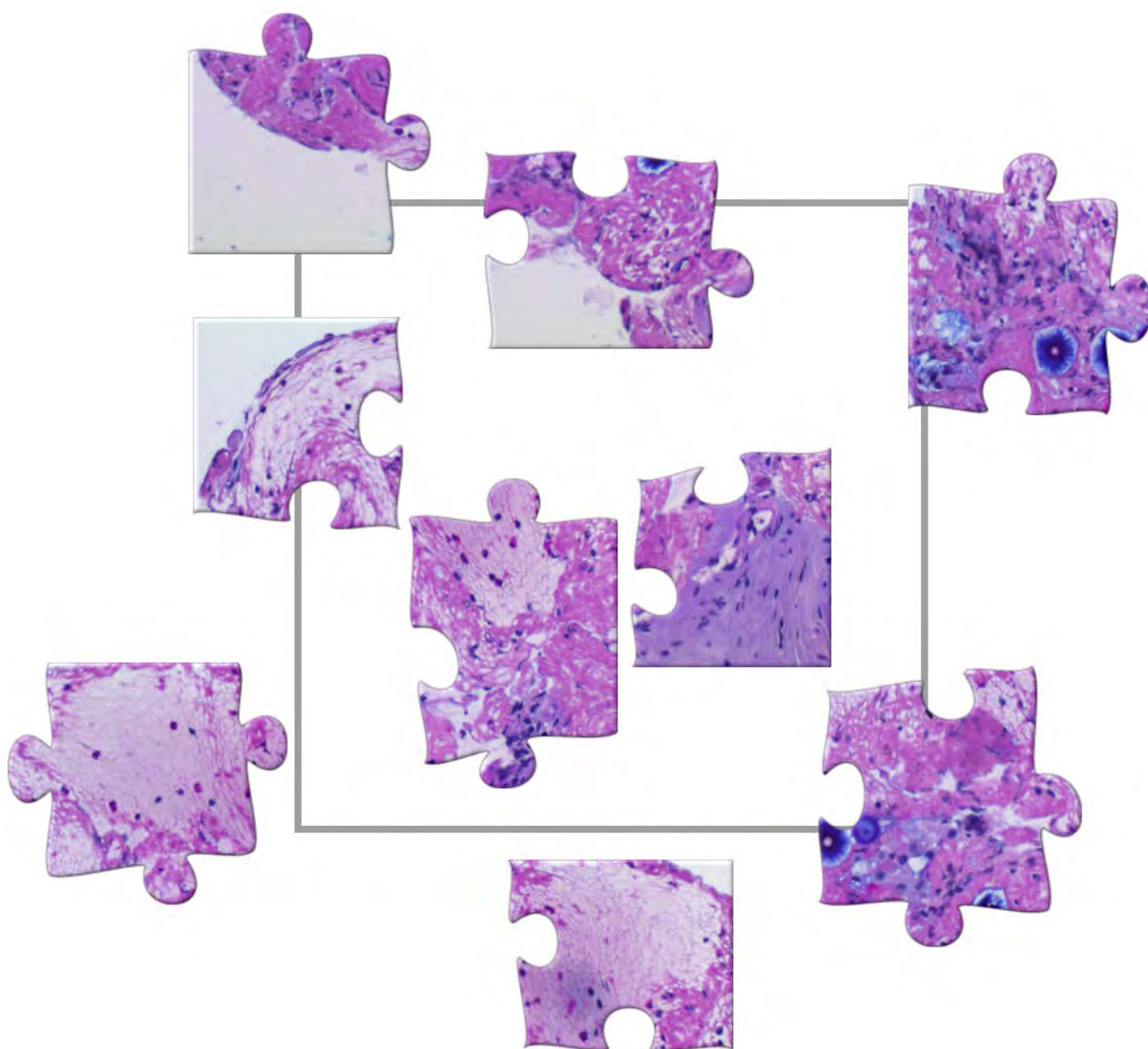


***HISTOTECHNOLOGY***

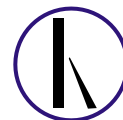
*SOCIETY OF NSW*

# HISTOGRAPH



**Newsletter No.1 2023**

**April**



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## Histograph Sponsors

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# HISTOTECHNOLOGY SOCIETY OF NSW

## JOIN NOW!

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Membership is open to anyone interested in Histotechnology or Histology who supports the objectives of the Histotechnology Society of NSW.

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- Access to members only educational content via the website
- HTS NSW members are eligible for a reduced rate off Australasian Professional Acknowledgement of Continuing Education (APACE) participation fees
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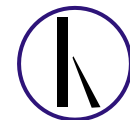
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### SCAN TO JOIN



*\*Terms and conditions apply  
Always refer to <https://histonsw.org.au>*





## Chairperson's Report

### Recent events

We kicked off the year with 3 presentations. Thank you to our presenters Dr Elizabeth Salisbury, Joanne La Malfa and Carlee Hill of NSW Health Pathology who presented on 'The value of cytology in pathological diagnoses,' Purpose of the mobile cytology lab - ROSE' and 'Benign gynaecology specimens.' We would also like to thank Agilent for sponsoring the March Surgical Cut-up webinar.

### 2023 webinar series, register now!

Click the links to register for our 2023 free webinar series.

Sat April 29<sup>th</sup> - [Hirschsprung disease](#)

Sat June 24<sup>th</sup> - [RCPAQAP presentation](#)

Sat August 26<sup>th</sup> - [Forensics presentation](#)

Thurs October 26<sup>th</sup> - [Annual General Meeting + surgical cut-up](#)

Sat November 25<sup>th</sup> - [Macroscopic triage and cut-up of placentas](#)

### 'Histo at the Beach' 29<sup>th</sup> July, save the date!

It is official! We are holding an in-person event in Newcastle on Saturday 29th of July 2023.



Join us as 'Noah's on the Beach' for a networking morning and afternoon tea, 2-course lunch and several quality educational sessions including 'Forensics on the foreshore' by Dr Allan Cala all while soaking up beautiful views of Newcastle Beach. Check out the next page for more details.

