HGVT

Org. No. A003523F

PARAFFINALIA NEWSLETTER

VOLUME 26, NUMBER 4
December 2021

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.

CONTENTS:

Committee Page

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

Name	Institution
Kerrie Scott-Dowell	Dorevitch Pathology/Leica
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
Bindi Bates	Leica Biosystems
Kitty Feng	VCS
Yashi Xie	St. Vincent's Hospital
Cristina George	Holmesglen TAFE
Linda Lu	Royal Melbourne Hospital
Alex Johnston	Leica Biosystems

Contact us at
http://www.hgvt.org.au/contact-us
Membership enquiries: membership@hgvt.org.au
General enquiries: secretary@hgvt.org.au
Sponsorship/Advertising enquiries: trade@hgvt.org.au
Newsletter enquiries: editor@hgvt.org.au

Advertising for the next edition of Paraffinalia closes: **26th February, 2022**

Paraffinalia Rates:

A4 Electonically Submitted **$400 (no GST) Flat Rate Per page**
$1200 yearly package
Positions Vacant- No Logo up to 75 words
FREE to email membership
FREE list on Website
Used Equipment- 50 words – no logos/no pictures
FREE
A4 with logo $200 (no GST)

Articles & Reports:
Author enquiries and readers wishing to contribute articles or reports can contact the Editor - editor@hgvt.org.au

Please email articles (preferably Microsoft Word format) for inclusion in the next edition to editor@hgvt.org.au All items submitted for publication will then become the sole property of the Histology Group of Victoria Inc.

Disclaimer:
Any opinions expressed in this publication are solely those of the contributing author and are not necessarily reflective of the Histology Group of Victoria Incorporated or the editor.

NOTE: No responsibility is assumed by the Histology Group of Victoria Incorporated for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. It is the user's responsibility to ensure that all procedures are carried out according to appropriate Health and Safety requirements.

President's Report – Behind the Bench

Season's Greetings Histo-Lovers,
The Committee has been busy all year delivering a diverse educational program and I would like to take the opportunity to thank all those people who have shared their knowledge and to all my wonderful Committee members for their support. Congratulations to those on the Committee who have found time to have a baby (Emma and Kellie) and get engaged (Sam and Mark), perhaps despite the pandemic or maybe because of the pandemic.
We continue to be impacted by corona and will hopefully be able to get some face-to-face sessions later in 2022. I will leave you with a little Christmas poem:

T 'was the month before Christmas and all through the lab,
Every scientist was stirring, who had gotten their jab,
The specimens were covered in formalin with care,
In hopes that fixation soon would be there.
The patients all nestled, all snug in their beds,
Visions of recovery danced in their heads
Blocks on the cold plate, and blocks in the chuck,
The microtomy will be easy, with any luck.
When out of their room, there came such a clatter
I jumped from my chair, to see what is the matter
Where are my slides and what is the reason?
My workload is so high, it is the Christmas Season

Kerrie Scott
(Leica/ Dorevitch Pathology)
HGVT President

EXCLUSIVE
Validated for chemical/ biological hazards*

eGROSS pro-x

Simply the most innovative, safe grossing workstation, built with user & patient safety in mind

- Designed with safety in formalin handling
- Ergonomic design – allows for standing or sitting
- EU certified biohazardous containment working area
- High powered extraction with down & backdraft

*The eGROSS pro-x is tested according to the European Norms UNI EN 12469, and satisfies the essential requirements for bio-safety aspects in the working area.

MILESTONE
HELPING PATIENTS

Distributed by Abacus dx - Contact Mick for a quote
Mick van Werkhoven
Territory Manager- VIC & TAS
0499 400 601 | m.vanwerkhoven@abacusdx.com | www.abacusdx.com

abacus dx

High-Performing Mismatch Repair FLEX RTUs for Autostainer Link 48

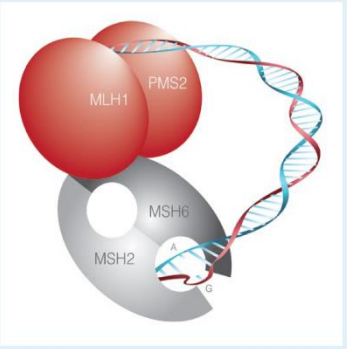
The Dako MMR FLEX RTUs for Autostainer Link48 are well accepted in the market and show strong performance in EQA schemes

Agilent
Dako

NordiQC performance: Dako FLEX Ready-to-Use (RTU) antibodies*

		Pass rate using vendor recommended protocol	Number of labs using Dako RTU
MSH2, clone FE11	Run 57, 2019	100%	66
MLH1, clone ES05	Run 56, 2019	100%	35
PMS2, clone EP51	Run 53, 2018	100%	41
MSH6, clone EP49	Run 52, 2018	100%	43

*EQA data based on Autostainer RTU data.



MMR and DNA string showing how the MMR proteins work in pairs.

Loss of one or more mismatch repair proteins is often associated with the loss of a coupled protein. MLH1 and PMS2 loss are often paired. MSH2 and MSH6 loss are often paired.

Learn more about Autostainer link solutions for IHC:
www.agilent.com/en/product/autostainer-link-solution-for-ihc

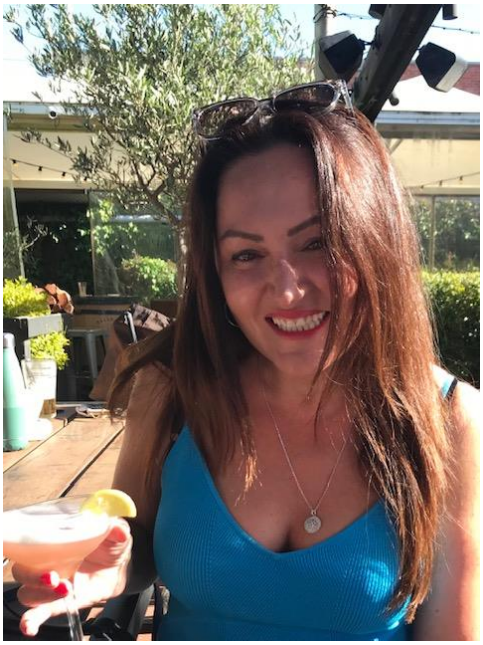
Ordering Details

Target	Product	Package Size	Part Number
MLH1	FLEX Monoclonal Mouse Anti-Human MLH1, Clone ES05, Ready-to-Use (Autostainer Link 48)	12 mL, 60 tests	IR07961-2
MSH2	FLEX Monoclonal Mouse Anti-Human MSH2, Clone FE11, Ready-to-Use (Autostainer Link 48)	12 mL, 60 tests	IR08561-2
MSH6	FLEX Monoclonal Rabbit Anti-Human MSH6, Clone EP49, Ready-to-Use (Autostainer Link 48)	12 mL, 60 tests	IR08661-2
PMS2	FLEX Monoclonal Rabbit Anti-Human PMS2, Clone EP51, Ready-to-Use (Autostainer Link 48)	12 mL, 60 tests	IR08761-2

This information is subject to change without notice.
D68764/01

Agilent
Trusted Answers

Under the Microscope with Effie Cozijnsen



What was your first part-time job?
My first part-time job was as a cashier at Red Rooster when I was 16.
There were lots of young kids working there so it was very social.
The cheesy nuggets were my favourite item on the menu.

