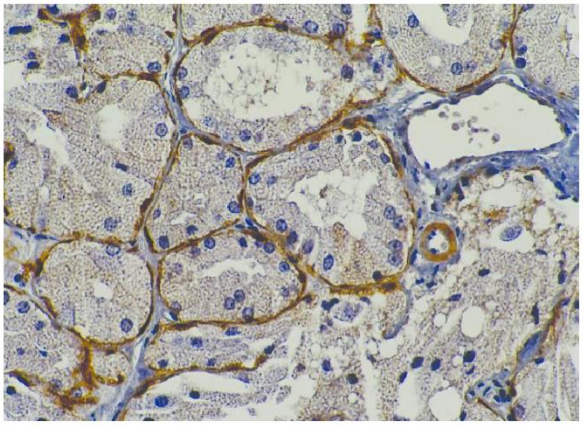
Basic calponin expression is reported to be restricted to smooth muscle cells and is a marker of the differentiated contractile phenotype of developing smooth muscle. Vascular smooth muscle cells convert to a synthetic dedifferentiated phenotype when this protein is lost and this is a key stage in both atherosclerosis and restenosis of coronary arteries after balloon angioplasty. (Calponin (Basic) - IHC Primary Antibodies, 2022)

Basic calponin mRNA is expressed in smooth muscle of prostate, bowel and aorta, whereas neutral and acidic calponin mRNAs are expressed in non-smooth muscle tissues such as heart, placenta, lung, kidney, pancreas, spleen, testis and ovary as well as in smooth muscle-containing tissues.

For further papers discussing Calponin refer to this link: https://www.sciencedirect.com/topics/medicine-and-dentistry/calponin



Human prostate: immunohistochemical staining for Calponin (Basic). Note staining of basal cells. Calponin (Basic): clone 26A11 (Calponin (Basic) - IHC Primary Antibodies, 2022)



Immunohistochemical staining of calponin on myoepithelial cells (V30, original magnification 400X)

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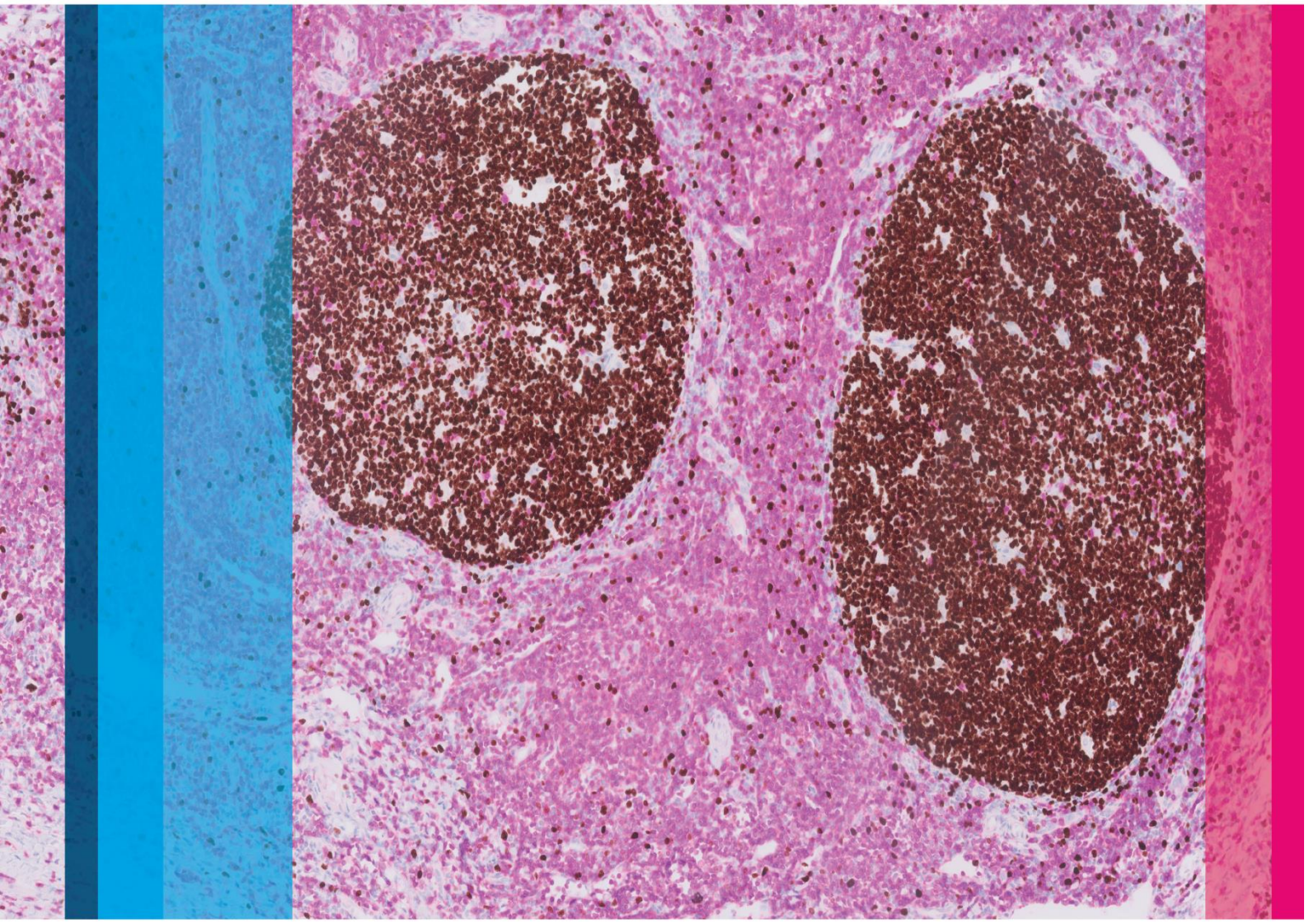

