Volume 57 / Dec 18th, 2021

TISSUEPAPER

Histotechnology Group of Queensland

President's Report- Mark Bromley

With Christmas fast approaching and the festivities ramping up everywhere you look, I welcome you to the fourth and final 2021 edition of TissuePaper.

Since the last edition, our ongoing tussle with COVID saw some wins and some losses. Unfortunately, the Workshop at QUT fell into the latter category, and we have had to postpone it into the New Year. At this stage we are looking at March to do some super sensational silvery stains, so bling it on in 2022! However, with COVID held sufficiently at bay for most of the time in the Sunshine State, we did manage to meet up face to face a couple of times. The 10th of September saw a gathering at the Normanby Hotel for a great Trivia Night, with the honours taken by RBWH. Following this, the AGM was also held in The Normanby on the 21st of October. Thanks go to Dr Myo Thu for a great presentation on breast cut-up.

In This Top Issue :

This brought a close to our 2021 program of scientific meetings and events. And what a year it proved to be. As we rung in the New Year nearly 12 months ago with a general feeling that 2021 can't possibly be as bad as 2020, little did we suspect that we would be shown in no uncertain terms that it absolutely could be and was! Still, we soldiered on, and as 2022 approaches, I think we once again have the feeling that next year will herald a return to normality, or at least something considerably closer to normality than this year has been. And if nothing else, we all now know far more of the Greek alphabet!

May I take this opportunity to thank the HGQ Committee for all of their hard work over the last year. Putting on Scientific Meetings, Trivia nights, producing the Newsletter and all of the things that go on under the bonnet of the HGQ take time and effort, and without the dedication of The Committee, none of this would happen. So thanks! Thanks to those who have renominated for 2022, thanks for those who decided not to, and thanks to those who have joined us for the first time in 2022. We look forward to putting on the Workshop in March, and border restrictions falling away as we have rebooted planning the Cairns State Histology Conference in October which is looking like it will be one not to miss.

So raise a glass to Santa and his reindeer as they fly overhead, book a session with a psychiatrist for early Jan because.. well, you just saw a flying reindeer! And enjoy Christmas, the end of 2021 and the launch of a fantastic 2022.

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Contributions Welcomed!

Journal, scientific article and antibody reviews all accepted!

Know someone who should be featured?

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Let us know!

We are always looking for contributions of scientific articles and news, or if you have improvements and techniques that make a difference in your lab!