How long have you worked in histology?
I have been working in Histology for over 20 years.
Having worked in a few different labs, I have gained varied perspectives on histology processes which has been very useful.
I have been with Peter Mac for the last 3 years. I'm extremely proud to be part of the team. Staff are exceptionally talented, the quality of the work is of a very high standard, and there are many opportunities for professional development.
I also have a keen interest in Health Safety & Wellbeing in the workplace, of which Peter Mac values and promotes.

When people ask, "So, what do you do?" How do you explain Histology?
I try to simplify it as much as possible and give examples of the type of tissue specimens we receive. I would tell them that we cut very thin sections and stain them so we can look for disease in the cells under a microscope.

What is a skill you'd like to learn and why?
A histology skill I would like to learn would be complex cut-up at Peter Mac. Our Pathologist Assistant, Courtney Saville, was trained in the US and has a wealth of experience handling a variety of complex specimens ranging from prostate and neck dissections up to mastectomies, colon resections and complex head and neck resections. Courtney operates the cut-up room and heads all the training for scientists and registrars at Peter Mac.
A personal skill I would like to master is playing the guitar, as music is one of my passions. It would also impress my teenage girls.

If money was no object, what would you do all day?
Spend most of my time outdoors in nature and travel to all the breathtaking places around the world.
I would also volunteer in programs that support disadvantaged young people as it's a cause I feel strongly about.

What's an ideal weekend for you?
A sleep in, a dog walk on the beach, a strong coffee, and a massage.
It's the simple things in life that are the most EXTRAordinary.

If you could take only THREE items with you to a deserted island, what would they be?
My dog Trixie
Coffee
A masseur

What's on your bucket list this year?
Catching up with my friends and family now that we are out of lockdown.

Where do you most want to travel, but have never been?
South Africa. I would love to go on safari in Kruger National Park and see the Big Five.

CHEERS AND BEST WISHES FOR THE HOLIDAYS TO MY FELLOW HISTOLOGISTS

DAKEWE

Laser Cassette Printer
SurePrint C100

* High efficiency, high throughput and stability.
* Clear and smooth printing, customized templates.
* Suitable with majority brand embedding cassettes.
* Patent RGB technology to recognize cassettes color.
* Fully compatible with LIS/HIS system.

Laser Slide Printer
SurePrint S200

* Printing resolution up to 2500 dpi.
* Double loading station loading up to 200 slides.
* Two work modes: Single ouput and batch output slides.
* Innovative top push method effectively avoid slides damage.
* Fully compatible with LIS/HIS system.

Tissue Processor
HP300

* Access to connect Cloud Platform, real-time monitoring.
* Electronic locking system, special teflon coating retort lid.
* Components level quality control, light indicator showing working status.
* Patent fluid control system distributing reagents in an efficient way, solve the problem of tube blocking.
* Reagent & Paraffin rotation function, paraffin easy loading and auto supply.

Cryostat Microtome
6250

* Good Sectioning Quality: High precision guide rails make transmission smooth, durable and capable of multiple tasks.
* Freeze 20 tissues at the same time.
* Semi-conductor rapid cooling peltier stations.
* The UV lamp allows to disinfect itself scheduled or manually.
* Smart temprature control system provides an ideal environment for the specimens.

Combo System
Automatic Staining and Coverslipping
DP360+CS500

* Intuitive and easy to use software, 100+ programs memory.
* Precise staining time for each step, quick robotic arm to achieve 1s differentiation.
* Unique AAT function delivers a perfect consistency on staining results while making the best of reagents.
* Reagent water-bath heating station to keep reagents in an active temperature.
* Reagent quality control & tracking system, recording & reminding reagents consuming status.
* Fully closure design and smart exhaust system to provide a healthy working environment.
* Easy connection combo system to achieve walk-away staining and coverslipping.

Authorized Agent:

DAKEWE

Dakewe (Shenzhen) Medical Equipment Co., Ltd.
www.dakewemedical.com

HURST SCIENTIFIC
2/36 Hensbrook Loop Forrestdale WA 6112
P: 1300 778 068 E: sales@hurstscientific.com.au
www.hurstscientific.com.au

Advancing Cancer Diagnostics
Improving Lives

Leica BIOSYSTEMS

HistoCore SPECTRA Workstation
Routine and Special Staining

Efficiency • Flexibility • Confidence

Contact Leica Biosystems for more information: (Aust) 1800 625 286 or (NZ) 0800 400 589

LeicaBiosystems.com

181208 Rev A 9/2018

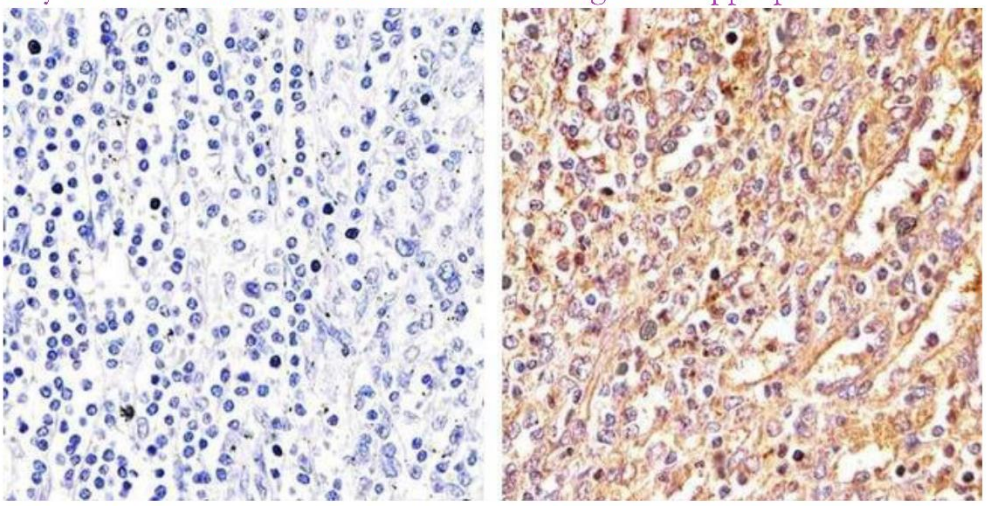
IHC ANTIBODY SPOTLIGHT:

Interleukin 6

Interleukin 6 is a multifunctional cytokine, secreted by lymphoid and non-lymphoid cells. Its production can be found in fibroblasts, activated T cells, activated monocytes or macrophages and endothelial cells. The cytokine has key roles in immune responses and hematopoiesis. [1] In addition, it is important for cellular proliferation and differentiation Primarily produced at sites of acute and chronic inflammation, IL-6 is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. [2] The functioning of IL-6 is implicated in a wide variety of inflammation-associated disease states including diabetes mellitus and systemic juvenile rheumatoid arthritis.

