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TISSUE PAPER

President's Report - Jerres Alcober

Welcome to the final edition of the Tissue Paper for 2019. This year has again provided members with regular continuing educational & networking opportunities. The final event of 2019 was the AGM & 3rd Scientific Meeting. A special mention to Dr Luke Vasanthakumar, Tony Reilly, Brett Harrison, Trajan & the Normanby Hotel for another informative and entertaining evening for our guests. Attending members also elected the 2020 HGQ Committee at this event – President: Jerres Alcober; Secretary: Amanda Marsden; Treasurer: Mark Bromley; Committee: Chris Cazier; Melissa Hillas; Greg Bowlay; Emma Hughes; Michelle Goddard; Lloyd Blundell; Brett Harrison; Chin Ho Suen & Jessica Ellis. I would like to take this chance to thank Kellie Vukovic & Arin Chandra for all their work during their time in the committee. The 2020 committee are looking forward to providing many more opportunities for continuing education and networking for our members again next year. Stay tuned to the following events on the 2020 calendar - 4 editions of the "Tissue Paper" (FEB, MAY, AUG, NOV); 1st Scientific Meeting – Envoi Pathology (MAR); 2nd Scientific Meeting (MAY); Social Event - Trivia Night (JUN); 3rd Scientific Meeting - AIMS (AUG), QLD State Histology Conference & AGM – Cairns (FRI 9th - SUN 11th OCT). Thank you again to our valued members and proud sponsors for your continued support during the year and hope that you all enjoy the upcoming festive season. To keep up to date with the HGQ, take advantage of free membership at www.hgq.org.au

Until the next edition, take care, stay safe and enjoy!! Happy reading ;)

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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 newsletter@hgq.org.au in digital format

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How to Cut It as a Dissectionist

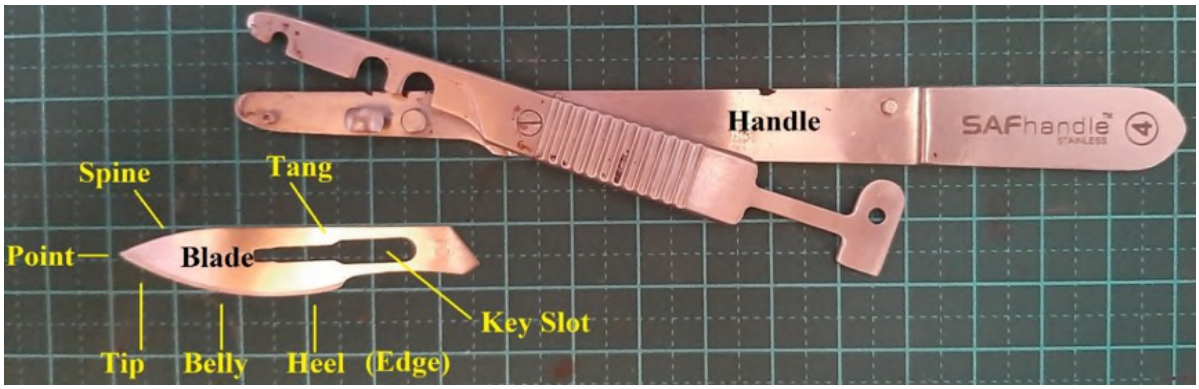
Surgical knives have come a long way since the days of Aztecan obsidian or Egyptian bronze. Earliest examples of medical blades were made of flint and date back to 8000 BCE. The first discovered records are from Hippocrates describing something similar to the shape still used today.

Histologists ought to know about scalpels as we use them daily. Scalpels have more components than merely a handle and a disposable blade. Non-disposable dissection knives can also be used but these are rarer and require special care. A scalpel must be used correctly as it can do a lot of damage to the specimen and the user when mishandled.

The Anatomy of the Scalpel

A scalpel has several components, all serving a purpose.

- The Blade is the sharp piece of metal to which the handle is attached to create a usable scalpel.
- A blade is attached to a Handle. Some handles can be quite advanced.
- The hole in the blade through which the handle is fitted is called the Key Slot. This is where most blade failures occur if the scalpel is misused (especially under torsion).
- The Tang is the bit which sticks into the handle. A "full tang" is for kitchen knives and hunting knives where long-life is desirable. In a disposable blade, the weakest point is the key slot so a full tang is pointless.
- The stabby bit is called the Point. A blade may or may not have a useable point. Accidentally pushing the tip into tissue is harder with a rounded point.
- The Edge is the entire cutting portion of the knife. The edge is divided into three sections (tip, belly and heel) but this distinction is irrelevant in a disposable blade. It is of importance only to those of us who still use non-disposable dissection knives. The Tip is the furthest part of the edge, roughly, the final third. It should be the sharpest part of the edge, used for careful slicing (delayed closures, strange shapes et cetera). The Belly is the middle edge and is used for general slicing where simple straight lines are needed. The Heel is edge closer to the handle, to be used for heavier work through tougher tissue (it is easier to control as it is closer to the hand and the spine is usually heavier at this point).
- The side opposite the edge is called the Spine.



Above: The components of a basic scalpel

Scalpel Blade Types

The blade is the business end. There are over twenty types, each with their own designation and purpose. The numerical designations are mostly consistent across manufacturers. Blades can be grouped broadly into basic shapes which I shall discuss. The other major consideration is the key slot which is usually Fitment 3 (smaller key slot) or Fitment 4 (larger).