Submissions can be sent to HGQ Tissue Paper in digital format




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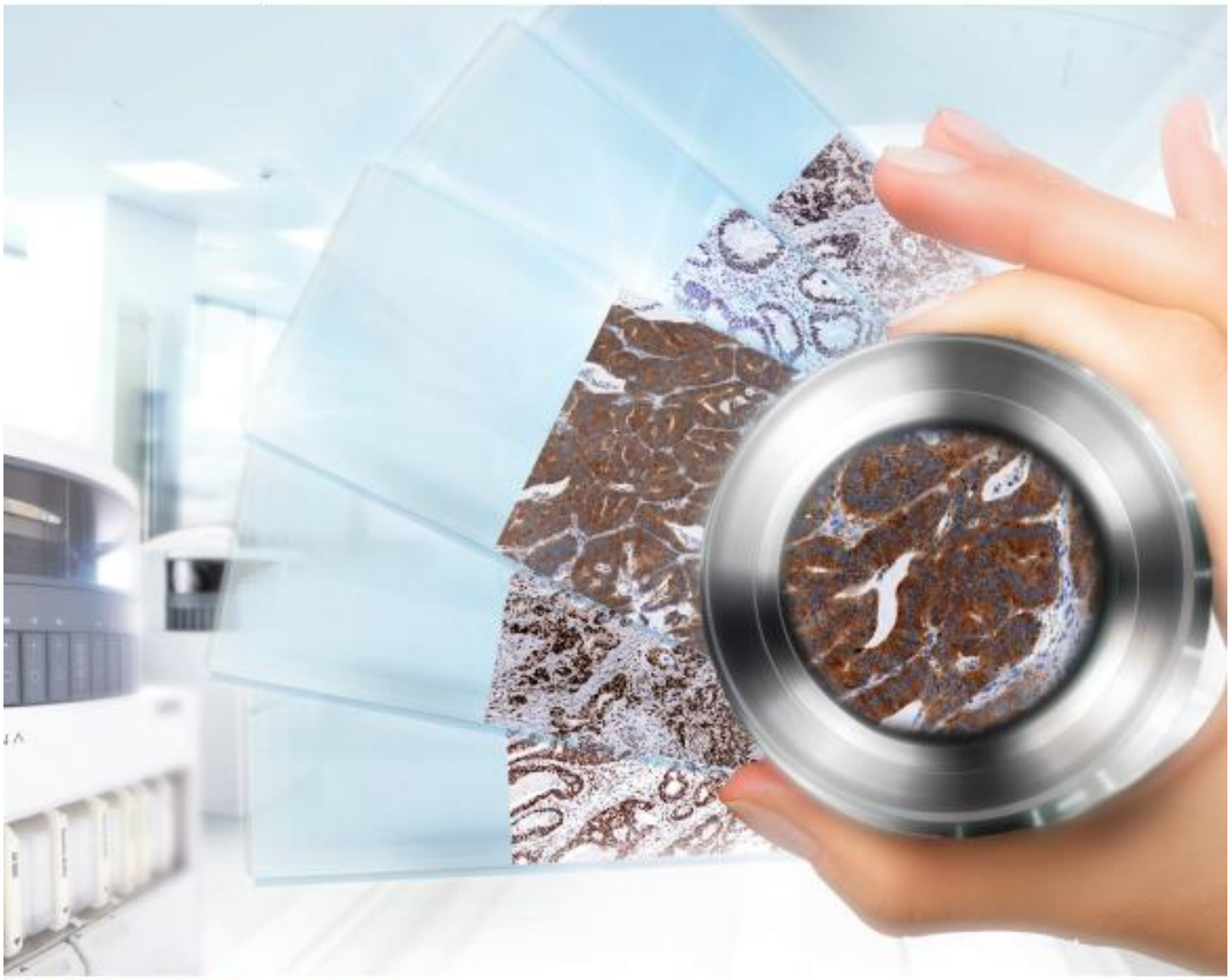

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Histotechnology Group of Queensland

2021 Trivia Night

Emma Hughes

On Thursday the 9th of September the latest HGQ trivia night was held. After unfortunately having to postpone once due to Covid restrictions, we still had a good turnout of nearly 50 people for Trivia at the Normanby Hotel.

We had five tables of 10 consisting of Histology people from all over Brisbane.

The Trivia was hosted by a wonderful host from Triviameisters with questions encompassing Music, Television, Advertisements and more.

With a special Histology round presented by HGQ President Mark Bromley and with questions written by Mark and Emma Hughes (Don't worry neither of us answered any questions Lol)

The special Histology round was won by a combined team of RWBH, TAFE Qld and me.

After a great alternate drop dinner of Steak or chicken and multiple rounds the winners were announced.

The winner with a clear lead was the combined team of RBWH/QUT/Emma Hughes.

There were two teams tied for second place PAH and the Mater.

After a tie breaker round with Higher and Lower the 2nd place went to Mater and third went to PAH.
It was a great night for everyone and lots of fun was had.

I hope to see everyone coming along to the HGQ Trivia in 2022.

HGQ 2021 TRIVIA WINNERS

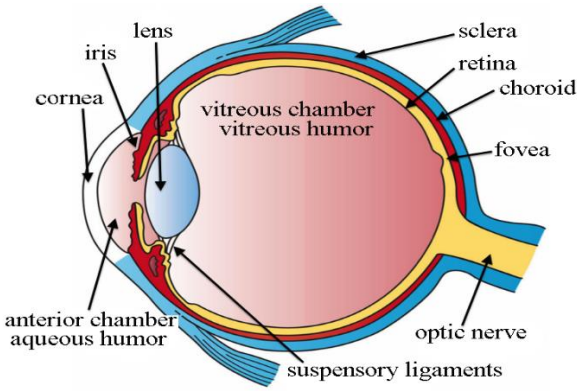
The eye: Anatomy, Disease & Histology

Emma Hughes

The human eye is a specimen sent in for Histology quite infrequently, but it is a very interesting organ.