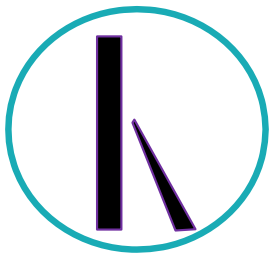
You could even make a weekend of it; Newcastle is full of lots of fun [things to do and see](#) :-)

I hope to see you all in-person again soon!

Warm regards,

**Leah Simmons**





**HISTOTECHNOLOGY**  
SOCIETY OF NSW



We are getting together in-person again! Join us for

# Histo at the Beach

**Saturday 29<sup>th</sup> July 2023**

**First presenter announced: 'Forensics on the foreshore'**

**Dr Allan Cala, Forensic Pathologist, NSW Health Pathology**

**Get out and about! Face to Face 10am till 3.30pm**

**Don't forget the food! 2 course lunch & drinks**



**Register now!**

<https://histonsw.org.au/event/histo-at-the-beach/>

Click [here](#), use the QR code or visit our website to register!\*

Registrations close 17<sup>th</sup> of July 2023

# HAPPY HISTOTECHNOLOGY PROFESSIONALS DAY

FRIDAY 10TH MARCH 2023

Histotechnology Professionals Day is on the 10th of March every year and is a day to raise awareness of the importance of Histology and to celebrate the amazing work of Histotechnology Professionals all around the world.

This year our theme was "Hats off for Histotechs", encouraging everyone to put a hat on so they could take it off for Histology Professionals.

Photos from Team Histo at the children's Hospital at westmead



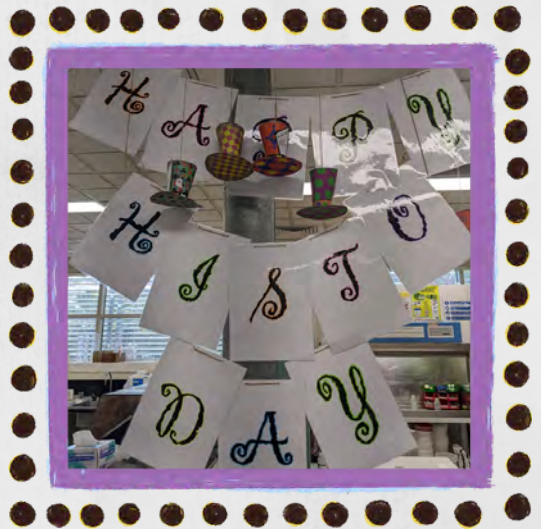
HATS  
ON!

HATS  
OFF!





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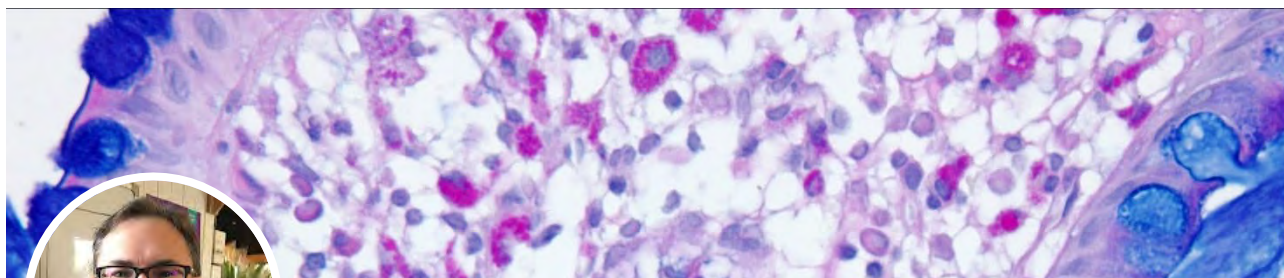
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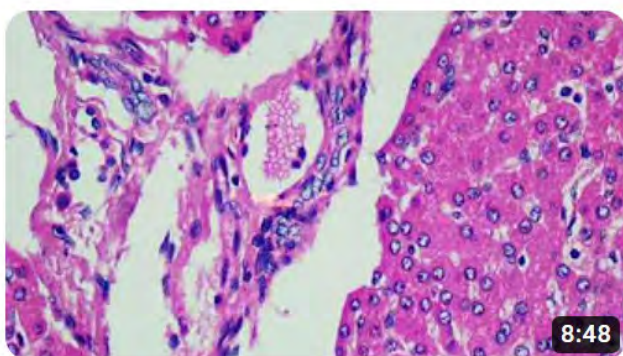


## Social Media Spotlight: Damien Harkin

By Momoko Sakaki, The Children's Hospital at Westmead

This issue we are spotlighting Damien Harkin, Professor at the Queensland University of Technology (QUT) and Visiting Senior Scientist at the Queensland Eye Institute. While he has social media presences on Instagram, Twitter, and Tumblr, YouTube is the platform where he is most prolific with 2.4k subscribers.

For a discipline like histology that relies heavily on the visual, quality instructional and troubleshooting videos are priceless. Professor Harkin's growing video collection includes topics such as fixation, microtomy, cryotomy, special stains and using Photoshop for Histology.



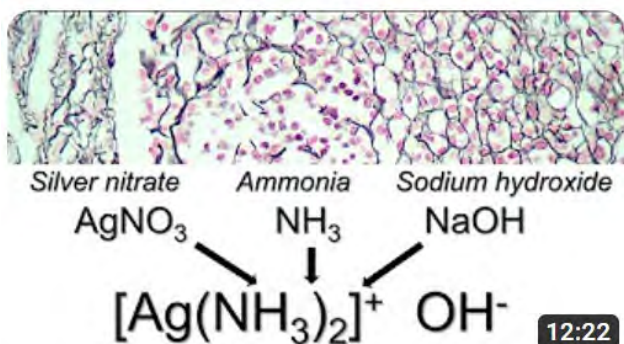
### Fixation Artefacts

1.8K views • 1 year ago



### Preparation of frozen tissue sections (Cryotomy)

42K views • 2 years ago



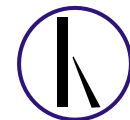
### How to prepare ammoniacal silver

2.5K views • 2 years ago



### Gram Staining Methods

821 views • 1 year ago



The videos that students and trainee histologists may find the most useful are the “Microscope Tutorial” series. In this series, Professor Harkin discusses the stain whilst showing exactly what he is looking at down the microscope. He evaluates student slides, troubleshooting the issues they had performing the stain and how that affects the staining of various tissue elements.