10 is the traditional scalpel but considered a bit too small for standard histology. The curve allows for an ergonomic rolling motion when cutting. This takes some of the strain away from repeated actions.

22, 23 and 24 are typical ones in dissection. They are a larger version of 10. The longer blade means they endure multiple specimens.

15 is a smaller version of 10. The small size allows for fine work with less risk of collateral damage. 15 is more for fine surgical work than dissection.

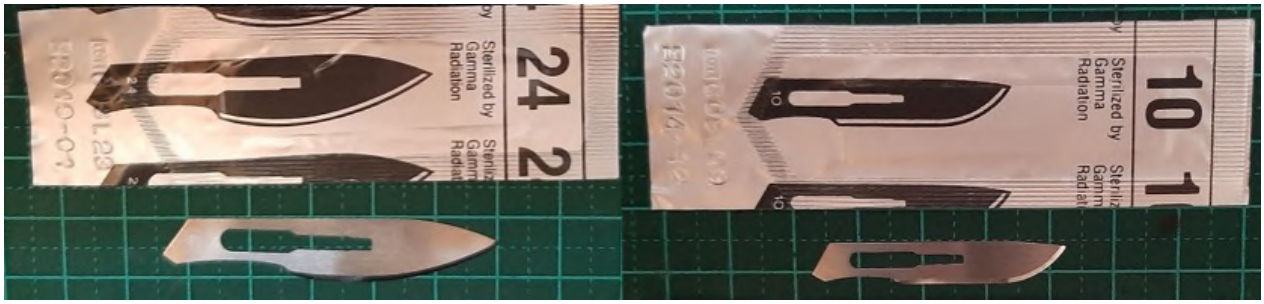
12 has a concaved edge, good for removing sutures without risking the underlying tissue but little else. Outside of histology, it is used for incising tubular structures.

11 is used for fine stabbing work (lancing boils et cetera) but from our point of view, it is ideal for precise non-linear cuts. Used carelessly, it can easily destroy a specimen.

14 is a flat blade without a point. It is safer but the flat edge makes it harder to get a clean cut all the way through.

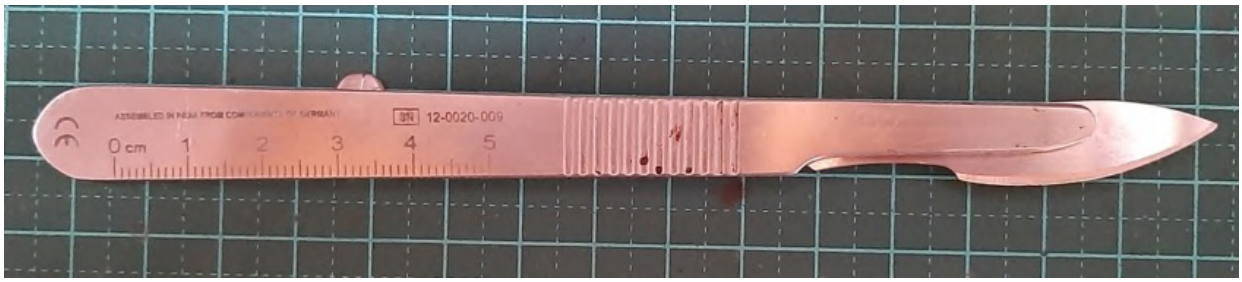
Most routine histology can be done without specialised blades but occasions arise when the right tool is critical. Bear this in mind when fitting your blade: Is this the best choice for the specimen?

Above: Various blade types. Below right: A 24 blade with a Number 4 Fitment. Below left: A 10 blade with a Number 3 Fitment.



Handles

Scalpel handles come in a variety of shapes. The ideal one depends on the user. They are usually not disposable. Try as many as you can to find which works for you.



Above: A scalpel handle with in-built ruler.

Features include quick-release actions, hexagonal cross-sections for oblique slicing, type of material used, rulers engraved into the handle and the fitment size (nowadays, reduced to Type 3 or Type 4). Dual end scalpel handles can take both types of blades, one at each end but using both at the same time would be considered unsafe by boring people. Other handles hold the blade at an angle for ergonomics. Fancier versions allow this angle to be adjusted for preference

Right: Some scalpel handles come with attachment points at each end for those who feel daring enough to wield them. Left: Being a Sith Lord is no excuse to ignore the comfort of an angled scalpel blade. Below: Find a handle which works for your personal style of dissection.

These variations have their own characteristics and it lies with the dissectionist (and his quartermaster) to decide if the money is worth the handle. My personal favourite was an eight-inch handle because I am used to wielding a larger tool.

Above: My favourite scalpel handle (bottom) with 24 blade and a standard scalpel for comparison with a 22 blade (top).

Permanent Dissection Blades

Permanent blades are either purpose-built or chosen by dissectionists according to personal taste. There are myriad of knives which can be sharp enough for dissection and easily cleaned/sterilised (a vital but often overlooked consideration). Most of my colleague's preferred style was a single piece of quality metal with no adornments. These styles are usually found in knife shows by small scale artisans and are rare as each tends to be unique.