Firstly, I will talk about the structure of the Eye and the main reasons that it may be sent in for Histology then I will detail how Eyeballs are dissected and processed by Sullivan Nicolaides Pathology.

Anatomy



When looking at an eyeball there a few main structures it pays to be aware of before processing an eyeball.

The Cornea. The cornea is the transparent front part of the eye that covers the iris, pupil, and anterior chamber. Along with the anterior chamber and lens, the cornea refracts light, accounting for approximately two-thirds of the eye's total optical power.

The Iris and the Pupil is a thin, annular structure in the eye, responsible for controlling

the diameter and size of the pupil, thus the amount of light reaching the retina. Eye colour is defined by that of the iris. In optical terms, the pupil is the eye's aperture, while the iris is the diaphragm.

The Lens: is a transparent biconvex structure in the eye that, along with the cornea, helps to refract light to be focused on the retina. By changing shape, it functions to change the focal length of the eye so that it can focus on objects at various distances, thus allowing a sharp real image of the object of interest to be formed on the retina.

Vitreous Chamber: is the space in the eye occupied by vitreous humor. The vitreous fluid, along with supporting the lens, also functions in maintaining the shape of the entire vitreous chamber and posterior cavity. It is imperative that the eye remains the proper shape to ensure that the light passing through the lens and the fluid can focus properly on the retina.

The Sclera, Retina and Choroid: The Sclera also known as the white of the eye, is the opaque, fibrous, protective, outer layer of the human eye containing mainly collagen and some crucial elastic fiber. The Retina is the innermost, light-sensitive layer of tissue of the eye The optics of the eye create a focused two-dimensional image of the visual world on the retina, which translates that image into electrical neural impulses to the brain to create visual perception. The Choroid is the vascular layer of the eye, containing connective tissues, and lying between the retina and the sclera. The choroid provides oxygen and nourishment to the outer layers of the retina.

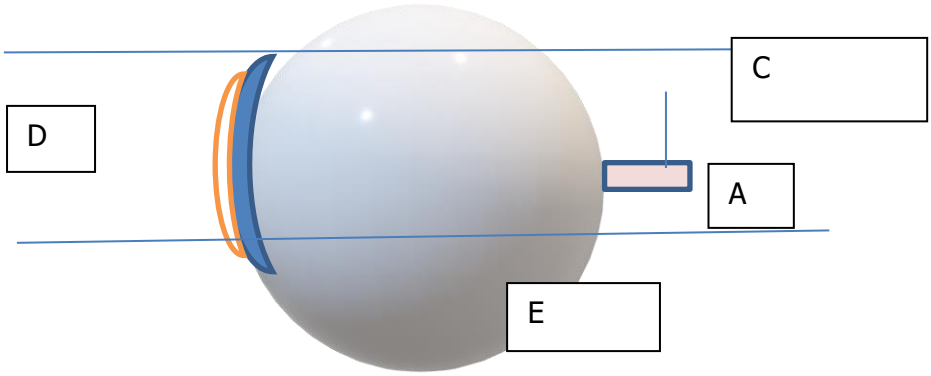
Optic Nerve: is a paired cranial nerve that transmits visual information from the retina to the brain.

Main Causes for Histology

There three main reasons an Eyeball may be sent in for Histology they are: Trauma, Cancer and Chronic Infections/Glaucoma/other Eye diseases.

Advantages for Histotechnicians

Dissection



1. Orientate if possible using the attachment of the internal oblique which is located just below the horizontal line on the postero/lateral aspect of the globe.

2. Measure globe in 3D and cornea in 2D.

3. Ink and measure optic nerve. Take shave of optic nerve and re-ink to assist Microtomy. (Block A).

4. Identify and sample vortex veins. (Block B).

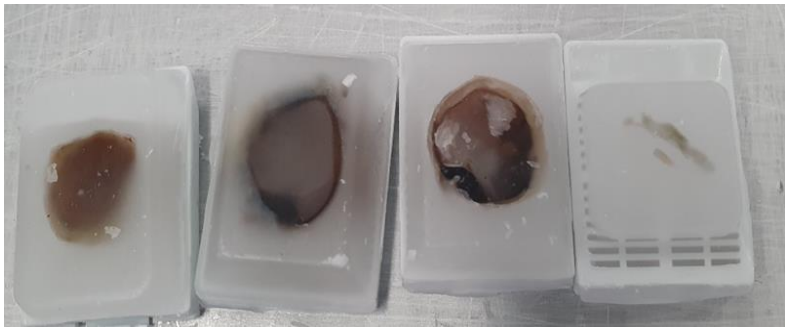
5. Trisect globe at margins of cornea in AP direction keeping optic nerve in middle third.