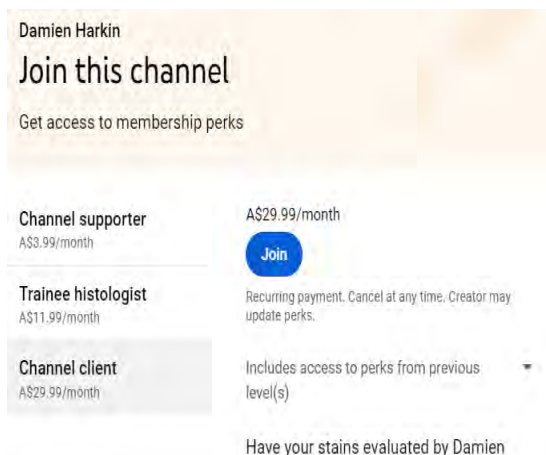
**Microscope tutorial - Troubleshooting the MSB stain**

1.4K views • 3 years ago

**Microscope tutorial - Troubleshooting the Van Gieson stain**

2.6K views • 3 years ago

A relatively new addition to the channel is the ability to join as a Member with three different membership levels. The first level is as a “Channel Supporter” which gives access to loyalty badges, custom emojis and priority replies to comments. The second level is “Trainee Histologist” which gives access to Professor Harkin’s online Essentials of Histological Technique course. The third level, “Channel client” gives access to the course and having your slides and protocol evaluated by Professor Harkin. This is all for a great cause as the profits go to support research at QUT.



If you would like to support a researcher and scientist who is obviously passionate about histology and teaching.

### Follow Damien!



YouTube Damien Harkin - @damienharkin



Instagram Damien Harkin - @damgerryharkin



Twitter Damien Harkin - @DamGerryHarkin



Tumblr Simply Histology - @histopics



# VEGA Laser Cassette Marker

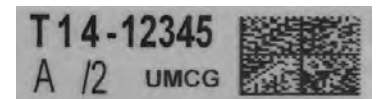


The Vega Laser Cassette Marker offers permanent indelible marking of tissue cassettes with text, linear barcodes, and two-dimensional barcodes.

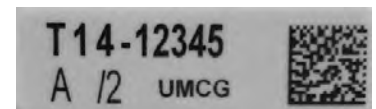


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## An observation on silver stains - Von Kossa Stain for calcium and AgNORS - argentaffilic?

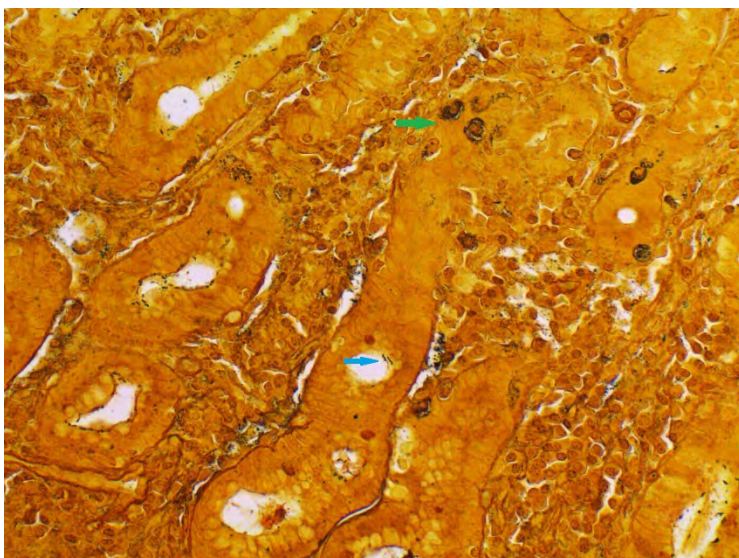
Tony Henwood, Principal Scientist, Histopathology,  
The Children's Hospital at Westmead.

When characterising silver reactions in histology, histochemists have routinely classified them into Argentaffilic, Argyrophilic and Argyrophobic (Grimelius 2004).

- The argentaffin reaction is positive in cells where the cellular proteins bind silver ions from the silver solution and reduce the ions to metallic silver. This technique is a “one-step” staining process that is based mainly on using an ammoniacal silver or methenamine silver solution. Enterochromaffin cells, lipofuscin and melanin are argentaffilic.
- The argyrophilic reaction is a “two-step” method, where the sections are first treated (impregnated) in a silver solution and subsequently submitted to an external reducing process. Most hormone-producing cells, including thyroidal C-cells. Pancreatic islet cells and gastrin cells are argyrophilic.
- Argyrophilia describes cells and structures that do not bind silver and therefore are argentaffilic and argyrophilic negative. Amyloid is (mainly) argyrophobic.

Many cells and cellular structures can be made argentaffilic or argyrophilic by employing selective pre-treatments. This is often achieved by creating numerous reducing groups in them, e.g., aldehyde groups produced from polysaccharides in cartilage or fungi by oxidation with periodic acid or hydroxamic acids produced from phosphoglycerides in myelin by alkaline hydroxylaminolysis (Gallyas 2008).

Pre-treating sections with periodic acid followed by methenamine silver allow the demonstration of basement membranes, whilst pre-treatment with chromic acid allows the demonstration of fungi. Both of these techniques require visual determination of the end-point to ensure specific staining with minimal background. This is a unique feature of argentaffin reactions.