It is possible important role as an autocrine growth factor in metastatic prostate cancer. [3] Furthermore, there has been an increasing number of reports that cytokines of the IL-6 family play an important regulatory role in heart physiology.[4]

IL-6 has a crucial role in cellular homeostasis under normal conditions. On the other hand, conditions that involve inflammation cause the concentration of IL-6 increase markedly. As such, it plays a role clinically, being a major alarm signal in humans in response to infections (sepsis/septicemia), inflammation, autoimmunity, and cancer. Overall, IL-6 cytokine is nonspecific biomarker of systemic inflammation that may have relevance in clinical decision making in the appropriate context. [5]



Cytoplasmic staining of spleen cells (right) compared to negative control (left) that does not include application of the primary antibody [1]

References

1. *Interleukin 6 - IHC Primary Antibodies.* [online] Available at: <https://shop.leicabiosystems.com/us/ihc-ish/ihc-primary-antibodies/pid-interleukin-6> [Accessed 28 November 2021].
2. IL-6 Antibodies [Internet]. Thermofisher Scientific. 2021 [cited 28 November 2021]. Available from: https://www.thermofisher.com/antibody/primary/target/il-6?ef_id=Cj0KCQiA7oyNBhDiARIsADtGRZY1oadiPILDl0Yp8F7x0c2Dk0OxYxQEBrWHu43ZIHvNe_IhKl9gGPMaAs6wEALw_wcB:G:s&s_kwcid=AL!3652!3!376537616829!b!!g!!%2Bthermo%20%2Bil%20%2B6&cid=bid_pca_aup_r01_co_cp1359_pjt0000_bid00000_0se_gaw_bt_pur_con&gclid=Cj0KCQiA7oyNBhDiARIsADtGRZY1oadiPILDl0Yp8F7x0c2Dk0OxYxQEBrWHu43ZIHvNe_IhKl9gGPMaAs6wEALw_wcB#
3. Culig Z, Puhr M. Interleukin-6: A multifunctional targetable cytokine in human prostate cancer. Molecular and Cellular Endocrinology. 2012;360(1-2):52-58.
4. Interleukin-6 Signaling and Anti-Interleukin-6 Therapeutics in Cardiovascular Disease [Internet]. Circulation Research. 2021 [cited 28 November 2021]. Available from: https://www.ahajournals.org/doi/abs/10.1161/CIRCRESAHA.121.319077
5. IL6 - Clinical: Interleukin 6, Plasma [Internet]. Mayocliniclabs.com. 2021 [cited 28 November 2021]. Available from: https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/63020

TekEquipment

ErgoTekMed 80/180 Microtome Benchtop

TekEquipment has designed an extremely stable, chemical resistant bench top supported by a four leg, electronically adjustable hydraulic system.

ErgoTek™

Designed to improve the workplace ergonomics for your staff!

- 70-120cm height range
- 400kg maximum load
- 40mm tabletop thickness
- Range of colours available
- 80 x 180cm benchtop, customised sizes available
- Dedicated height control panel
- Hidden cable tray with optional powerboard
- Made in Australia
- **Excellent stability for microtome use**

Down-Draft GrossPath GP Workstations

- Integrated active carbon air recirculation filter (+ HEPA option)
- No ducted ventilation system needed
- Height adjustable
- Simple installation
- Full range of accessories
- Widths available: 125cm, 150cm, 175cm, 200cm

Ventilated Tissue Storage Cabinets

- Ducted or integrated fan & filter
- Stainless steel or glass doors
- Stainless steel or coated laminate construction
- Height adjustable shelving
- Roll out trolley option

bsi. ISO 9001:2015 Quality Management FS 521104

Contact Us today to learn more!

1300 368 138 | tekequipment.com.au | info@tekequipment.com.au

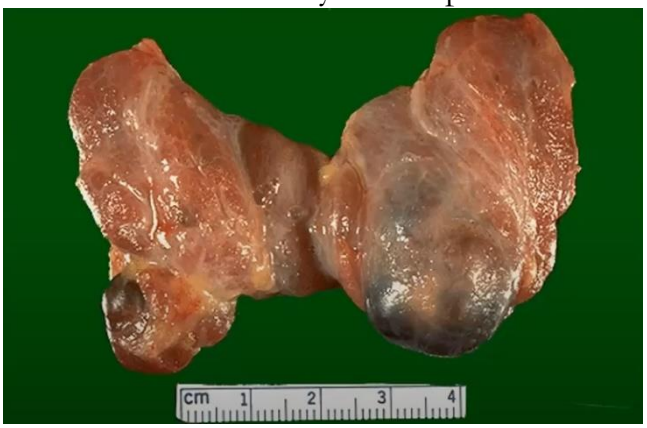
ONLINE SCIENTIFIC MEETING

'Thyroid Cut-Up' presented by Donna Lenghaus

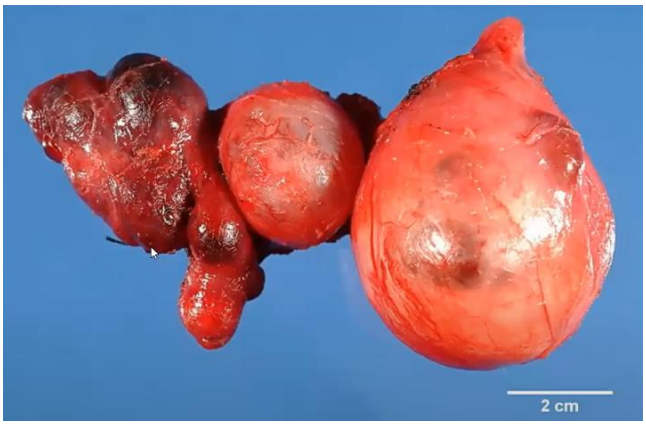
14th of October, 2021

The HGVT were fortunate enough to have Donna Lenghaus take the time to share her expertise regarding cut-up in the context of the thyroid.
She emphasised the importance of referring to the RCPA manual as well as carrying out cut-up in a systematic manner so that one can rely on their work patterns in situations where recall is required.
Moreover, a sound knowledge of anatomy was iterated throughout the presentation, setting up her poetic description of the cut up process, making sure your 'blocking tells a story' with each block being likened to a chapter in a book for that specimen.
Part of this relies on RCPA guidelines as well as an awareness of the questions that you are attempting to answer through the process. In relation to thyroid lesions, Donna relayed the questions she asks in her approach:

- What type of lesion is it?
- How big is the lesion?
- Does it involve the surface of the thyroid?
- Does it cross the isthmus from the left side to the right side or vice versa (One or two lobe involvement?)
- Is there any extracapsular extension?