As the blade is permanent, it needs to be robust. Avoid anything which claims to be Damascus steel as that is a blatant lie. Knives with whorls are suspect. High-carbon steels are good if kept clean (difficult for an entire shift). Fancy edges are difficult to hone so choose straight or slightly curved styles. Ceramic and titanium are (currently) inferior to steel but are durable so they will be nearly as sharp for less effort and for longer.

The ultimate choice is for the dissectionist. Ego is a major force behind selecting a non-traditional dissection knife but there is much to be said for finding that perfect knife, especially when it is to be used for hours each day.

Above: Examples of the styles of knife I have seen used in dissection

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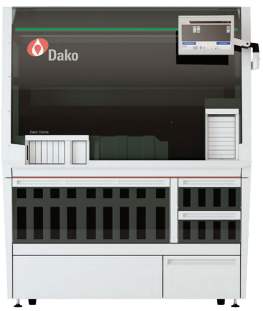

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Most laboratories no longer pay for sharpening services so it falls the dissectionist to maintain their own equipment if disposables are eschewed.

Both factory and bespoke knives must be honed to a sharp and durable edge. This is usually done using a flat grinding surface (a stone) to remove anything which is not a blade. Over time, the edge can become folded (as it is very thin and easily bent). A steel is used to restraighten the edge by dragging the knife along the steel. This is quick and done during a shift as often as needed.

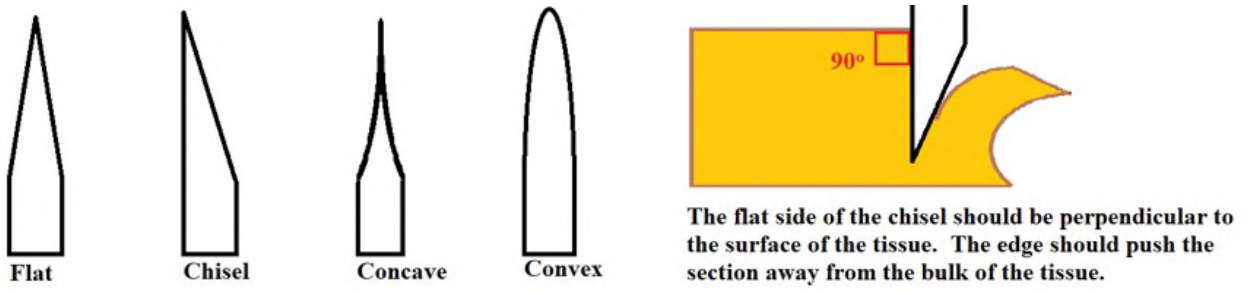
There are four common types of edges and further multitude exist as variations of these four.

Flat: A standard isosceles triangle cross section, good all-round edge. The longer the triangle, the sharper the edge but the more vulnerable it is to dulling.

Chisel: An asymmetrical edge created by honing only one side. Sharp, easy and fast to create and maintain but the cuts tend to curve away from the honed side. See diagram for the best way to use a chisel edge.

Concave: Very sharp but prone to rapid dulling. Difficult to create and maintain.

Convex: Durable and moderately sharp. Difficult to create/maintain.



Right: Four basic cross sections of a blade. Left: Using a chisel edge properly.

Knife sharpening instructions can be found on the internet but the basic plan is to grind the sides of a blade into a triangular cross-section and keep it that way.

Lab Hack: The underside rim of a saucer and a smooth pair of forceps can be used to hone and straighten a blade in a pinch.

Non-disposable blades are an option for dedicated dissectionists but require a thought and effort when selecting and maintaining a dissection knife.

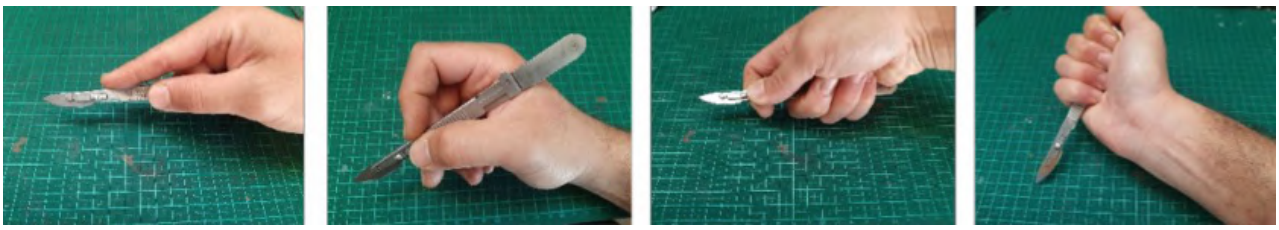
How To Hold a Scalpel

There are three basic ways to hold a scalpel (of which I know–feel free to teach me a fourth) and innumerable variations. The "correct" grip depends on the circumstances.

Table Knife Grip is the instinctive grip into which most people stumble. The scalpel is held like a table knife (if one from England or the Continent) with the index finger on the spine. It allows a simple long slicing motion, good for taking fast and easy transverse sections without canting the blade. The index finger is used only to control the blade not to apply force. Cutting motions/force come from the arm rather than fingers, hand or wrist. Be certain which side is the blade before using this grip.

The Pencil Grip is like holding a pencil (unsurprisingly). It gives excellent control when the tissue is immobilised but not when the specimen is free to move. This is more for dermatologists (or artists) who need to cut with precision. Gripping too close or far from the blade can cause cramping. Full control requires resting one's hand on a solid surface. Controlling the cuts is done through the fingers like controlling a pencil.