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Image Caption: Tonsil, sequential staining of Ki-67 (DAB, nuclei) in germinal centers and, CD3 (HRP Magenta, cytoplasm and membranes of T cells) in the mantle zone.

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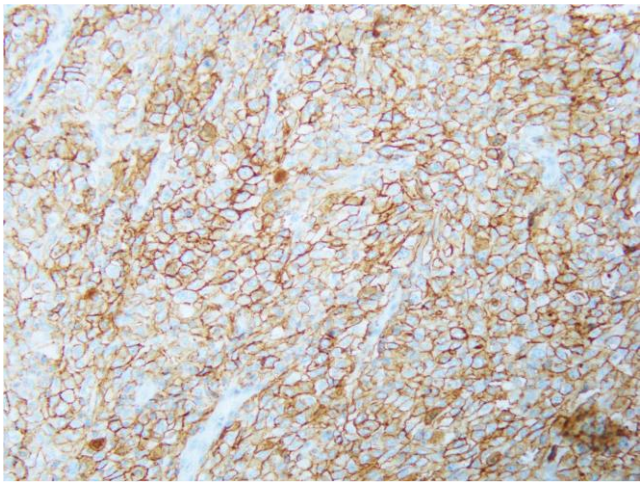
Challenges of Procuring Control Material for Immunohistochemistry

BY KITTY FENG

Immunohistochemical (IHC) validation has many challenges, from choosing the antibody clone to optimizing the staining quality. Yet this process cannot truly begin without a suitable control material to test on. This alone proves to be quite the challenge, as discussed in the review article "Immunohistochemical Validation of Rare Tissues and Antigens with Low Frequency of Occurrence: Recommendations from the Anatomic Pathology Patient Interest Association (APPIA)" by Robert Lott et al. The article highlights the issues laboratories face in procuring control material in conjunction with the recommendations and guidelines provided by the College of American Pathology (CAP), as well as providing some solutions to the problem. This review will discuss the issues and solutions the article introduced, and will compare them to the practical setting of a diagnostic laboratory.

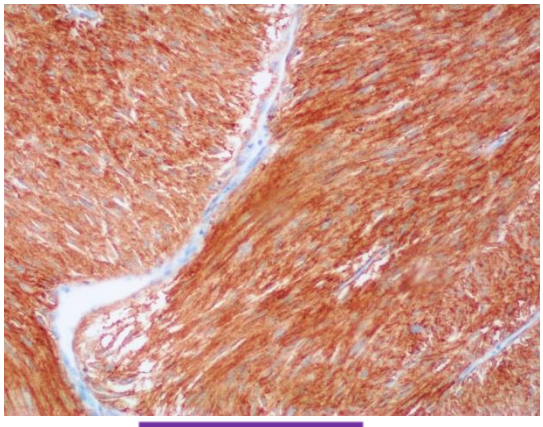


PD-L1 stain (10x)