Normal 'happy' thyroid



Abnormal 'unhappy' thyroid

One of her most important notes that we could all learn from is to ask for help when needed. There are many experienced, approachable members in laboratories who can make terrific mentors for new or ambitious members.

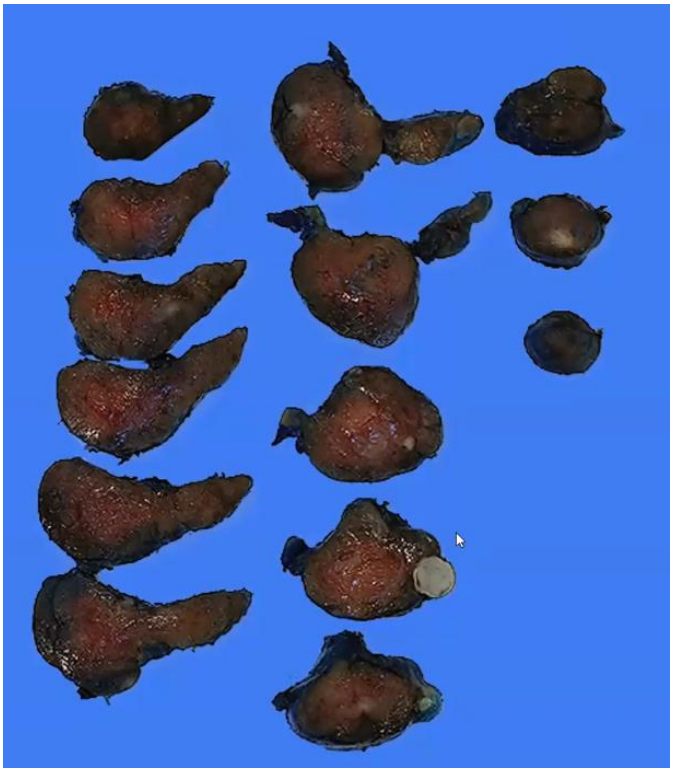
ONLINE SCIENTIFIC MEETING

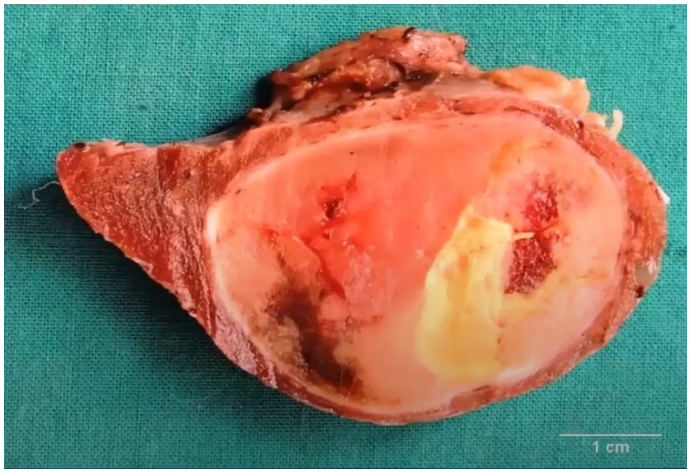
'Thyroid Cut-Up' presented by Donna Lenghaus

14th of October, 2021

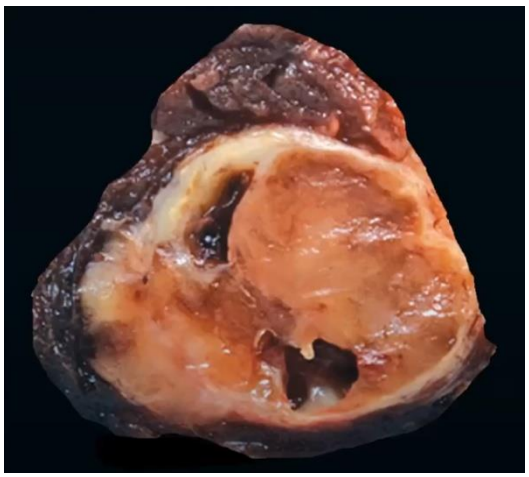
Points from Donna regarding Thyroid slicing:

- **Separate the lobes if total thyroid received**
- **Always cut the lobes from superior to inferior in the horizontal plane**
- **3mm slice thickness**
- **Lay out slices from superior to inferior when taking photos**





Example of adenoma from presentation



Example of carcinoma from presentation

Overall, Donna's insight was a delightful scientific cocktail combining interesting, engaging and educational material. For those interested in hearing her full presentation in greater detail which we highly recommend please use the link here: Donna Lenghaus - Thyroid Cut-Up Presentation

See more with the VENTANA MMR IHC Panel

Featuring the exclusive VENTANA BRAF V600E (VE1) antibody

The VENTANA MMR IHC Panel consists of VENTANA anti-MLH1 (M1), VENTANA anti-PMS2 (A16-4), VENTANA anti-MSH2 (G219-1129), VENTANA anti-MSH6 (SP93) and VENTANA BRAF V600E (VE1) and aids in the identification of patients at risk for Lynch Syndrome in patients diagnosed with CRC.

The ready-to-use VENTANA MMR IHC Panel is optimised for use with the OptiView DAB IHC Detection Kit, OptiView Amplification Kit and ancillaries on the fully-automated BenchMark ULTRA and GX systems.

Roche Diagnostics Australia Pty. Limited
2 Julius Avenue North Ryde NSW 2113 Tel: +61 2 9680 2222 ABN 29 003 001 205

www.roche.com
rochediagnosticsaustralia.com

7222A-4 1217 RTDPC-ASFASA-0173

Optibodies™ - When Good Is Not Enough

A series of monoclonal antibodies for immunohistochemistry from Nordic Biosite

Optibodies™ are a series of monoclonal antibodies for immunohistochemistry developed by Nordic BioSite (Sweden). Created to boost quality and options for users of IHC biomarkers in clinical pathology, Optibodies™ work for different protocols and platforms with standard buffers.

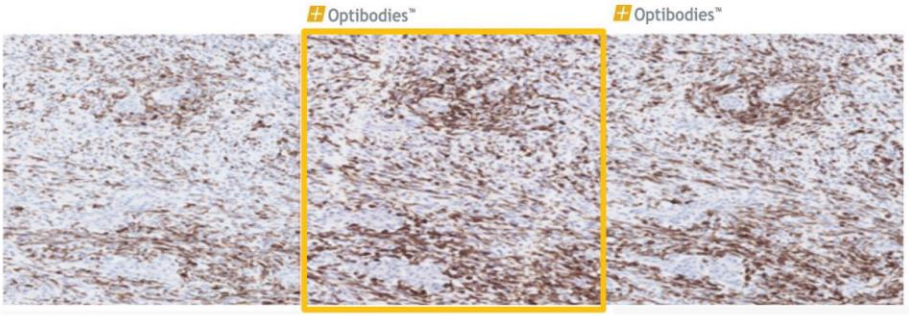
Optibodies™ receive high scores in NordiQC assessments, a confirmation of their high quality.