The reticulin stain and the Warthin-Starry stain for bacteria use pre-treatments to improve the sensitivity and specificity of the reactions. With respect to reticulin staining, permanganate oxidation and ferric alum are often used whilst uranium nitrate is regularly used for spirochetes. Several investigators have replaced uranium nitrate with lead nitrate or phosphotungstic acid, especially for the demonstration of helicobacter (El-Zimaity et al 1999, Buesa 2001).

Figure 1 Gastric biopsy stained with Warthin-Starry for helicobacter pylori (blue arrow). Note the demonstration of argyrophilic cells in the base of the crypts (green arrow) (WS x40).



## An observation on silver stains - continued

### AgNORs

Nucleolar organizer regions are loops of chromosomal DNA which contain clusters of ribosomal RNA genes. The silver staining method for argyrophilic nucleolar organizer region-associated proteins (AgNORs) has been used to visualise NORs in chromosomes and nucleoli. The number and size of AgNORs correlate with the level of rDNA transcription, the degree of cell proliferation, evidenced by the percentage of cells in S-phase, and the growth fraction determined by Ki-67 monoclonal antibody (Nikicicz & Norback 1990). The AgNOR silver technique has been successfully applied to a wide variety of neoplastic lesions on pathological material in order to distinguish benign from malignant lesions (Solymosi et al 1996).

The first silver-staining methods employed for AgNOR protein visualisation consisted of two successive phases: an impregnation step with silver nitrate and a development step with a reducing agent (such as ammonia or formic acid) (Goodpasture and Bloom, 1975). Howell and Black (1980) introduced a "one-step" silver-staining method in which impregnation and development were performed simultaneously (Trere 2000). The AgNOR technique would be classified as an argyrophilic reaction since an external reducer (formic acid) is employed.

Thiebaut et al (1984) and Foucrier et al (1990) noted that if sections were pretreated for one hour in Schiff's reagent prior to staining in 60% silver nitrate (at 60°C), then an external reducer was not required. This AgNOR reaction had thus become an argentaffin reaction. Foucrier et al (1990) have suggested that after the Feulgen reaction AgNO<sub>3</sub> may react with Schiff's reagent, which is a halogenated compound. The strong avidity of silver for halogenated species leads to silver salt formation accompanied by aromatization and decolouration of the Schiff's reagent.

### Von Kossa Stain

The Kossa method for demonstrating significant calcium phosphate and/or carbonate deposits has been regarded as being an argentaffilic reaction by several investigators because the calcium ions are converted first into large light-sensitive silver phosphate and/or carbonate deposits, then to large metallic silver deposits when sections are exposed to light (Gallyas 2008). According to Meloan and Puchtler (1985), von Kossa's technique is a two-step reaction. In the first step, silver cations react with components of calcium deposits. In the second step, bound silver is reduced to black metallic silver by organic material with the aid of light or by photographic developers. Therefore, von Kossa's technique should be classified as an argyrophilic reaction.

Von Kossa regarded only the yellow colouration of calcium deposits during early stages of the reaction as diagnostic for calcium phosphate and ascribed the blackening to organic matter. Efforts to prevent this blackening were unsuccessful. Further studies showed that bright light, generally regarded as essential for von Kossa's reaction, only causes the irreversible blackening of organic matter that masks the yellow silver phosphate. When the reaction is performed in subdued light, yellow to yellowish brown silver phosphate is visualized selectively. Silver carbonate dissolves in thiosulfate and cannot be demonstrated with von Kossa's technique (Meloan & Puchtler 1985, Schneider 2021).

Since we can detect yellow calcium deposits, should we consider this the argentaffilic reaction whilst reducing the yellow silver phosphate to black metallic silver with physical developers (such as hydroquinone) or visible light renders the von Kossa stain argyrophilic?





## An observation on silver stains - continued

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# LaserTrack™ FLEX™ Cassette Printer

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The FLEX features LaserTrack's proven laser printing technology that produces clean lines and high-definition text and barcodes. The FLEX also introduces innovative new enhancements that make it a must-have for a modern histology lab workflow.

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### ■ Small Footprint + Workflow Layout Flexibility

The footprint of the LaserTrack FLEX measures only 13" L x 19" D. Cassettes can be loaded on either the right or left side, so you can position the FLEX where it best fits your lab's layout and workflow.

### ■ LIS Connectivity Flexibility

The FLEX software has a user-friendly interface for full operational control of the printer. It also easily interfaces with LIS systems including Epic® Beaker®, Cerner® CoPathPlus®, Sunquest CoPathPlus™, SoftLab® and others.



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## Cut-up Case Study: Uterine Carcinosarcoma

### Case history

54F HMB, fibroids, previous endometrial curettages = benign (late secretory endometrium)

**Clinical indications:** no clinical indications to suspect carcinoma

**Specimen collection method:** Hysterectomy

**Tissue type:** Uterus + cervix + bilateral fallopian tubes

**Specimen type:** Whole organs and/or peripherals

**NPAAC complexity classification:** Complex

### Cut-up technique / grossing pattern

- 1x anterior and 1x posterior cervix
- 1x anterior and 1x posterior uterine wall
- 1x full face of uterine wall
- Both tubes all embedded
- Right and left cornu and parametrium



Figure 2 Uterus and cervix as received

### Macroscopic description

The specimen consists of a uterus and cervix received in two pieces together with separate bilateral fallopian tubes with fimbriae. The uterus weighs 190g and measures 126x62x67mm.

The serosa is smooth. The cervical os is patent and transverse. The endometrium is soft and thickened and measures up to 8mm. The myometrium measures 21mm and 26mm in thickness anterior and posterior respectively with areas of trabeculation throughout.



On further examination, at the fundus there is a pale area present to a depth of 12mm and measuring 7mm from the closest serosal surface. The anterior margin is inked blue, posterior margin is inked black and orange ink marks the false margin created by the removal of the parametrium. The first tube measures 70mm in length by 7mm in diameter. The second tube measures 62mm in length by 7mm in diameter. Representative sections.