Sourcing control material is challenging on its own, but proves to be particularly difficult with low frequency occurring biomarkers. The article provided some examples of these, including ALK, BRAF, TLE-1, INI-1, myogenin, Myo-D1, ckit, DOG- 1 and PDL1. In the case of ALK positive lymphomas, this rare type of anaplastic large cell lymphomas are only found in 2-3% of all adult lymphoma diagnosis and 10-30% of all paediatric lymphoma diagnosis. Within these cases, only 50-60% stain positive in ALK immunohistochemistry. Only 4-5% of all non-small cell lung cancers contain the ALK gene rearrangements. The BRAF V600E activating mutation is present in approximately 7% of all solid tumours, and varies from 1-3% in lung cancers to 45% in papillary thyroid carcinomas. TLE-2 is a marker for synovial sarcoma that has an estimated occurrence of only 900 cases a year. INI-1 is a biomarker for malignant rhabdoid tumours and wilms tumour, which has low incidences of approximately 0.19 per million for renal tumours, 0.89 per million for atypical/rhabdoid tumour, and 0.32 per million for tumour of other sites. Occurrence of Wilms tumour is also low, with only 650 cases diagnosed per year. Myogenin and Myo-D1 are both biomarkers used to identify rhabdomyosarcoma, a cancer that only occurs in 4.5 cases per million children and adolescents per year. DOG-1 and ckit are sensitive biomarkers for gastrointestinal stromal tumour, which only occurs in more than 1% of all gastrointestinal tumours and around 4000-5000 cases diagnosed per year.

Lastly, PD-L1 is positively expressed in 13-70% of lung cancer, within those cases only a small number meeting certain percent-positive cut off. It is important to note that these statistics are from North American research, although it does give an idea of the infrequency of these cancer types and biomarker presence across the general population.

The article discussed some of the recommendation and guidelines for IHC test verification and validation released by the College of American Pathologists (CAP). A particular focus was on the number of positive and negative cases/controls that the new antibody needs to be tested on. The CAP guidelines recommend a minimum of 10 positive and 10 negative cases for non-predictive tests, and double that for predictive tests. Verification on protocols that alter a FDA-approved predictive assay require no less than 40 positive and 40 negative cases to be tested on. Note that predictive tests are assays that can have an effect on treatment (eg. PD-L1, HER2) while non-predictive tests are assays for diagnostic purposes (eg. Ki67, TTF-1). Unsurprisingly, statistics from a CAP sponsored survey on IHC testing laboratories showed that most laboratory validations were not meeting those case number requirements. This survey conducted on 714 laboratories showed that while 75.4% of the laboratories fulfil the 10 positive and 10 negative case testing recommendation for non-predictive markers, only 45.9% of those laboratories fulfil the case number testing recommendation for predictive markers. The survey also demonstrated that more than 50% of laboratories used less than 25 cases to validate predictive tests, much less than the recommended 40 cases (20 positive and 20 negative). In the 75% of laboratories that report having recently validated a new antibody, the median number of cases tested on was only 15. Lack of availability of control material, as well as low time and personnel resources are suggested to be reasons for the laboratories inability to meet CAP guidelines. The CAP recognise the challenges of fulfilling these guidelines especially with low occurrence biomarkers, and address this by stating that the extent of the validation is at the discretion of the medical director. Therefore, laboratories can validate with less than the recommended case numbers and still be within compliance, so long as the rationale of the decision is documented.



DOG-1 stain

Antibody validation within an Australian diagnostic laboratory must comply with NPAAC requirements in order to maintain NATA accreditation. As stated in NATA document *General Accreditation Criteria: ISO 15189 Standard Application Document,* new examination procedures need to be verified by evaluating the clinical sensitivity and specificity of such procedure in a local population such as a hospital when possible. NPAAC document *Requirements Quality Control, External Quality Assurance and Method Evaluation* expands on this topic with an emphasis on documentation. NPAAC requirements include documentation for the introduction of the new test, the results of the validation/verification must be approved as fit for purpose, concordance study with an existing validated method, and identification of sources of uncertainty of measurement.