6. Identify, measure and describe tumour.

7. Place in mega cassettes. (Blocks C,D,E). Dictate cassette containing optic nerve (usually block D).

Embedding

When embedding you want to place the cut surface of the top and bottom of the eyeball face down as seen in the picture below. You then want to place the middle section into the mold as flat as possible preferably with the optic nerve towards the bottom. A special deep mold is used to embed blocks C-E if required (see picture). Care should be taken to ensure the Optic Nerve shave and the Vortex nerves are blocked as flat as possible.



Microtomy

The Blocks A,B,C and E usually only require one good full face section. The importance with Blocks C and E is to ensure a full section of the entire outside of the eyeball.

When cutting Block D, the middle of the Eye including the Optic Nerve often twenty slides are required. This may vary slightly as indicated by the Dissectionist.

The levels through this block will be deeper than usually taken. The idea is to make sure

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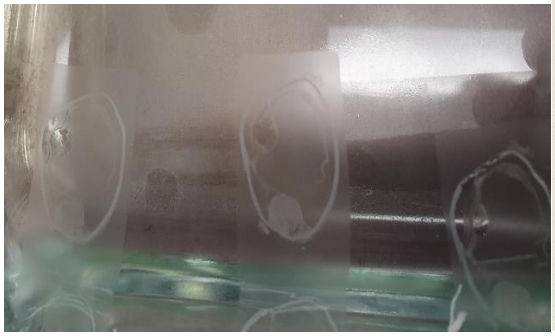
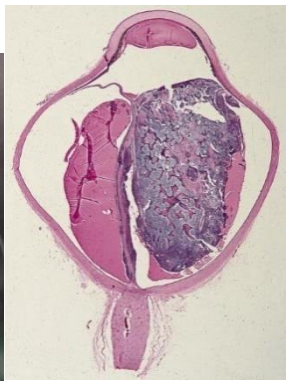
that ultimately you end up with a full face of the eye including a good full face of the Optic Nerve. This will usually require multiple levels to produce. Usually you want the Optic nerve to present on the sections by at least level 17 and a full face preferably by level 19.

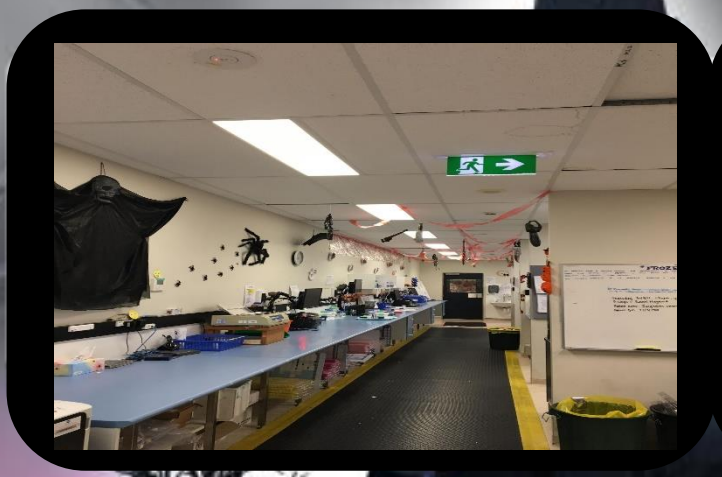
Sections from the Eyeball are placed onto Superfrost Slides to ensure good adhesion to the slides. You especially want to ensure this when cutting the middle of the eyeball to make sure you don't lose any important pathology in the middle of your levels.