Kitchen Knife Grip is when the scalpel is pinched at the border between blade and handle. The remaining fingers are wrapped around the handle some distance from the pinch. This grip is good for long incisions. It is a compromise between control and ergonomics but is slightly dangerous as the scalpel is not a kitchen knife and therefore has no bolster. However, scalpels are never used with force so the danger is minimal. This can be tiring as the fist can grip too tightly when controlled slices are needed.



From left to right: The table knife grip, the pencil grip, the kitchen knife grip, the Norman Bates grip (for salary negotiations).

Which is the best? The answer is simply whichever gets the job done for the dissectionist. Experiment (we are scientists after all) with these and others if you are unsure of which will work best for you and the task you are performing. And never be committed to a single type of grip as each person, specimen and situation is different.

Scalpel Safety Axioms

A final few words on safety:

- A sharp knife is a safe knife. It will act the way you expect it to act.
- A falling knife has no handle. Do not try to catch it, just get out of the way.
- Put down the scalpel in the same place and orientation each time.
- Never recap/reuse a scalpel, just throw it out. It is not that expensive. It really is not.
- Secure the cutting board.
- Remove the blades every time you stand up from a bench—do not let someone else pay in blood for your apathy.
- Hold the specimen with forceps not your fingers.
- It saves more time to change a dull blade than to tolerate it for one more specimen.

Below: A basic movie prop for autopsy scenes.

In the nineties, InGen redesigned the tang for fast and safe blade changes. Unfortunately, InGen's transport company went bankrupt so the blueprints could not be exported.

Their new tang plan had no-one to truck with.

There are many choices when it comes to dissection including blade type, handle type (if one opts for disposable equipment) or knife and edge type (if one prefers non-disposable knives). Proper grip and safety are important considerations too. The best combination is a function of what is available, what works, personal preferences and safety.

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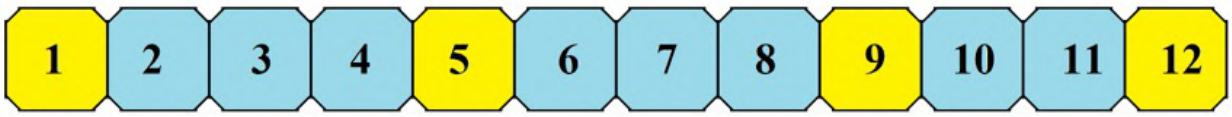
How to Cut TRUS Biopsies: Cores and Effect

TRUS prostate cores are annoying. The need for serials on such small tissue and to keep/stain only a few creates a tedious task with too many slides for efficiency. There are ways to reduce the labor of the task with simple techniques and care. It helps to remember that cores are not at all difficult, merely tedious and painstaking. With care, anyone can section TRUS cores.

The basic plan is:

1. Embed the cores flat and tight (reduces wasted tissue)
2. Pare off excess wax around the tissue (more sections per slide and easy ribbons).
3. Cut a ribbon of the required number of serials
4. Take the sections as needed

Spare slides are cut at the start because the cores are hard to obtain and easy to destroy. It is safer to cut the spares than risk facing out the tissue later if IHC slides are needed. This also ensures the tissue between the H&E sections does not go to waste. Typically, the order is 9 or 12 serial sections, 3 or 4 of which are stained with H&E and the remainder kept for later. This can result in over 100 slides if multiple cores are taken. The H&E sections, if embedded and sectioned properly, can be put onto a single slide to reduce this number.

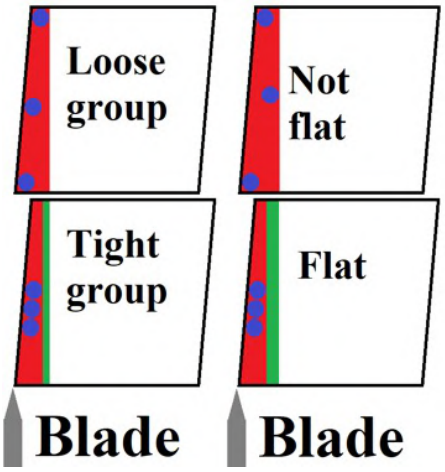


A typical pattern is 9 or 12 serial sections with some stained as H&E (1, 5, 9 and 12 in this example) for microscopy and the remainder kept as spares if needed for IHC analysis.

1) Embed the cores flat and tight (reduces wasted tissue)

Given the diameter of a TRUS core, it is imperative that all measures are taken to ensure they are not faced out. This means alignment, embedding and grouping must be as exact as possible.

Alignment is never perfect so some degree of facing is necessary. Embedding as flat and as tight as possible makes it easier to get the full face with minimal loss of tissue. Cores are one of the rare examples where using a tamper is justified to push the tissue flat. Whilst this applies to all blocks, take extra care with TRUS cores as they are so small.