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Challenges of Procuring Control Material for Immunohistochemistry **Continued…**

Unlike the CAP guidelines previously discussed, NPAAC does not give a testing case number threshold to fulfil. Rather, the laboratory has to provide documentation fulfilling the requirements mentioned previously. Validation using in-house cases is also preferable. In practice, IHC validation involves and requires approval from all relevant personnel, usually consisting of the principal scientist, pathologist and scientist performing the validation. Another important requirement is the continuous quality control of IHC assays by using and checking of control material on all IHC slides, and participation in RCPA quality assurance program.

Some solutions for low frequency biomarker validation have been proposed by the CAP, such as retrospective search, purchasing of control material, external quality assessment participation and literature search. Retrospective search into older cases within the institute/hospital is one of the most common and routine methods of sourcing control material. This method is also most ideal as the control material will have the same fixative and processing methods as the test tissue. Purchasing of control material from qualified vendors is also an option, with some companies able to produce engineered cell lines with gene-editing platforms to produce a cell button similar to a cell block. However, most diagnostic laboratories are unwilling to purchase control material as it can be costly. External quality assessment can assist laboratories in re-evaluating the quality of already validated IHC assays. In Australia, the Royal college of Pathologists of Australasia runs the quality assurance program (QAP) that diagnostic laboratories participate in. However, participation of the QAP does not help with the initial validation of a new antibody, nor assist in sourcing control material. Lastly, literature search was suggested to help in finding the expected staining results, but once again does not solve the lack of control material.

Two methods for sourcing control material that are commonly used in diagnostic laboratories but not mentioned include using remaining specimens that are to be discarded, and working with other institutes/hospitals to source material that cannot be found in house. Tissue not sampled during cut up can be retained for control use at the point of discard. This method requires foresight; optimal control material can be noticed and noted down by scientists checking and sending out immunohistochemistry, or by pathologists when reporting immunohistochemistry. Specimens deemed usable for control material can be marked, therefore the staff performing tissue discard can leave the specimen aside for control use. It is also not uncommon for laboratories to share control material between another laboratories. Laboratories can ask for control tissue from a larger laboratory with more archival material, or from a more specialized institute such as a children's hospital. Forming a strong network with other institutes and hospitals by sharing control material can benefit all laboratories involved.

Control material for immunohistochemistry validation can be challenging to procure for some laboratories, especially in validating low frequency biomarkers. These challenges are highlighted by Robert et al in "Immunohistochemical Validation of Rare Tissues and Antigens with Low Frequency of Occurrence: Recommendations from the Anatomic Pathology Patient Interest Association (APPIA)". Unsurprisingly, survey conducted by the CAP showed that most laboratories do not fulfill the recommended case number to be tested during IHC validation.

Lack of control material, as well as insufficient staffing and time are attributed for not meeting the CAP recommendations. The review article mentioned solutions such as retrospective search, purchasing of control material, external quality assessment participation and literature search. In practice, laboratories can also source control material in excess tissue intended for discard, and by sharing control material with other laboratories. Hopefully, these solutions can assist laboratories in sourcing control tissues for IHC validation.

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L E I C A B I O S Y S T E M S