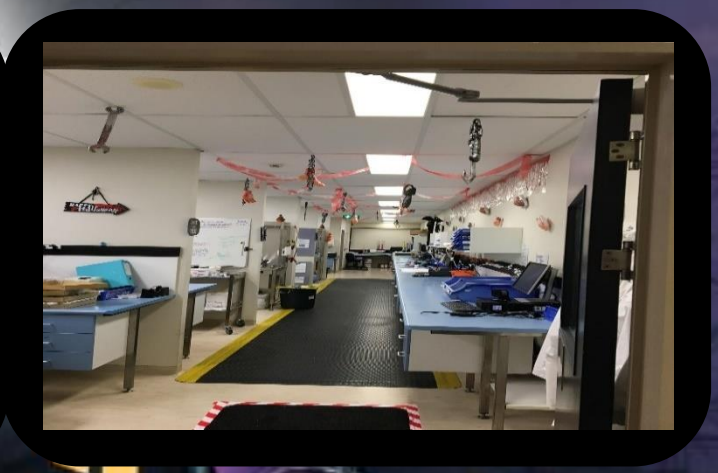
The importance of having a good section of the optic nerve is especially importance in cases of Tumour. This is to confirm/exclude the presence of tumour in this nerve and the possible metasis into the brain/headspace.





HALLOWEEN





Fixing the World with Ethical Ethanol

Evva Järvinen

"Excellent security." André commented after a minute of staring at the Histolo-Sea Laboratory. "Paranoid."

"When you're producing illegal chemical warfare agents, top-tier security isn't paranoia."
"Evva, I can get us in but there will be a police response. Are you sure Histolo-Sea is worth it?"

"Remember, I want the evidence of anthopleurin extraction from sea anemones *and* the ethanol fixative formulas." I told him. VA—Variant Anthopleurin—was the smoking gun. A cardiac toxin to be loaded into artillery shells and used against insurgents in Latin America. VA was made by the Histolo-Sea Laboratory we were looking at. "Proving they make WMDs is worth it." Ethanol fixative recipes were just an extra.

"Evva, weaponised sea anemone toxin makes sense," André agreed, "but why is ethanol fixative a good reason to distract me from saving the world with my sea sponge research?"

"Because ethanol fixatives…wait, what? How does sea sponge histology save the world?"

"Sea sponges can fight sea-level rise."

"Because they capture carbon?"

"No, they absorb water."

Figure 1: Histolo-Sea Laboratory Secret WMD Production Facility

"*Voi kyrpä!* Forget your sponges, focus on their anemones. Get me inside."

"Tonight, Evva. Fill me in on this chemical weapon VA toxin while we wait."

We headed to a nearby café to discuss things and ordered dinner.

"What do you know about alcohol?" I eventually asked.

"Alcohol?" He paused a moment. "One half-shot each of lemon juice, Malibu, Stolichnaya, Gordon's, Cointreau and Bacardi in a colada glass with ice. Top with Coca Cola. The Lisa D Iced Tea. Quite refreshing but it has the knock-out power of a fifty calibre meteorite strapped to Mohammad Ali's fist."

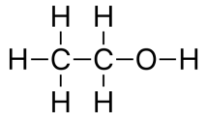


Figure 2: Ethanol molecule

"It's also a fixative." I said to get him back on point. "Histolo-Sea has a few new ideas on that." The basic ethanol fixative recipes all gave the basic results. Better H&Es, specials, identical IHC results for at least the common antibodies, probably all of them. But with side effects.

"I thought ethanol fixative damaged tissue?" He was right. André's sea sponges were usually fixed, decalcified and then subject to careful dehydration and desilication using graded ethanols and hydrofluoric acid.

He had to be careful as too much time in ethanol hardened and shrank the tissue. Conversely, not enough time in hydrofluoric acid left the silica and other hard minerals in the tissue which also made microtomy difficult. André's work was a balance between the two forces.

Ethanol was not a good fixative on its own which is why most methods used formalin instead. Formalin was far from perfect but had the twin advantages of being good enough and well-established. Research on improving histology was mostly derived from formalin methods.