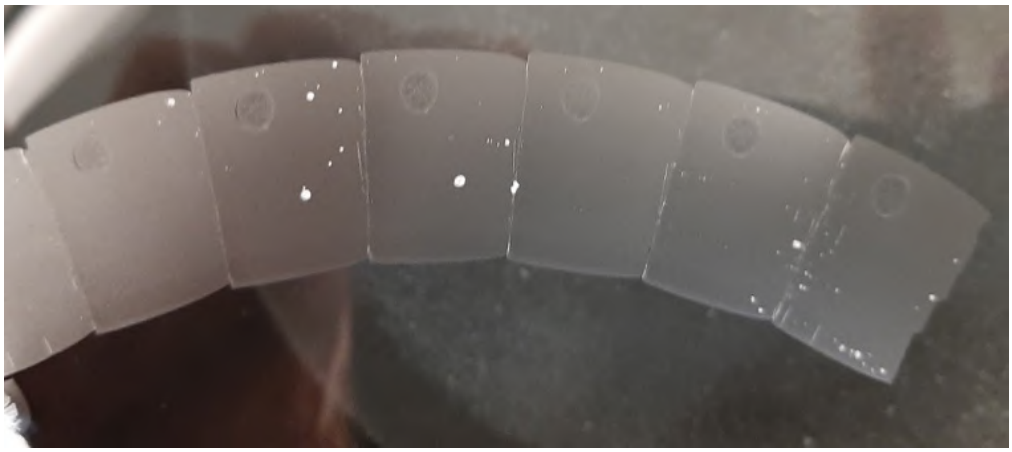


If the block is not PERFECTLY aligned…The wider group of cores (blue) requires deeper facing than the tighter group. The red area represents the full depth of the cores relative to the microtome blade. The green area is the difference. Likewise, for cores which are not embedded flat. Ideally, the block will be perfectly aligned and the cores will be perfectly flat.

2) Pare off excess wax around the tissue (more sections per slide and easy ribbons).

Paring the block is a matter of cutting off the empty wax around the tissues. This creates ribbons which can break apart as needed and allows more sections onto a slide/waterbath.

Paring is best done with a sharp knife and care needs to be taken that tissue is not removed by mistake. Aim for roughly 45 degree dihedral angles of slope and be sure to get a straight edge. Each section adheres to the next via these edges so straight cuts give more contact area and less chance of breaking the ribbon. Remember: Clean straight edges give stronger ribbons.

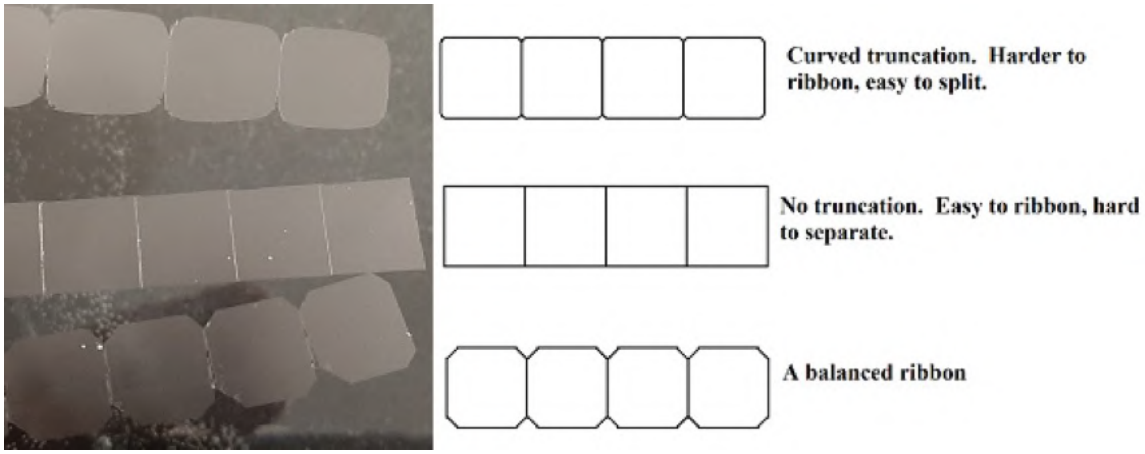


If the sides of the block are not cut parallel, the ribbon will curve.

Stronger ribbons are good for laying out onto the waterbath but we also need to separate the sections. Truncating the corners of the block creates break points which to separate the sections at will.

The trimmed block is ready for sectioning. A quadrilateral with truncated corners.

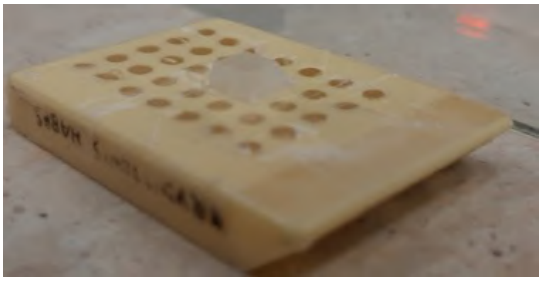
If the corners are not truncated, the sections are harder to split. The sharper the angle, the weaker it is. A curved corner of a mold creates a very acute angle which is rather weak. The angle of incidence acts as an artificial crack from which a real crack can propagate. By choosing the angle we want, we can control how strong or weak the break point is. Balancing this is what allows for easy ribboning of easy to separate sections

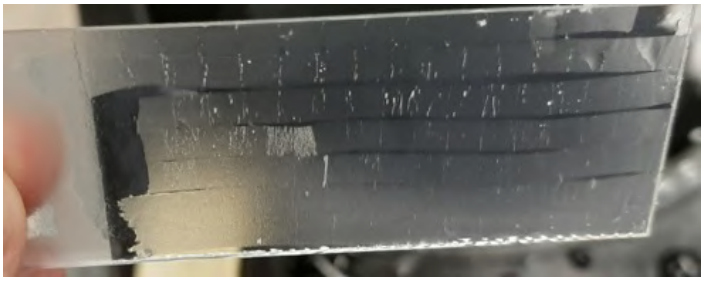


Top: Standard sections from an uncut block. Middle: Sharp corners create a strong easy-to-section ribbon. Bottom: Truncated corners balance a strong ribbon with easy separation of the individual sections.