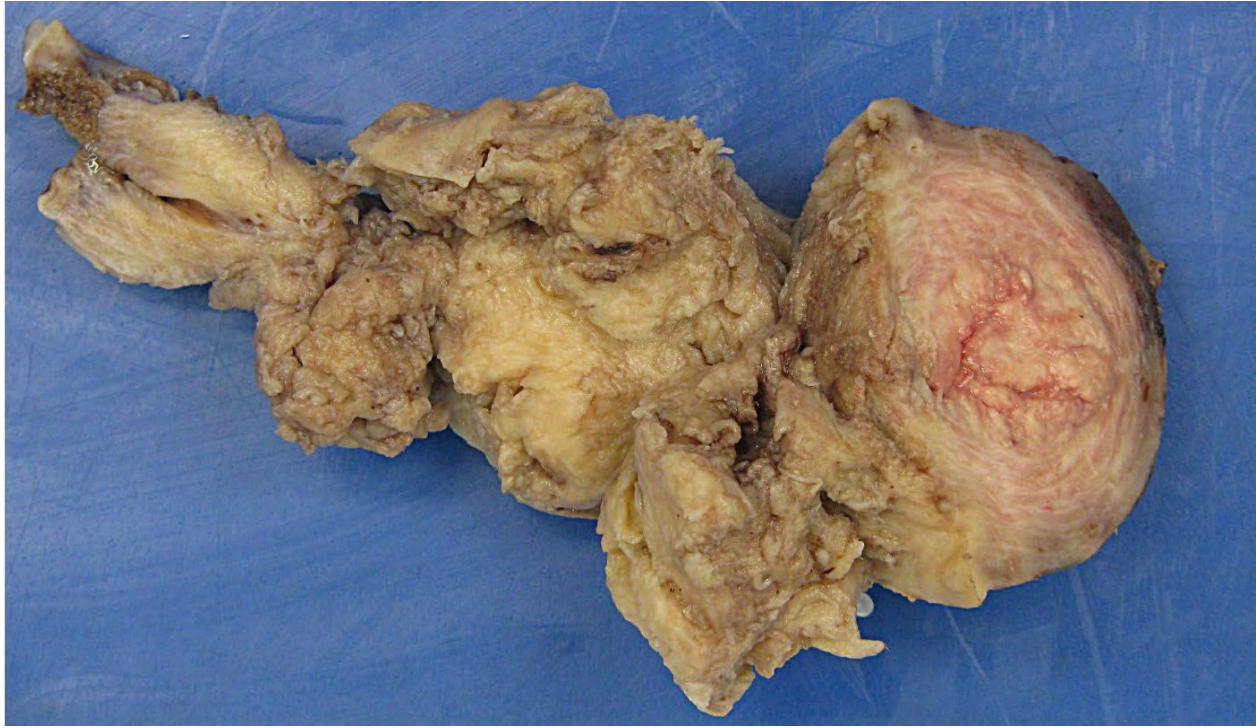


Figure 3 Bisected uterus

**Block key:** (Block A - anterior cervix, B - posterior cervix, C and D - anterior uterine wall, E to H - posterior uterine wall (E and F - one composite slice, G and H - one composite slice), I - fundus, J and K - first tube and fimbria, L and M - second tube and fimbria, N - right cornu and parametrium, O - left cornu and parametrium, P to U - full face of uterine wall and fundus (P to S - right side, T and U - left side)).

**Inking/orientation:** Blue = anterior margin, black = posterior margin, orange = false margin created by removal of parametrium

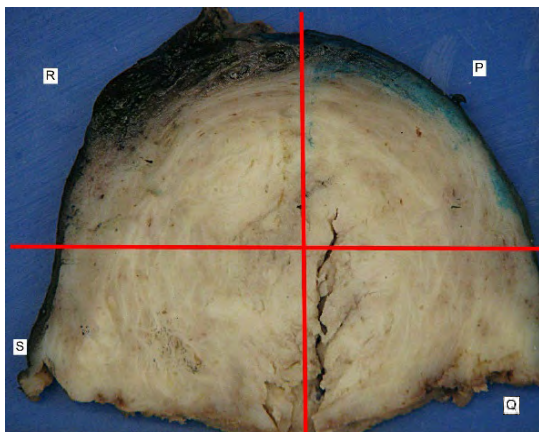


Figure 5 Right side

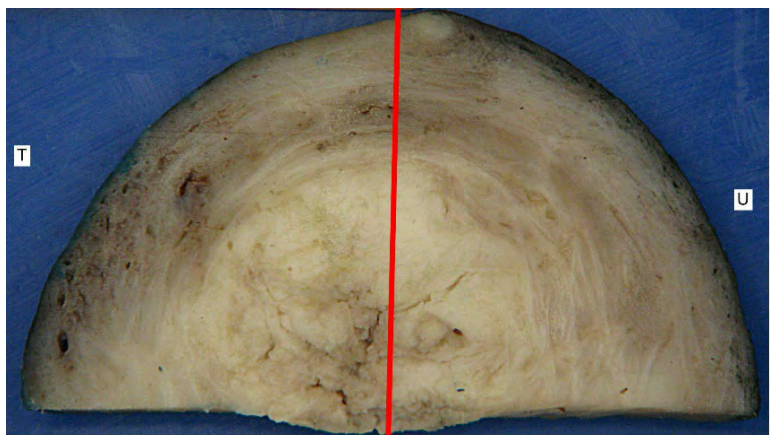
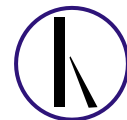


Figure 4 Left side

**Pathologist guidance provided:** No Pathologist input (was contacted). Consulted with cut-up Team Leader. Specimen returned for further blocks to be taken.