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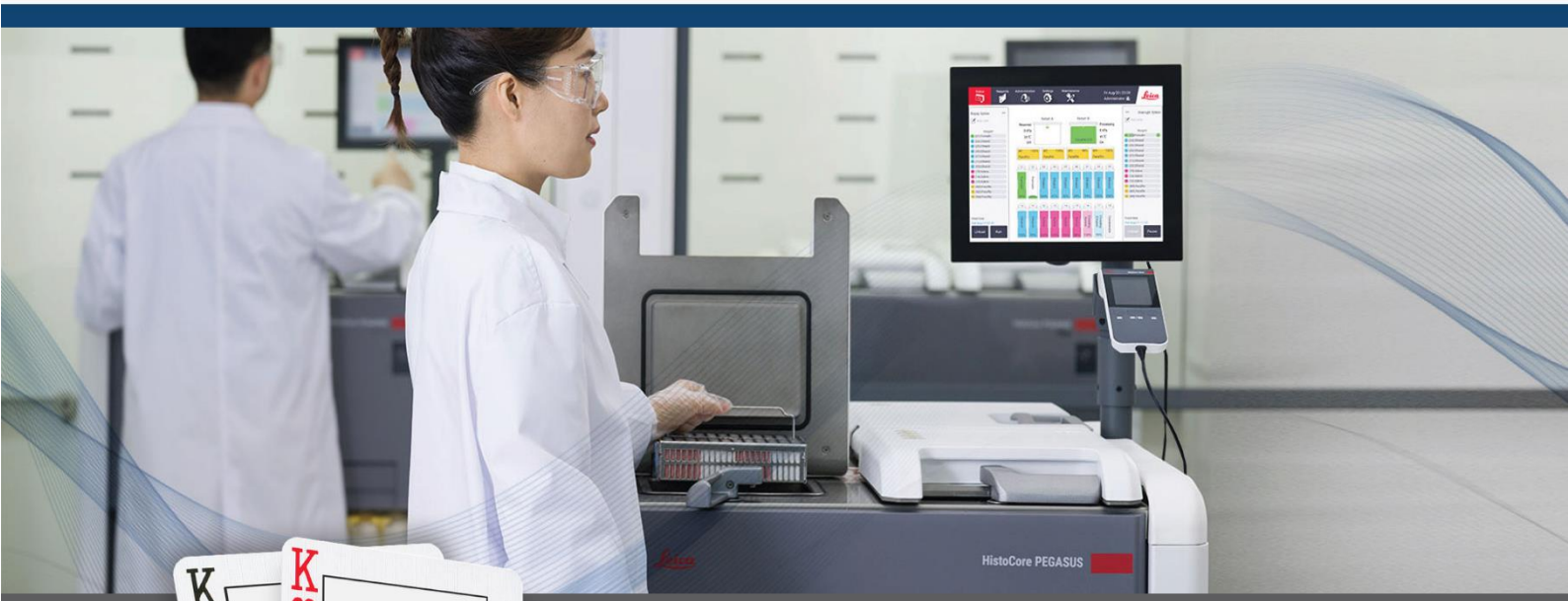

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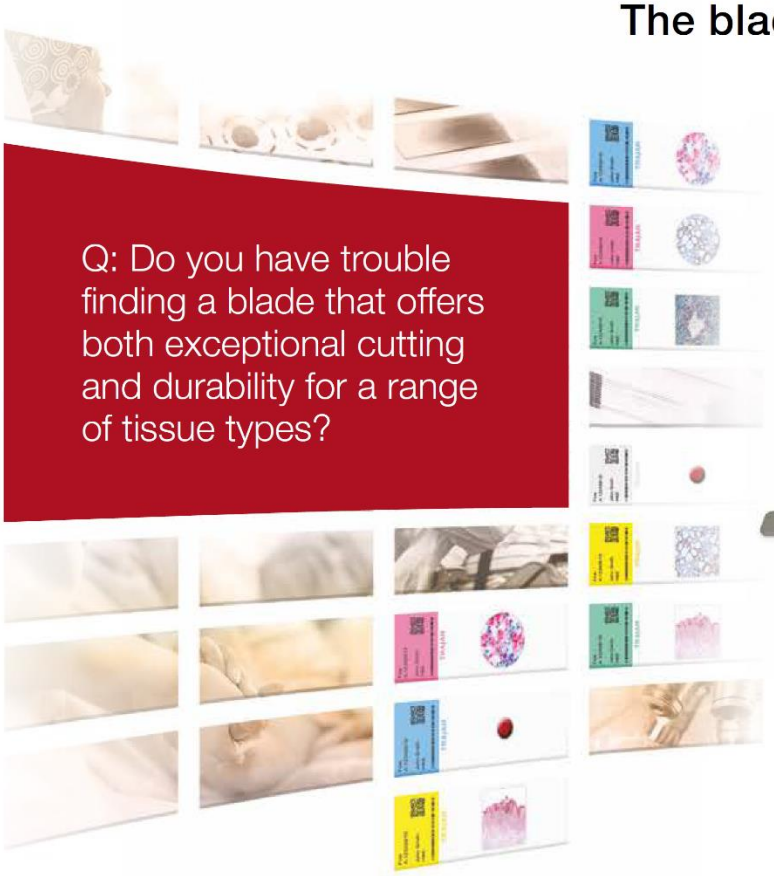

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A: Designed for durability and precision, Trajan microtome blades are the blade of choice for any type of tissue.

Trajan microtome blades offer exceptional cutting and longevity for a range of tissue specimens.

A unique coating offers exceptional durability while enabling smooth and precise tissue sectioning for both hard tissue and bone samples in general paraffin.

They are compatible for use with most microtomes and cryostats, and are available in low and high profile.