Essentially, we are recreating the shape of the original mold but with two important differences: The block is now the size we want it to be and the break points are not as delicate.

An effect of paring a block is that it is faster to get the full facet of the wax and tissue.

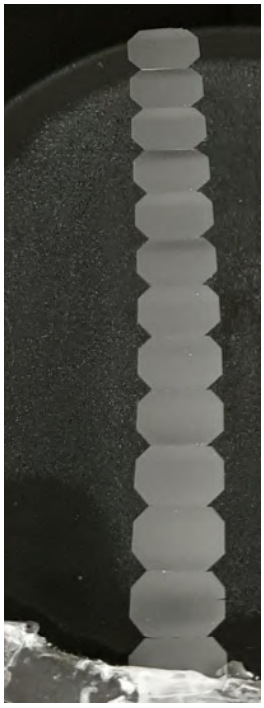


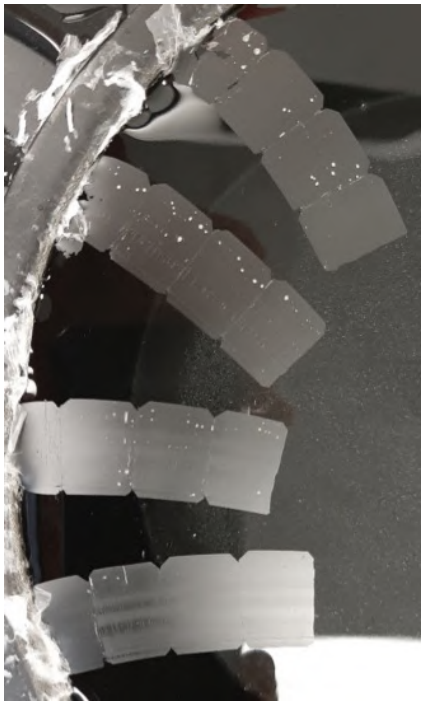


It is possible to put over 100 serial sections onto a single slide by paring wax off a block. This is used only for serials through a biopsy but shows what can be achieved by paring down a block.

3) Cut a ribbon of the required number of serials

Having pared the block, sectioning a single ribbon is quite easy. The individual sections can be collected as needed. If necessary, the serials can be taken as 2 or 3 ribbons. This is easier and loses only 3 sections of the serials.

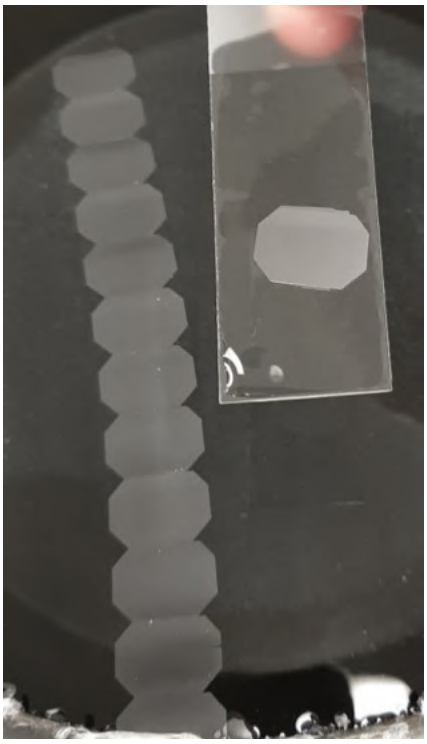




The H&E sections can fit onto one single slide rather than 3 or 4 which, for a 12-core case, reduces 48 H&Es to a manageable 12.

4) Take the sections as needed

The sections are then taken as needed. Obviously, it is important to know which is the first and last section to maintain the order. Break the sections apart and collect them onto the appropriate slides. Aim for the center of the slide. The H&Es should fit onto a single slide if the block is embedded and pared correctly. Just take an H&E section starting at the top of the slide, take the spare sections, take the next H&E just below the first section until all 3 or 4 H&E sections are on a single slide. If a second slide is needed, so be it but the vast majority of H&E sections can fit onto a single slide using this technique.



Individual IHC sections can be placed on their own slide. All 3 or 4 H&Es can fit onto a single slide for convenience.

TRUS cores need not be difficult or cumbersome with proper technique. Embedding them flat and tight means they are quick to face. Paring the excess wax makes for fast, strong ribbons which are easy to handle. The sections can be combined onto a single slide to reduce the number of H&Es needed. All of these combined creates a simple procedure for reducing TRUS cores into a quick and easy task for the microtomist.