Like the QWERTY keyboard, it might not be objectively the best but everyone used it. Some versions of QWERTY's origin story claim that the QWERTY keyboard was *intentionally* slow and difficult to prevent typewriter jams (common letters and words are typed by the left hand *et cetera*). Dvorak (and other) keyboards were faster but most medical typists train on QWERTY, so it made paradoxical sense to stick with the worse keyboard because it is better. This is the legacy problem of formaldehyde. I knew that to be accepted, any new ethanol fixative must work at least as well as formalin *and* in all the same ways before it could be adopted. It had to fit into existing methods for a seamless transition.

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And my friend was right. Ethanol fixatives cause shrinkage, hardening and erythrocyte lysis. This erythrolysis was so bad it even occurs *in vivo* in alcoholics due to unmetabolised ethanol in the bloodstream. But ethanol had some advantages. It preserved morphology, gave better staining, caused less nucleic acid damage, needed less antigen retrieval for IHC methods and was cheaper to buy and use.

Ethanol fixation works by disrupting protein shape through dehydration. The proteins lose their affinity for water and precipitate out of solution. This is also why the tissue shrinks—it is chemically drying out. Additives such as acetic acid or chloroform cause swelling and could be used to counter the shrinkage.

Formalin cross-linked the proteins, so shrinkage is avoided. However, these linkages sometimes needed to be undone like the Gordian Knot prior to immunohistochemistry. Hiding antigens inside the cross-links meant that formalin often needed annoying antigen retrieval prior to IHC staining.

Interestingly, ethanol-fixed tissue sometimes needed antigen retrieval for formalin-fixed methods, but this was a simple act of soaking a slide in formalin for 30 seconds prior to staining. New antibodies could be created if ethanol fixatives became common, but this is mostly unnecessary given how easy it is to use existing antibodies on ethanol tissue.

In short, a proper ethanol fixative could replace dangerous formaldehyde if any laboratory had the right recipe and the will to use it. I hoped the promise of ethanol fixatives for André's sea sponges would be enough inducement to help me. That, and the moral issue of WMDs.

Histolo-Sea was growing sea anemones for their toxins, and I needed proof of that. VA, found in *Anthopleura* species of sea anemone, acted like digitalis and could stop a human heart in sufficient doses. When weaponised, the effect over a square kilometre was more horrific than an international Tim Tam shortage triggering a nuclear war with China during a Justin Bieber concert. I wanted the proof and, along the way, the ethanol fixatives they used. A moral goal with industrial espionage as a bonus.

I explained this to André over a terrible café dinner. My carbonara so overcooked it was almost carbon and André had ordered a medium-rare chicken and was surprised to find the waiter had

taken him seriously.

I stressed the morality of fighting WMD production plus the advantage of ethanol fixatives but he didn't need much convincing. We went to his home to get the tools we needed and were back by midnight.

André held up a bottle I had made for him that afternoon. "Evva, will this work?"

"The weapon of Finnish resistance." I assured him patriotically. It was a Molotov cocktail containing ethanol, tar, kerosene and $KClO_3$ (derived from boiling hypochlorite bleach to supersaturation then adding sodium-free salt [yes, it is a thing]) with unextinguishable lifeboat matches instead of a burning rag. It was not completely-filled so it was more likely to break when thrown. Sealed, safe, portable, and effective. My people had named and perfected the Molotov Cocktail decades ago and used them against Soviet tanks during the Winter War. "It'll work." I promised him. If André's plan needed fire, Finland could provide it.

Figure 3: The Russian Army capturing Vyborg on March 12th, 1940, 1941. Artist: Blinkov, Alexander Alexandrovich (1911-1995). Figure 4: Molotov Cocktail

André lit the match and hurled the bottle through the window. There was smashed glass, a brief burst of flame and then fire-suppressant flooded the room. The fire went out eventually as the $KClO_3$ tried to keep it alive in the absence of atmospheric oxygen.

A moment later the alarm screeched and that was our cue to leap inside. It was all a bit anticlimactic for someone who, as a child, was made to think that Stop, Drop and Roll would be needed far more often than it has been.