Top-performing Optibodies™

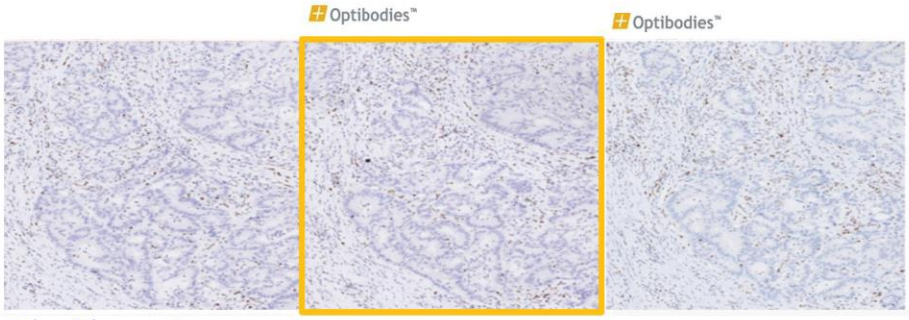
Top performing Optibodies™ include Desmin, MSH6, Pan Cytokeratin, EpCAM, and Napsin A, all CE/IVD marked. Protocols are available for Ventana Benchmark Ultra and Dako Omnis.
See below examples.

Desmin(BS21)

Desmin is an intermediate filament expressed in smooth and skeletal muscle cells and used as a marker to distinguish tumors of myogenic origin. Our desmin antibody (clone BS21) has great analytical and technical sensitivity.



Desmin, Rhabdomysarcoma 2



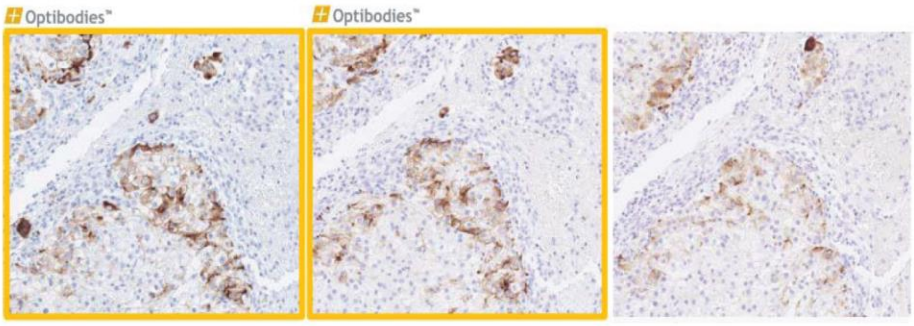
Colon Adenocarcinoma

MSH6 (BSR100)

MSH6 (MutS Homolog 6) is a member of DNA mismatch repair system and it is associated with certain colon cancer, colorectal cancer, and endometrial cancer. Loss of MSH6 expression can be visualized in these cancer types by our MSH6 antibody (clone BSR100)

Pan Cytokeratin (BS5)

Cytokeratins are expressed in a wide variety of epithelial tissues and are of great importance for carcinoma diagnostic. The challenge is to stain all the 20 different cytokeratins optimally. BS5 clone meets this challenge as one of the best clones on the market.



Pan Cytokeratin, Renal Clear Cell Carcinoma (RCC)

Interested in Optibodies™ ?

Contact us for more information or a quote.

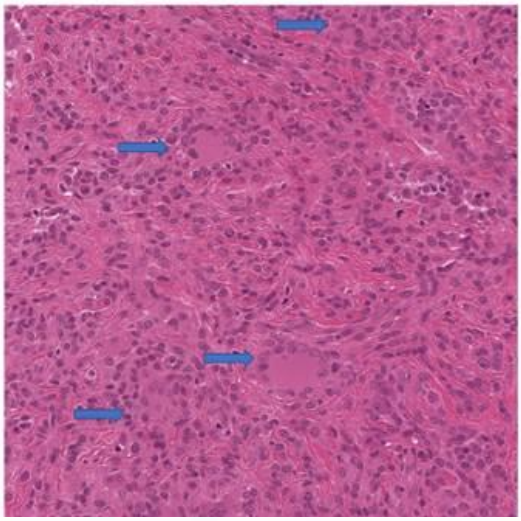
www.metagene.com.au - 1800 788 498 - admin@metagene.com.au

CASE STUDY

Giant Cell Tumour

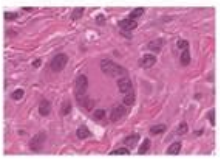
By Kerrie Scott

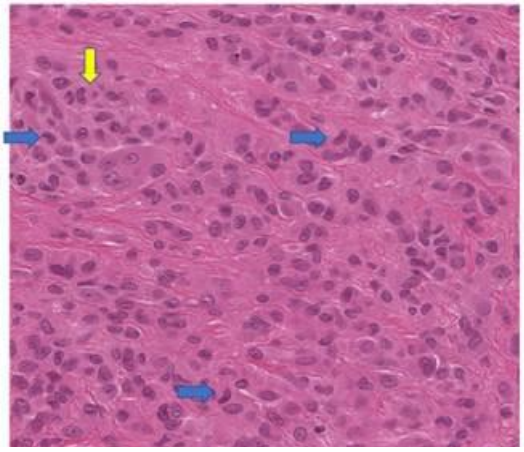
As is true for most soft-tissue tumours, the aetiology of giant cell tumours of the tendon sheath is unknown. Pathogenetic theories have included trauma, disturbed lipid metabolism, osteoclastic proliferation, infection, vascular disturbances, immune mechanisms, inflammation, neoplasia, and metabolic disturbance.
Giant cell tumour of tendon sheath (GCTTS) is the second most common tumor of the hand (the first is a ganglion cyst), typically presenting in the third to fourth decade of life. Also known as localized nodular tenosynovitis, GCTTS is characterized by diffuse presence of multinucleated giant cells and proliferation of synovial-like cells.



Giant Cell Tumour -H&E

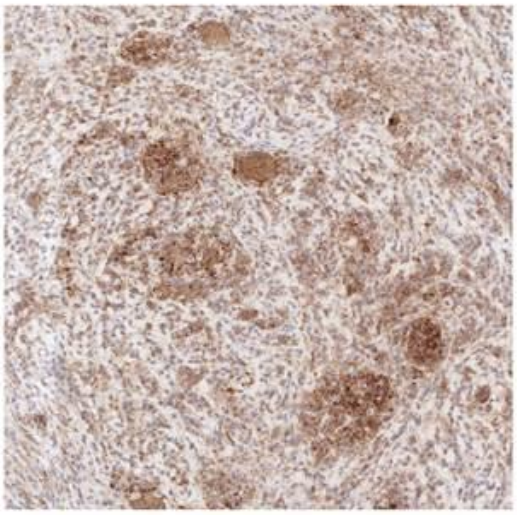
- Some of the Multinucleated giant cells can be described as horseshoe shaped





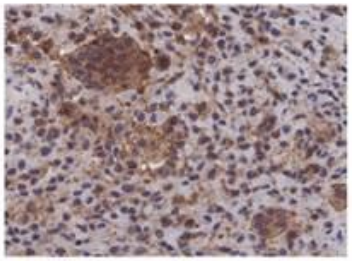
Giant Cell Tumour

- The macrophage-like mononuclear cells formed most of the background cells. These had folded kidney shaped or "coffee bean", grooved oval, or occasionally spindle shaped nuclei with very small or no nucleoli.