Emily Warré

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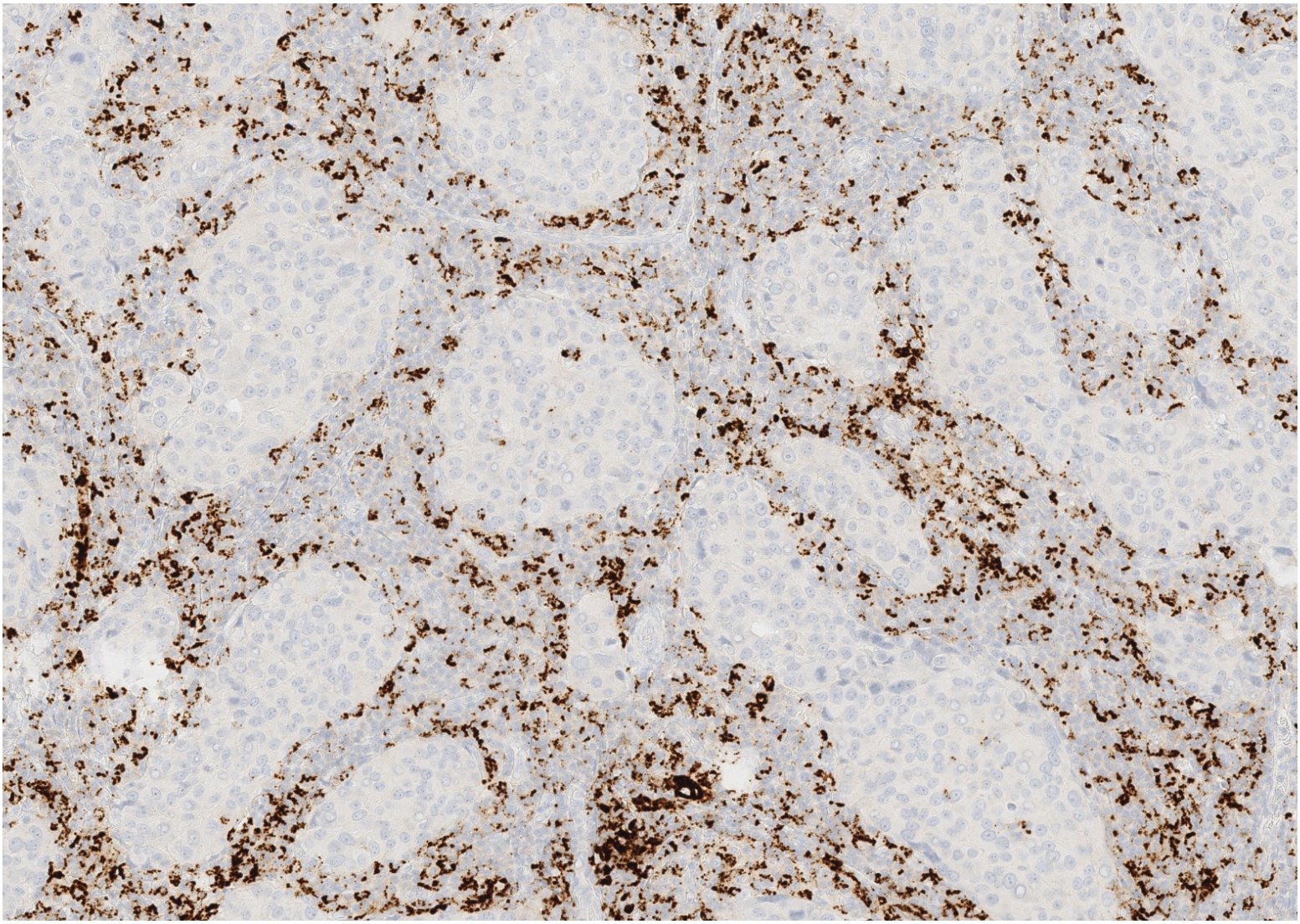


1. Ventana Medical Systems Inc. VENTANA PD-L1 (SP142) Assay Package Insert.
2. TECENTRIQ Prescribing Information.

Roche Diagnostics Australia Pty. Limited 2 Julius Avenue, North Ryde, NSW, 2113
Tel: +61 2 9860 2222
ABN 29 003 001 205

www.roche.com
rochediagnosticsaustralia.com

What's new with the HGQ?

Upcoming Events

February 2020

- **Newsletter - Tissue Paper (Vol 50)**

March 2020

- **Scientific Meeting - Laboratory**
  - Venue - Envoi Pathology
  - THU 26 MAR

May 2020

- **Newsletter - Tissue Paper (Vol 51)**

- **Scientific Meeting - Laboratory**
  - Venue - Brisbane (TBC)
  - THU 07 MAY

June 2020

- **Social Event - Trivia Night**
  - Venue - Brisbane (TBC)
  - FRI 26 JUN

August 2020

- **Newsletter - Tissue Paper (Vol 52)**

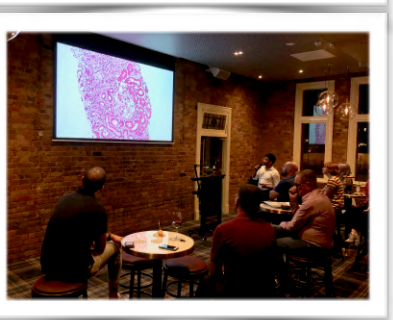
- **Scientific Meeting - Joint AIMS/HGQ**
  - Venue - Brisbane (The Pineapple Hotel)
  - THU 13 AUG

October 2020

- **Conference & AGM - QLD State Histology**
  - Venue - Cairns (TBC)
  - FRI 09 - SUN 11 OCT