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Performing Histological Procedures in An Educational Laboratory

BY CRISTINA GEORGE

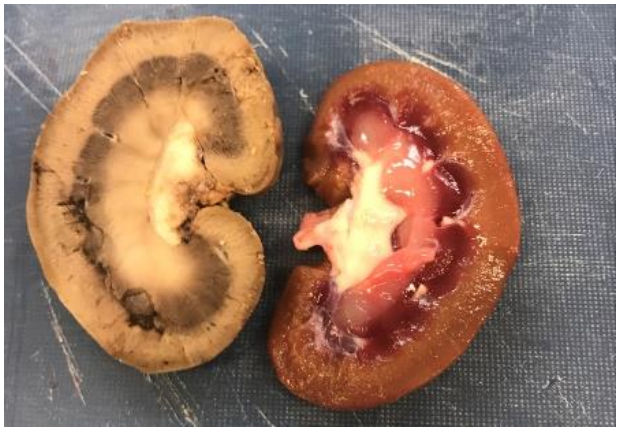
Holmesglen is a leading Australian provider of vocational and higher education and one of the largest government-owned TAFEs in the state of Victoria. Since its opening in 1982, Holmesglen has provided education and training that enables learners to be work, life and world ready.

Delivered at the Moorabbin campus in a specialized pathology and biotechnology education facility, the MSL40118 Certificate IV in Laboratory Techniques emphasises practical training. The instrumentation is reflective of what is being used in industry and prepares students to start a career in a laboratory.

One of the course's elective units, *MSL973020 Perform histological procedures*, aims at developing the important skills and knowledge to perform straightforward histological procedures involving processing and sectioning (by hand or rotary microtome) of plant and/or animal tissues in paraffin wax. The unit runs for 17 weeks, of four-hour classes, in semester two of the course.

Students are also introduced to the notion of "*cut up*" in the first weeks of the unit. During this training, they receive fresh organs with request forms, which they need to record in a simulated LIMS (using Google forms). The *MSL954003 Relate anatomical and physiological features to laboratory samples* unit, which is also delivered in the same semester, correlates with the delivery and application of histology.

In both units, we use fresh animal organs from different body systems, and, before dissection and grossing, students take basic measurements (dimensions and weight), describe the macroscopic appearance (internal and external) general appearance, contour, colour, texture, lesions, and distribution (if any). They also must also be able to identify if the organs are fresh or fixed.

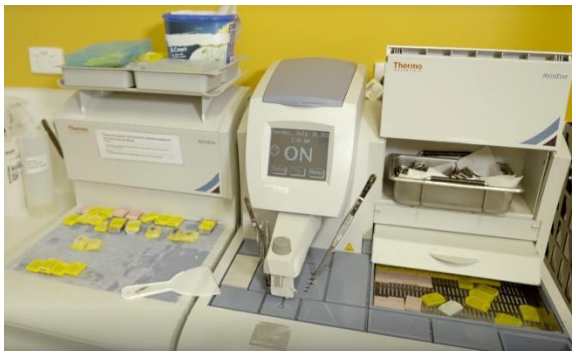


The "*cut up*" exercise continues with transferring the tissue to cassettes, on which students have their personal code with three identifiers – name initials, organs/species, and a third identifier.

The unit requires students to process the tissue, after pre-use checks on the instrument (deterioration and adequate volume of reagents, and/or any items requiring replacement or errors signaled).

We use Shandon Excelsior ES on which a routine overnight and a rapid biopsies program have been set up, for our most common used sample types.
In each class, students inspect the instrument, keeping a record in their manuals. This inspection of the instrument is part of assessments where each student must inspect the reagents of the tissue processor and load samples and select a program (processing, or flush) at least twice during the semester.