Giant cell tumour

The cellular constituents comprised four cell types, namely: macrophage-like mononuclear cells, epithelioid histiocyte-like cells, osteoclast-like giant cells, and xanthomatous cells.
Most cells stain for CD68



epredia Enhancing precision cancer diagnostics

POWERED BY SHANDON MICRON MENZEL-GLÄSER Richard-Allan Scientific

PrintMate™ AS Cassette Printer

Lean Workflow and Error Reduction in Your Laboratory

The PrintMate AS™ cassette printers are the third generation of labeling equipment, that when used with PrintMate cassettes, are designed to maximize accuracy while streamlining workflow and improving efficiency.

Small Footprint with Big Impact

- Ideal for on-demand labeling right at the grossing station or microtome, leading to lower error rates than traditional batch printing
- Fast print speed matches your grossing workflow
- Print as few as one cassette and avoid mismatched pairing
- PrintMate AS optional collection system captures cassettes in trays for organized batch printing
- The small size of the PrintMate AS™ cassette printer allows it to fit easily next to the grossing area, providing greater flexibility in determining the optimal workflow solution for your laboratory

Solid Security

- The simple method of communicating with the Laboratory Information System provides a secure method of obtaining the details to be printed
- PrintMate AS can be used as intelligent stand-alone printers capable of accepting data from a bar code or typed input
- Cassettes are pre-heated for the ultimate print performance
- High-quality print, every cassette is clearly marked with easy-to-read, permanent black type
- PrintMate AS cassette printer can be used as an on-demand or batch printer in any size laboratory

Use with PrintMate cassettes from Epredia

Trade in | Trade Up

EPRNA83800002 ~~$36,020~~ ***$28,500***

For any PrintMate model, working or not
Valid until 30 June 2022

bio-strategy
delivering technology

Bio-Strategy PTY Limited
T 1800 008 453 | sales.au@bio-strategy.com
www.bio-strategy.com | shop.bio-strategy.com

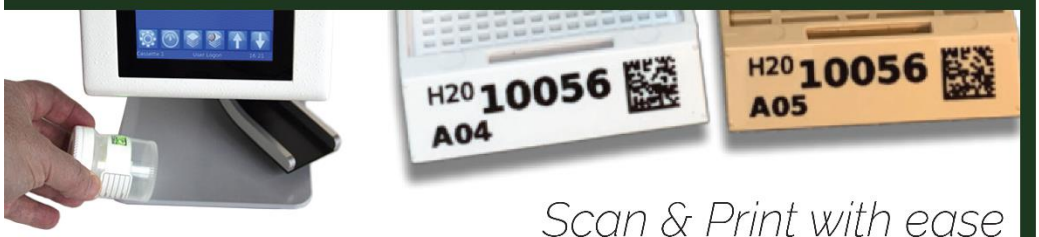
VETO MEDICAL TECHNOLOGY

When you need to know

0400 289 318

SALES @ VETOMEDTECH.COM.AU

Pyramid Innovation's **Smart Printer Range**



Scan & Print with ease

Track & Trace your lab workflow in real time

Twin Hopper Slide Printer

Print standard slides, IHC charged, specials like ThinPrep or control slides in the same print job in the right order!

Six Hopper Cassette Printer

Pedigree

Built by the team that invented the industry standard Microwriters.

Performance

Fast - 4 second thermal labelling for cassettes & microscope slides.

Future-proof

Not 'just a printer' - Full Track & Trace suite to digitise your workflow with lifetime warranty options available.

Thin & LEAN

Perfect for labs starved of bench space.

Touchscreen

Quality of life features at your fingertips.

Tracking

Complete chain of custody handling. Rapidly search and report on your lab in real time - live as you work.

Scanner

Scans all forms of barcodes including 2D, linear and QR code.

LIS / On Demand

Compatible with all LIS / LIMS as well as offering true 'On Demand' LEAN.

Stellar Service

Included preventative maintenance & lifetime product support with additional training, customisation and live support when you and your staff need it. Find out why our clients love us.

To find out more or arrange a demonstration.

SCAN ME

Histology History Bites

Cryotomy: Cold as Ice, to Take a Slice

By Alex Johnston

As keen connoisseurs of all things histology, we are all familiar with the process of microtomy. You take the wax block containing tissue and cut a very thin section so that light may shine through and demonstrate the wonderful cellular architecture. Personally, I describe this as a bespoke, hyper engineered version of a deli slicer to family and friends and it hasn't yet failed to get the concept across.

You may also be familiar with microtomy's 'cooler' cousin in cryotomy a.k.a. frozen sectioning. Whether you have been responsible for this task in your own work or have simply heard of the technique in passing, if you are like me, the first question (before several others) I asked when introduced to the method was **'Why?'**:

'Why are we going to the trouble of freezing tissue when we have something that works with wax embedding and processing?'

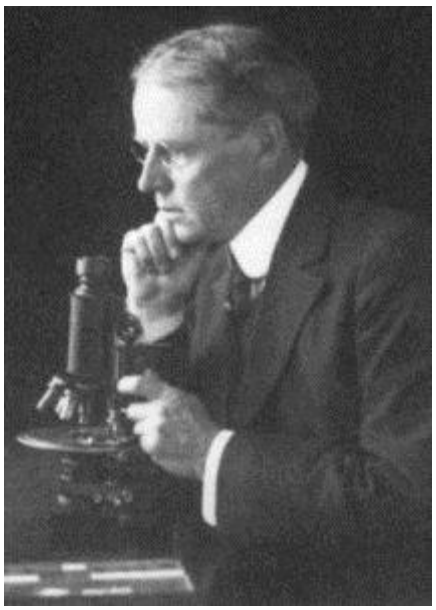
'Who thinks of such a concept in the first place?'

'How is it different to microtomy?'

'Is it hard to do and how can I make it easier?'

In examining the answers and providing some tips from my experience, we will discover that the technique is not only valuable scientifically but also professionally if the skill is honed.