November 2020

- **Newsletter - Tissue Paper (Vol 53)**

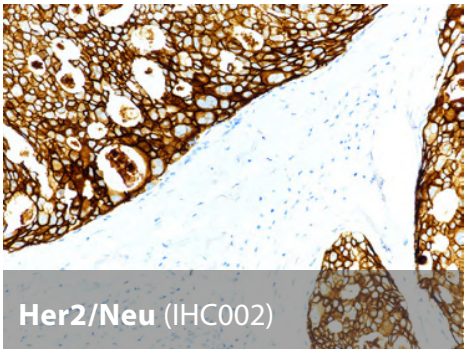


GeneAb™
Molecular Pathology Antibodies

METAGENE GenomeMe CANADA

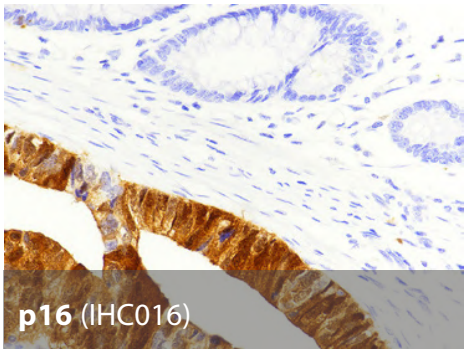
Free samples available:

CE IVD



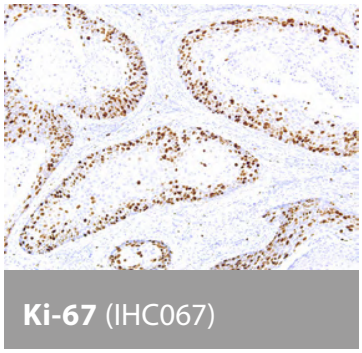
Her2/Neu (IHC002)

The Her2/Neu (c-erbB-2) proto-oncogene is a transmembrane receptor tyrosine kinase that is clinically indicated in a number of carcinomas, including ductal breast cancer as well as pulmonary and gastric adenocarcinomas.

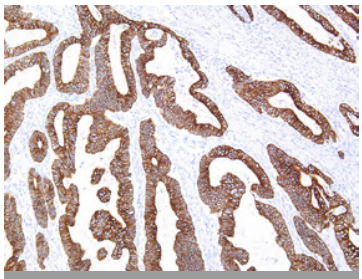


p16 (IHC016)

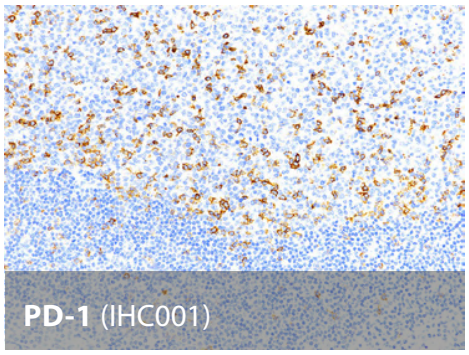
p16 is a tumor suppressor that is a key marker in several human cancers including head and neck cancer, as well as carcinomas of the esophagus, pancreas, lung, biliary tract, liver, colon, and urinary bladder.



Ki-67 (IHC067)

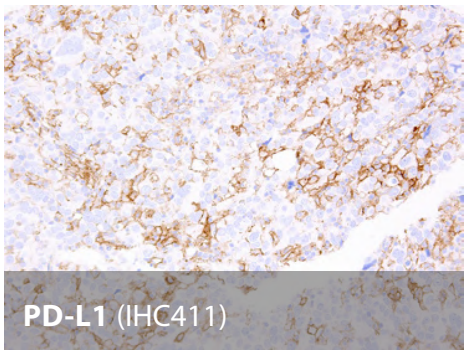


Cytokeratin 18 (IHC018)



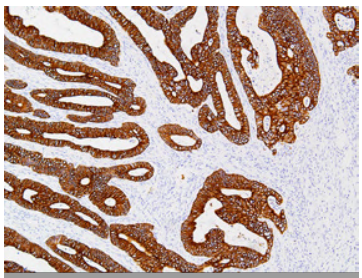
PD-1 (IHC001)

PD-1 is a co-receptor on the surface of activated T-cells, B-cells, and macrophages. Therapies targeted toward PD-1 have shown remarkable clinical responses in patients with non–small-cell lung cancer, melanoma, and renal-cell cancer.



PD-L1 (IHC411)

PD-L1 is involved in immune suppression and anergizes cytotoxic T cells through binding of the PD-1 receptor. Overexpression of PD-L1 may allow cancer cells to evade the actions of the host immune system.



Cytokeratin 19 (IHC019)

Available Formats:

Free Sample:
- Concentrate (1:100-1:200): 10µl
- Pre-dilute: 1ml

Product Size:
- Concentrate (1:100-1:200): 0.1ml and 1ml
- Pre-dilute: 7ml and 25ml*

**25ml available for some antibodies only*

Source:

Mouse Monoclonal:
- Her2
- p16
- PD-1
- Ki-67
- Cytokeratin 18
- Cytokeratin 19

Rabbit Monoclonal:
- PD-L1

Contact Us:

METAGENE

Website: www.metagene.com.au
Email: admin@metagene.com.au
Phone: 1800 788 498