## Synoptic report for uterine carcinoma

**Clinical:** Uterus, cervix, bilateral fallopian tubes

### Microscopic

<b>Histologic type:</b>	Carcinosarcoma
<b>Histologic grade (FIGO grade):</b>	High grade
<b>Myometrial invasion:</b>	Outer half at fundus
<b>MELF type of invasion:</b>	Present
<b>Depth of invasion:</b>	13 mm (R)
<b>Myometrial thickness:</b>	19 mm (R)
<b>Lymphovascular invasion (LVI):</b>	Substantial (eg C, D, I, R, T, U)
<b>Cervical stromal involvement:</b>	Not seen
<b>Uterine serosa involvement:</b>	Not seen
<b>Other tissue/ organs involvement:</b>	Not seen
<b>Fallopian tube or ovary involvement:</b>	Fallopian tubes not involved. No ovaries received.
<b>Additional pathological findings:</b>	Adenomyosis

**Lymph nodes:** No lymph nodes received

### Diagnosis

<b>Uterus</b>	<ul style="list-style-type: none"> <li>• Carcinosarcoma</li> <li>• Adenomyosis</li> </ul>
<b>Cervix and both fallopian tubes</b>	<ul style="list-style-type: none"> <li>• No evidence of malignancy</li> </ul>

**Case provided by:** Alan Huynh, Douglass Hanly Moir Pathology

**Edited by:** Leah Simmons, NSW Health Pathology

# TekEquipment

**Equipment for a safer, cleaner & more efficient laboratory.**

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- Quick charging battery powered options available



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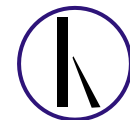


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## Webinar Review: Cytology

By ROY Yesudas, NSW Health Pathology

The cytology webinar co-presented by Dr Elizabeth Salisbury and Joanne La Malfa on 18/2/2023 was very informative.

### **The value of cytology in pathological diagnoses - by Dr Elizabeth Salisbury**

The history and basics of cytology and its divisions explained by Dr Salisbury, notably the history of pap stain was interesting. It was fascinating to know that a cytological technique can give us rapid on site/bedside results with minimal invasive procedure and that cytology is the best tool for initial diagnosis of breast cancers. They also introduced the modern techniques used in the cytology sample collection and used images of Mona Lisa and eggs to convey the importance of expertise to make a diagnosis.

Dr Salisbury clearly explained the power of cytology and ancillary testing in modern oncology specifically in lung cancer diagnosis and personalised treatment with a minimal invasive procedure. To me, it is new knowledge that cytology is the only minimal invasive technique used for the diagnosis of advanced lung cancers along with the help of immunohistochemistry and molecular genetic testing.

The presentation displays how far the cytology has advanced and helped me to realise the significance of cytological samples compared to histological samples for performing several molecular methods. Moreover, the real-life stories of how cytology helped with diagnosis and led to saving lives were touching.

### **Purpose of the mobile cytology lab - ROSE - by Joanne La Malfa**

Joanne La Malfa's presentation about Rapid on-Site Evaluation (ROSE) was very educational. There was thorough explanation of techniques and procedures involved in ROSE testing, including those relating to request form details. I was thrilled to understand about the fine needle aspirate (FNA) procedure. The slides were self-illustrating. I agree with Joanne that ROSE can be deemed as 'cytology on wheels'. It provided good insight into the different types of collection procedures and trail of a cytology specimen.

To conclude, both presentations were enlightening along with the question-and-answer session which included real life experiences. For a histology technician/scientist, especially working in a big lab most of the things presented are new experiences. I look forward to attending similar webinars from experts in the time to come.

### **Missed the presentation?**

[Click here](#) to watch the recording

Password: A#.34m+@

# Coeliac Disease

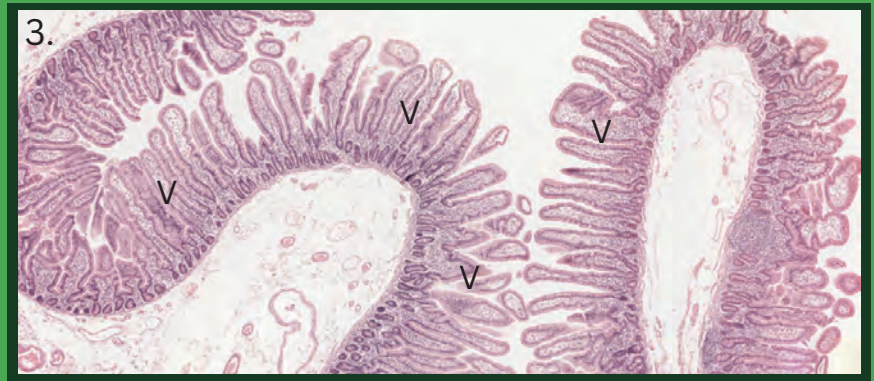
Coeliac disease is a **gluten sensitive enteropathy**. It is an autoimmune disorder in the small intestine caused by gluten protein. The inflammatory cascade results in changes to the anatomy of the bowel, creating a variety of health conditions.

## Healthy

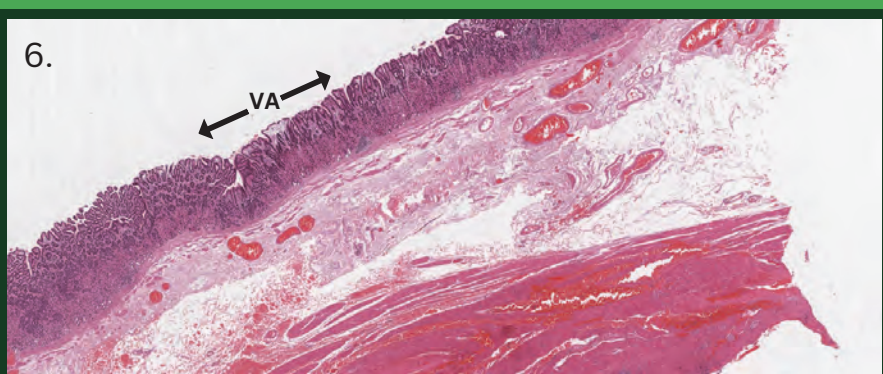
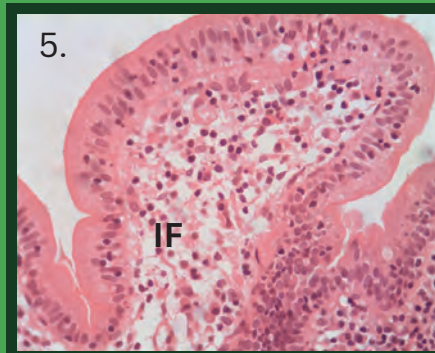
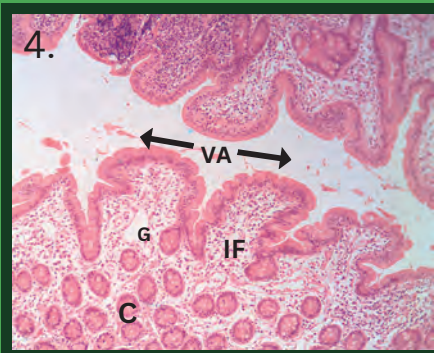
The villi (V) are finger-like projections into the lumen of the bowel [image 1,3]. They are folds which increase surface area, allowing an increase of absorption of nutrients. They have enterocytes (E) and goblet cells (G) lining the villi [image 2]. The enterocytes role is absorption of the contents of the bowel and their nuclei stain dark purple. Goblet cells are the mucus-secreting cells, there are fewer compared to enterocytes and they are a pale-stained cell.