The first instance of intraoperative frozen section was performed in 1891 by pathologist William Welch. Welch had studied pathology extensively in Europe and had established the first hospital pathology laboratory in Bellevue Hospital Center, New York, prior to being recruited to Johns Hopkins Hospital. The case in question was a suspected breast cancer case, with a slide being prepared using a carbon dioxide freezing microtome.[1]



Dr. Louis Wilson

Over the following decade other rapid frozen section methods were published in Europe, including Thomas Cullen, who employed a technique for freezing formalin-fixed tissue, published his method in the Johns Hopkins Bulletin in 1895. Despite this innovation, the fact the a fixation step was used before the freezing meant that the technique took nearly an hour overall to complete. The standard cryostat method used today is generally accepted to be published in 1905 by Louis Wilson of the Mayo Clinic.[1] He used a dextrin solution to embed the tissue and a carbon dioxide freezing microtome to produce the sections. With the use of methylene blue and interpreting slides without permanent mounting involved, his technique took only a few minutes. This was a tremendous innovation on Cullen's method which took nearly an hour. Following introduction of the procedure, surgeons saw a drop in inoperable cancer rates.

Histology History Bites

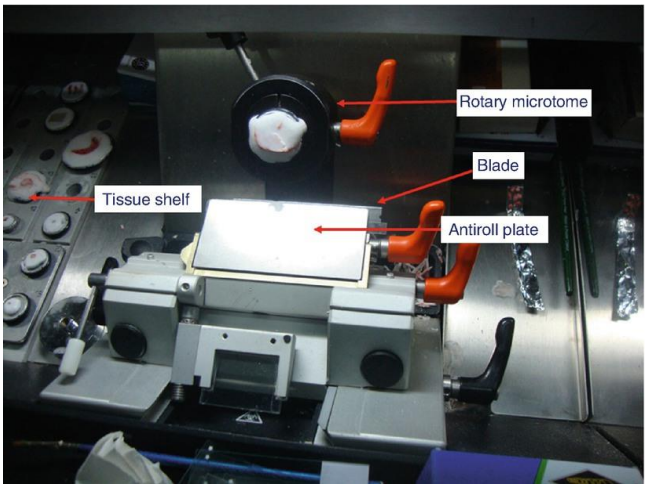
Cryotomy: Cold as Ice, to Take a Slice Continued…

At John Hopkins Hospital, for example, the rate dropped from 50% around 1900 to less than 5% in 1920. Increasingly, surgeons and gynecologists accepted frozen section as a reliable diagnostic tool. Simultaneously, they became increasingly comfortable with the role of the pathologist as diagnostician and consultant. [2]
Most centers today include a brief fixation step before staining in hematoxylin and eosin, followed by permanent mounting, but the technique at its core has largely remained unchanged for over 100 years.

So now that we have a crash course history on the origin of the technique, why do perform it at all?
There are two main reasons to consider frozen sectioning:

1. It is extremely valuable for cases that require rapid interpretation by a pathologist.
2. It is a useful technique to examine tissue features that may be impacted by conventional processing methods

For the former, rapid diagnosis presents as intraoperative specimens received by the laboratory. To use an example, examination of local lymph nodes surrounding a breast cancer is important to determine whether metastases has occurred. This examination may happen intraoperatively as the surgeon wants to determine this outcome in real time and make a decision as to whether surgical investigation needs to proceed at that time. Given that it is infeasible to have a patient waiting for traditional tissue processing which can take hours at a minimum, this is where frozen sectioning excels at delivering valuable information quickly.
In consideration of reason two, certain tissue components can be affected by traditional processing which impacts downstream special stains. Compared to paraffin-embedded sections, frozen tissues are thicker, lowering microscopic resolution and the ability to capture tissue morphology in detail. However, cryopreservation is thought to better preserve antigen and antigenicity. The study of post-translationally modified protein, DNA, or RNA is also recommended on frozen tissue. [3] Furthermore, in cases when staining for fat cells using Oil Red O staining, it is essential to employ frozen sectioning as conventional processing removes fat from tissue samples.
While cryotomy is used for different purposes, it is not completely alien to those who have previously experience in microtomy; though there are several considerations to be aware of. The first and foremost is safety. Frozen tissue is unfixed and therefore, poses a greater biohazard risk in the unfortunate event of injury. Like with any new instrument, take the time to become familiar with all the functions of your cryostat as well as develop a spatial awareness for the chamber space. Typically, cryostats at their core are not so different from one another and several features resemble those on a microtome. With this familiarity, it can be easy to be overconfident at first however exercising caution while learning leads to happy sectioning AND happy fingers.



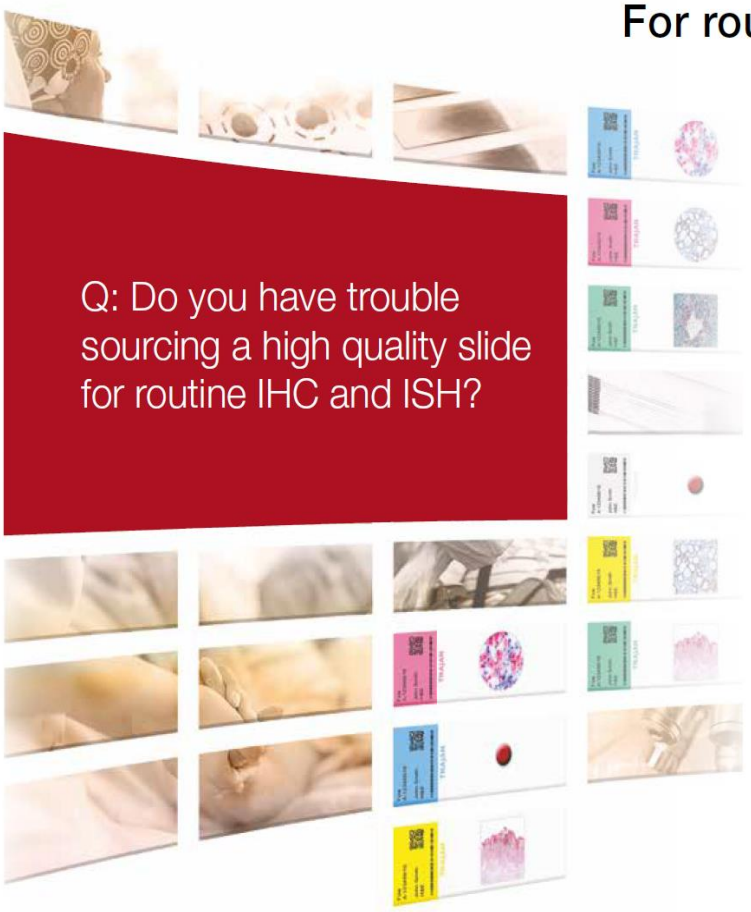
Inside of a typical cryostat

TRAJAN

Introducing

Series 2 Enhanced Adhesive microscope slides

For routine IHC, ISH and special stains



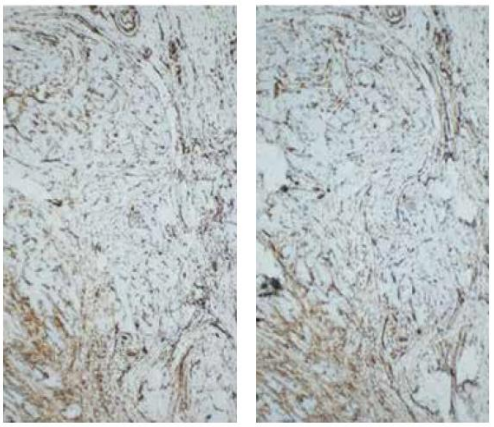
Q: Do you have trouble sourcing a high quality slide for routine IHC and ISH?