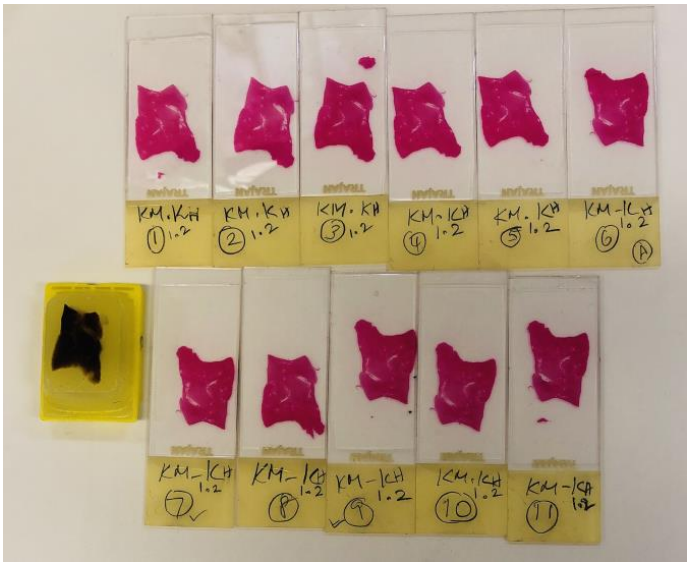
For embedding and microtomy of their own samples, students take turns using the HistoStar embedding centre. They ensure the correct orientation of samples but also minimise cross contamination of samples and cut sections using the Shandon Finesses 325 microtome.



Throughout the semester and prior to their microtomy assessments, students experience cutting a wide range of tissues like kidney, lung, trachea, liver, biopsies, ovary, spleen, testis, and heart.
During the course, we emphasise traceability of blocks vs. their slides, cross contamination in between tissue and students, troubleshooting difficult blocks and quality of sections (quality and quantity of slides is achieved mid to end of semester). After microtomy, all slides are recorded in a student's manual and each learner macroscopically self-assesses the quality of their slides.
At the beginning of semester, students practice H&E staining on slides from previous cohorts and then, as they practice and improve their microtomy skills, they stain and coverslip their own slides.
The assessments provide learners with opportunities to demonstrate they have attained the required skills and knowledge; using a range of practically based activities and tasks, as required by recognised standards:

- Assessment task 1 is a work health and safety knowledge task and was design to make students aware of the hazardous nature of histology (compared with their other units)
- Assessment task 2 is an observational checklist on tissue processor inspection and embedding, and student need to satisfactory perform the practical tasks twice
- Assessment task 3 is an observational checklist and knowledge on microtomy. Each time they are assessed, learners need to safely use the microtome to section and mount 5µm sections (minimum three slides) from three different tissue types, sections that are macroscopically free of wrinkles, scores and folds or other microtomy artefacts. In 2021, we chose to focus on animal
  tissues as they're more representative of the industry in which students will work (hand cutting plant tissue is performed in their Biology unit).

- Assessment task 4 is an observational checklist, knowledge, and project on H&E stain. In the practical task, students must stain and coverslip at least a full rack (24 slides), which are then checked by the assessor on the quality of stain and cover slipping, to ensure expected staining outcomes have been achieved. The project part asks the students to choose two organs and, using a camera microscope to take pictures, insert and identify five structural elements of the tissue/organ type and two microscopic artefacts. For the artefact, students must also explain why they appeared and how it could be rectified next time.



Learning histology – a rewarding experience for students, teacher and industry!
Students enjoy this unit. They gain invaluable hands-on experience and practice, which will support them in a workplace; should they pursue a job in Histology.
General skills in WH&S, sample preparation and processing, sample quality observations, reproducible techniques, microscopy, problem solving, and teamwork are all consolidated in this unit, which are transferable skills to many applications.

JOB ADVERTISEMENTS

MEDICAL SCIENTIST GRADE 2

ANATOMICAL PATHOLOGY

Histology/Cytology

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A senior position within Anatomical Pathology at Hobart Pathology to assist the Head of

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A minimum of 5 years recent laboratory experience as a Medical Scientist within an Anatomical

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Applications to Pip Rose, Human Resources Manager at Pip.Rose@dspl.com.au.

For further information please contact Susie Green, Head of Department, Anatomical Pathology (03) 6237 1231.

Closing date 14th March, 2022.

Future Events: 2022

Org. No. A0035235F

Date: 21st April, 2022

Scientific Meeting

Topic: Lung Cut-Up

Venue: Zoom Meeting (streamed and recorded)

Date: 16th June, 2022

Scientific Meeting

Topic: TBA

Venue: Zoom Meeting (streamed and recorded)

Date: August, 2022

HGVT Trivia

Topic: TBA

Venue: Zoom Meeting (streamed and recorded)

Date: 15th September, 2022

Scientific Meeting

Topic: TBA

Venue: Zoom Meeting (streamed and recorded)

Date: 27th October, 2022

HGVT AGM

Topic: TBA

Venue: Zoom Meeting (streamed and recorded)