The lamina propria (LP) is the core of the villi in the sub mucosa (SM) [image 1]. It is loose connective tissue which provides support for the villi and the crypt epithelium.

The anatomical structure of the healthy small intestine has definitive features that are altered by Coeliac disease.



## Coeliac



Once the gluten activates the inflammation cascade, there are a number of responses. The villi undergo atrophy (VA) and lose their arrangement, structure and organisation [image 4,6]. This leaves a flat surface compared to the healthy folds. The flat surface decreases the surface area which greatly impacts the absorption role of the small intestine. The submucosa becomes packed with densely staining inflammatory infiltrates (IF), in an attempt to relieve the stress that is being put on the enterocytes [image 5]. Crypt hyperplasia (C) occurs in response to the high turnover of the enterocytes [image 4]. There is also an increase in the number of goblet cells (G) present, to produce more mucus to also relieve the stress on the tissue barrier [image 4].

Malabsorption and maldigestion are the main results of coeliac disease. Symptoms also include changes in stool, such as watery stool or diarrhoea, stomach pain, bloating, weight loss, iron deficiency and others.

Removal of the allergen, gluten, allows the lining of the gut to be able to repair itself and return to a healthy functional state.





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## Student Poster References: Coeliac Disease

**Author:** Oliver Nash, University of Newcastle

### References

Dickson BC, Streutker CJ, Chetty R. Coeliac disease: an update for pathologists. J Clin Pathol. 2006 Oct;59(10):1008-16. doi:

10.1136/jcp.2005.035345. PMID: 17021129; PMCID: PMC1861744. Holtmeier W, Caspary WF. Celiac disease. Orphanet J Rare Dis. 2006 Mar 1;1:3. doi: 10.1186/1750-1172-1-3. PMID: 16722573; PMCID: PMC1435993.

Setty, M., Hormaza, L. & Guandalini, S. Celiac Disease. Mol Diag Ther 12, 289–298 (2008). <https://doi.org/10.1007/BF03256294>

Slide 3. Small intestines: U Michigan: Slide # 168 (H&E ileum) [https://histologyslides.med.umich.edu/Histology/Digestive%20System/Intestines/168\\_HISTO\\_40X.htm](https://histologyslides.med.umich.edu/Histology/Digestive%20System/Intestines/168_HISTO_40X.htm)

Slide 6. Coeliac disease - U Leeds H&E7173o

[https://www.virtualpathology.leeds.ac.uk/slides/library/view.php?path=h%2FResearch\\_4%2FTeaching%2FEQA%2FGeneral\\_Histopathology%2FHisto\\_EQA\\_CircJ\\_Set4A%2F7173.svs](https://www.virtualpathology.leeds.ac.uk/slides/library/view.php?path=h%2FResearch_4%2FTeaching%2FEQA%2FGeneral_Histopathology%2FHisto_EQA_CircJ_Set4A%2F7173.svs)

## Upcoming Events

**Sat 29<sup>th</sup> Apr** Webinar - [Hirschsprung disease](#)

**Sat 24<sup>th</sup> Jun** Webinar - [RCPAQAP presentation](#)

**Sat 29<sup>th</sup> July** Face to Face Event – [Histo on the Beach, Newcastle](#)

**Sat 26<sup>th</sup> Aug** Webinar - [Forensics presentation](#)

**Thus 24<sup>th</sup> Oct** Webinar - [Annual General Meeting + Surgical cut-up presentation](#)

**Sat 25<sup>th</sup> Nov** Webinar – [Macroscopic triage and cut-up of placentas](#)

\*Dates, type of event, and topics subject to change

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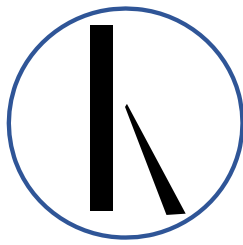


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# **HISTOTECHNOLOGY**

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### **Virtual Educational Session**