A: The new Series 2 Enhanced Adhesive slide is suitable for a wide range of applications.

Trajan's new Series 2 Enhanced Adhesive microscope slide is the first of its kind. Designed to provide both strong tissue adhesion and minimal background staining, the Enhanced Adhesive is the first to feature a hydrophobic surface, making it suitable for a wide range of applications.

Complementing the existing Series 2 and 3 range, the Enhanced Adhesive was designed with routine IHC and ISH, as well as special stains, in mind. Providing superior surface flatness and instant tissue adhesion, the Series 2 Enhanced Adhesive slide is suitable for use with a wide range of immunostainers.

472042481	Trajan Series 2 Enhanced Adhesive Microscope Slide 76 x 26 mm with clipped corners, 100 pcs



Human mammory gland stained on BenchMark ULTRA with Vimentin 9.

Showing the right path

Contact us on 1800 257 213 to learn more about our range of microscope slides.

www.trajanscimed.com

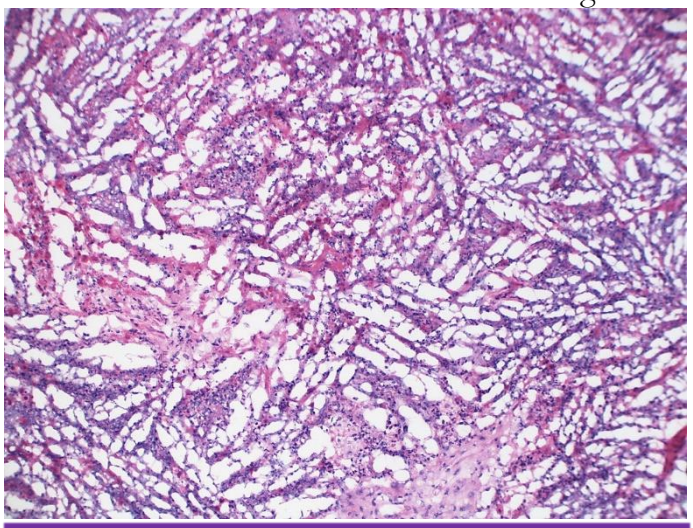
Histology History Bites

Cryotomy: Cold as Ice, to Take a Slice Continued…

The following video provides an example of the steps taken when preparing to cut a sample as well as addressing some of the factors about to be brought up below: Preparation of frozen tissue sections (Cryotomy)

On a technical level these are some pointers to be aware of:

- **Embedding/freezing:** Some samples may be fresh, others may be received snap frozen prior to getting to you. In cases where tissue is fresh, there are several approaches to freezing. This video provides just one technique of freezing in the cryostat however it's important to know there are other methods where freezing occurs outside of the cryostat, with some companies even developing instruments specifically to aid the freezing process: Pathology Frozen Sections: What We Do. Like with wax embedding, this is where you want to decide how you would like your section to appear on the slide and what the easiest way to achieve this goal is when considering tissue variables like keratin, calcification and fat.



Ice crystal freezing artifact: Slow freezing allows water within tissue to form large crystals, crystals melt when sections are above 0C° leaving 'holes' in their place.

- **Orientation:** This important factor relates to both where the tissue is positioning within your frozen block and which direction the tissue will move across the blade. In the above video you may have observed the central placing on the sample. This is good because it gives us good clearance to interact with the section as it comes off the block without risking unnecessary damage to the sample that may occur if the sample was towards the edge of the block. Deciding the cardinal direction for the cutting of the tissue relates to the above point about making the process easy for you. For example, if cutting skin it's best to ensure the harder epidermal layer strikes the blade last.

- **Cutting temperature:** Depending on the context of your frozen sectioning, you may be cutting several different tissue types. However, different tissue types can have different sensitivities for optimal cutting temperatures. While the difference from a really cold temperature like -20C° (common temperature for cryostats) to a really, really cold like -30C° may seem trivial, it could mean the difference between nice sections or shattered and compressed sections.

Histology History Bites

Cryotomy: Cold as Ice, to Take a Slice Continued…

- **Cleanliness:** Always a goodie to have in mind. As big application of frozen sectioning in modern time involves DNA and RNA downstream testing it is critical the cutting chamber remains clean. This means cleaning the stage (always brushing away from the blade) with 100% ethanol (always 100% as any water present will crystallise) is a necessity between samples as well as ensuring to use a fresh area of the blade.

- **Post-sectioning factors:** Being conscious of the testing to be performed on your slides following sectioning is important for good downstream results. For example, slide intended for H&E staining will typically have a brief formalin fixation step before skipping to the haematoxylin step as the clearing step reserved for paraffin embedded sections is not required. Conversely, samples for nucleic acid analysis will need to be stored at -80C° instead order to prevent DNA/RNA degradation prior to testing.

The best way to navigate the above factors is to always ask questions when unsure as the troubleshooting aspects of frozen sectioning are distinct from those found in microtomy and warrant their own separate write-up.

Personally, I have found cryotomy to be one of the most enjoyable and challenging aspects of histology and would highly recommend you take the opportunity to learn it should it arise.
As with many histological methods, the hands-on learning approach works best, so hopefully, this short tour of the method will inspire you to seek a good mentor within your laboratory.

References

1. The Frozen Section: Historical Background, Technique, and Quality Assurance [Internet]. Basicmedical Key. 2021 [cited 29 November 2021]. Available from: https://basicmedicalkey.com/the-frozen-section-historical-background-technique-and-quality-assurance/#R8-1
2. Development of Frozen Section Technology is Subject of Newspaper Story Highlighting the Value Pathology Brings to Medicine [Internet]. Dark Daily. 2021 [cited 29 November 2021]. Available from: https://www.darkdaily.com/2014/09/19/development-of-frozen-section-technology-is-subject-of-newspaper-story-highlighting-the-value-pathology-brings-to-
3. IHC Sample Preparation (Frozen vs. Paraffin) [Internet]. Novus Biologicals. 2021 [cited 29 November 2021]. Available from: https://www.novusbio.com/sample-preparation-for-ihc-experiments

‘Slice of Life’

Kellie Vukovic welcomed a baby boy (Fraser Jack Carroll) 3 weeks early on Saturday 4th September. Big congratulations Kellie!

Future Events: 2022

Org. No. A0035235F

Date: 24th February, 2022

Scientific Meeting

Topic: TBC

Venue: Zoom Meeting (streamed and recorded)

Date: 21st April, 2022

Scientific Meeting

Topic: TBA

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)