But the fire had done its job. I tested the internal doors and found them unlocked. The alarm

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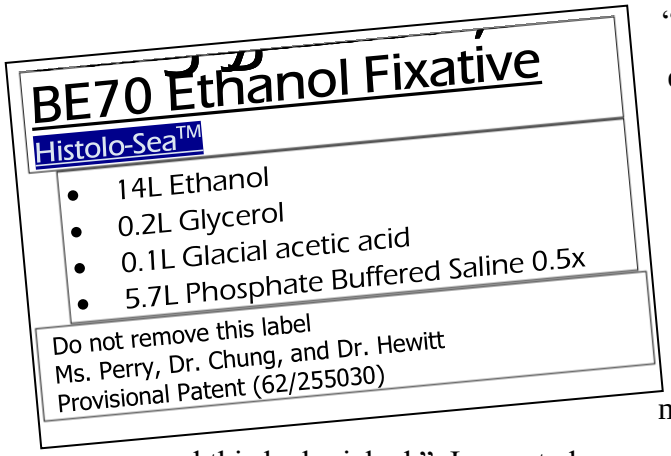
had unlocked every door in the laboratory. A security feature, not a flaw, designed to let people out and emergency crews in…emergency crews which would arrive in minutes to arrest us, so I needed to get moving. Time to find WMD evidence and ethanol fixatives. I ran through corridors until I found a filing cabinet which looked promising.



"I need you to pick this lock!" I shouted over the din. Suddenly, the alarm stopped. I discovered later that André had simply opened the laboratory's fire indicator panel and flicked every switch labelled **Isolate** or **Silence** until the siren stopped. He appeared a moment later carrying a 20L drum. "I need this lock picked." I repeated.

"No, Evva, you don't." He dumped the 20L drum on the floor. On the label, as it should be, was the recipe for the coveted ethanol fixative. It even listed the inventors. "They call it BE70." "Idiot. How far did you walk carrying that?"

"Pretty far." André admitted sheepishly while rubbing life back into his arm. He peeled off the label and handed it to me.

"That's just the current version. I want them all." I tapped the cabinet. "Quickly. Your *Queenslandin poliisi* will be here any second."

Figure 5: 003 key. Used to open fire control panels in Queensland

"Easy." He removed a ridiculously large set of keys from his pocket, looked at the filing cabinet lock, searched through his keys and chose one. "Cheap lockers and filing cabinets have the key number engraved on the lock. I just need the right numbered key…For instance, a 003 key will open any fire-control panel in Australia." Well, that explained the fire alarm. "You can get them at a hardware store for five dollars." He paused. "Not even that. The locks are so bad an approximation of the key will work".

Histolo-Sea had obviously spent their security budget on big fancy doors

and left nothing for filing cabinets. By the time he had stopped talking André had found the correct numbered key and the cabinet was opened. I got back to my search while André found the ethanol fixative data and perused it.

EthMeth

- 15L Ethanol
- 5L Methanol

Minor tissue contraction

Sections well

Fast fixation

Methacarn

- 12L Methanol
- 6L Chloroform
- 2L Glacial Acetic Acid

Sections well

Fast fixation

Better morphology, staining intensity and cellular detail than formalin.

Not as much hardening and shrinkage as Carnoy's

Basic Ethanol Fixative

- 4.2L Ethanol
- 8.4L Isopropanol
- 6L Water

Tissue hardening and shrinkage

Carnoy's

- 60L Ethanol
- 30L Chloroform
- 10L Glacial Acetic Acid
- 1g Ferric Chloride

Fast

Preserves glycogen

Good nuclear preservation

Tissue hardening and shrinkage

"Carnoys—you once told me it had formalin in it." André smirked. "Interesting. A few of these use chloroform." He glanced around and found the chloroform. He opened it and inhaled deeply.

"Yes, that smells like chloroform…Oh hells."

André slumped to the ground and looked woozy. He shook his head to clear it. I didn't worry too much. Contrary to Hollywood lore, chloroform takes several minutes to put someone to sleep.

I had made the Carnoy's error in a rush to deliver a manuscript as a favour to an editor-friend who was desperate for material under a publishing deadline. If André was going to tease me about it then he could pick himself up off the floor. André was back to his normal usual self a

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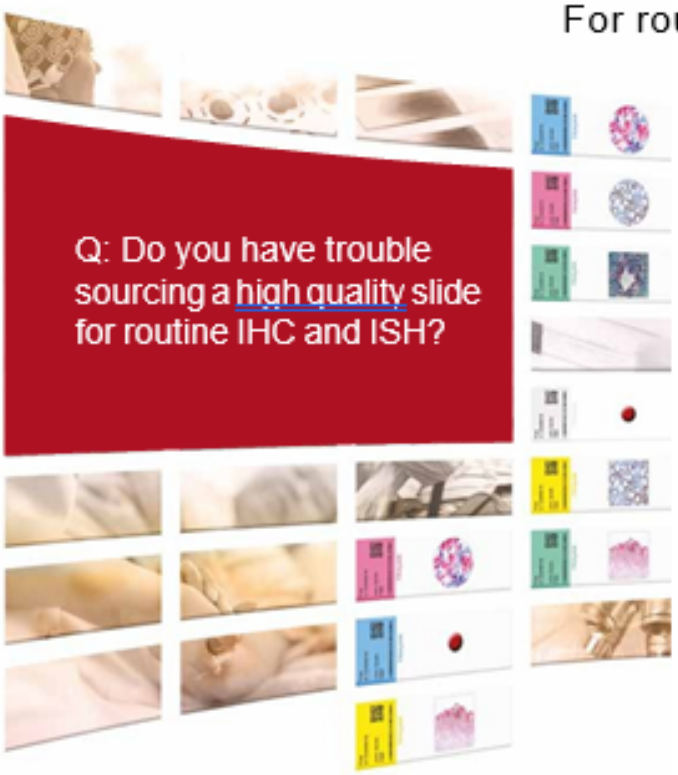

A: The new Series 2 Enhanced Adhesive slide is suitable for a wide range of applications.

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.

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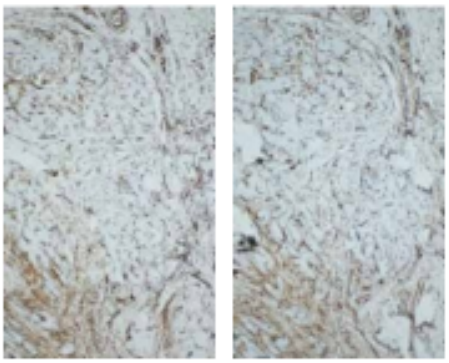
472042481	Trajan Series 2 Enhanced Adhesive Microscope Slide 76 x 26 mm with clipped corners, 100 pcs



Human mammary gland stained on BenchMark ULTRA with Vimentin 9.

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"Dammit." I flipped through the files in anger. "They're missing the WMD stuff." Indeed, they were. The folder which should have been full of incriminating VA toxicity data, Draize test reports and LD50 results contained just a single reference to Project Epiphyre. What the hell was that? Getting the ethanol fixatives was half a victory but I could not find the evidence of chemical weapons. "Look for something called Project Epiphyre! Kiire!"

"I found it, Evva." André scanned the papers. "Histolo-Sea isolated the VA toxin in a species of rainforest fern…A type of epiphyte…They cancelled the sea anemones and moved to growing ferns under Project Epiphyre."

VA was found in a fern as well as an anemone? Histolo-Sea had stopped the anemone harvesting because it was easier to grow plants than sea creatures. My shoulder's sagged as I realised this whole endeavour was like the flight of a chicken. I had no evidence.

"Evva, we have half a victory and I hear sirens." André pocketed the fixative notes and pulled me away from the filing cabinet. "We need to run like biro ink in a tissue processor." He pushed me out of the building and onto the street as I considered what had just happened. If Variant Anthopleurin was found in ferns then of course they would end the project.

Because with fronds like those, who needs anemones?

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In my last article, I confounded Carnoy's with FAA, implying Carnoy's used formaldehyde. This was embarrassing and I apologise.

Ms. Perry, Dr. Chung, and Dr. Hewitt invented the BE70 ethanol fixative according to patent application (62/255030). They do not make WMDs (to the best of my knowledge—I don't watch them all the time).

Anthopleurin is real, VA is not. Nor is Histolo-Sea according to Google. The rest is true (apart from the obvious) but please do not misuse anything in this story. Cops have no sense of humour.

PS: Try the Lisa D Iced Tea. It's quite good.