**Saturday 29th April 2023 10:00am - 11:30pm**

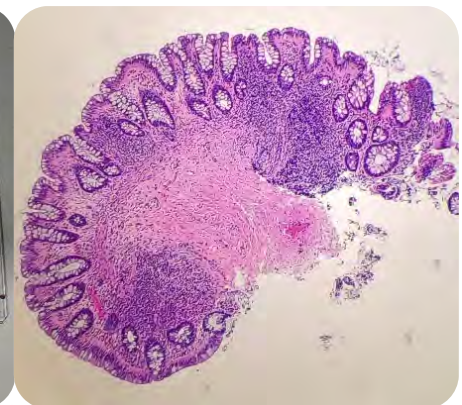
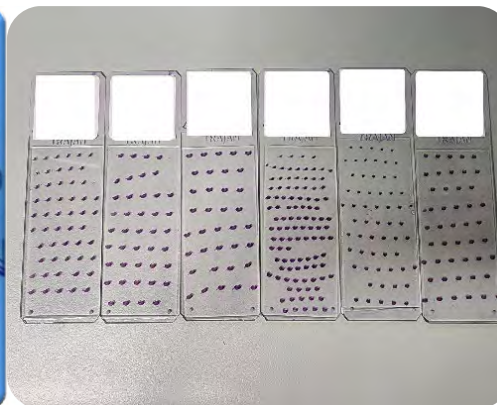
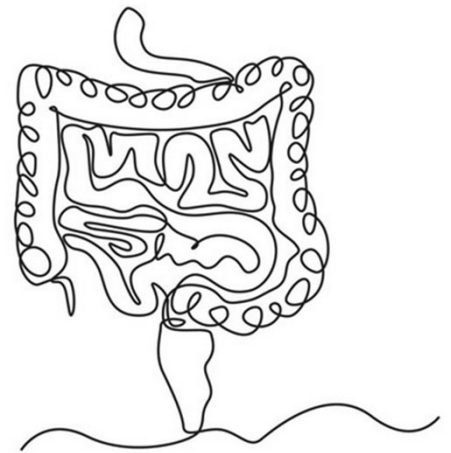
## **Hirschsprung Disease**

### **- Stool shall not pass**

**By Dr Joanne To**

**Registrar, NSW Health Pathology**

**Join us for the presentation**



Please register online via the QR code or the following link

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**Where:** Virtual Meeting Via Zoom

**When:** Saturday 29th April 2023

**Meeting opens:** 9:45am AEST (GMT+10:00)

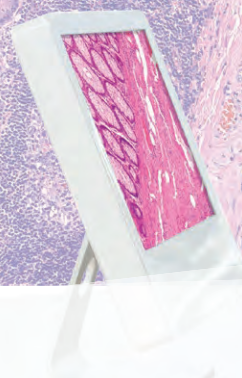
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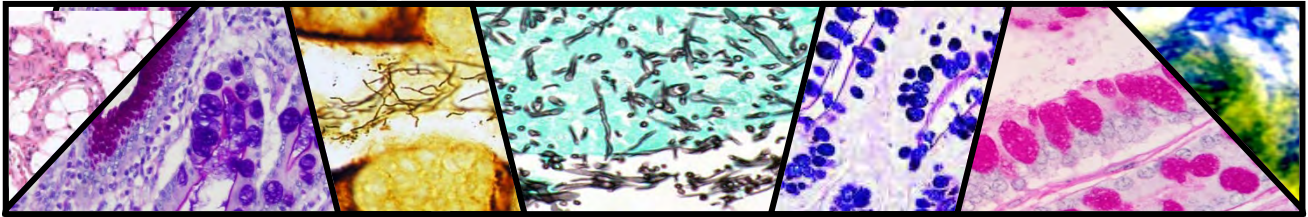
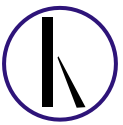
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## The Histochemical Chronicles: The Notebook 2

**Author:** Mike Rentsch

Laboratory Assistant to Medical Laboratory Scientist; On the bench 1969 to June 2019.

The best methods of improving our techniques and knowledge, comes from learning from our mistakes and trying to explain aberrant results. The Histochemical Chronicles are a sample of these experiences captured in my personal notebooks between 1979 and 2019.

### April 1980: Cloudy Neutral Buffered Formalin

#### Issue:

Recent deliveries of commercially prepared Concentrated Formaldehyde (37/7), prone to being very cloudy and not always being clarified upon mixing with buffer salts, with the cloudiness becoming more prominent as the cold weather progressed.

The formation of Paraformaldehyde advanced to such an extent, that the gelatinous material cannot be removed from the 20 Litre drum of Formaldehyde. Ordered in more but was already grossly affected. Material returned to the supplier. Material sourced from alternate supplier was 37/12 and was only slightly affected but by July was also becoming a serious problem.

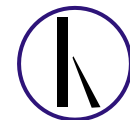
Mortuary Store frequently got down to 4°C overnight, and the Lab temperature down to 12°C (no heating or cooling, but I did have a window for ventilation).

#### Contact Advice: Scientist in Charge Dorevitch Pathology (Tutor RMIT)

Cloudiness in Neutral Buffered Formalin (NBF) can be eliminated in almost all instances, so long as the formation of paraformaldehyde is not too far advanced/severe. The guidelines given are based on a 20 Litre Drum.

- Make sure drum of Formaldehyde 37/12 is thoroughly mixed by rotation to evenly suspend the Paraformaldehyde.
- Weigh out Disodium Hydrogen Orthophosphate (DSP) and dissolve in about 3-5 Litres of boiling water. Avoid getting splashed with hot material by making sure you use a wide neck vessel as it may rapidly bubble.
- Measure and decant 2 Litre of formaldehyde 37/12 into drum.
- Add hot dissolved DSP to drum, cap immediately to avoid fumes. Mix by rotation. Repeat at intervals until such time as the drum sides start to collapse. Solution should now be clarified. (it's important to wait until drum is collapsing, to avoid a blast of formaldehyde fumes).
- Dissolve Sodium Dihydrogen Orthophosphate (MSP) in warm water, add to drum, recap and again mix by rotation.





## The Histochemical Chronicles - continued

- When drum sides again begin to collapse, remove cap and add water to 20 Litre mark. Recap and give final mix.
- Label with name, preparation date and expiry date (give a two-year expiry).

### Results:

No sign whatsoever of any cloudiness. Clarifying occurred in as little as 2-3 minutes.

### Conclusion: (remember this is 1980)

- Make sure operations are carried out in a well-ventilated area.
- Rotation of drum method of mixing i.e. swirling clock and anti-clockwise, is only suitable for those drums with a “U” inverted profile, as this base now acts like the rotor of a washing machine. This requires little effort or strength as opposed to inversion (which I used to do up till this point).
- By dissolving the Disodium Hydrogen Orthophosphate first, the initial solution is quite alkaline, and this together with heat de-polymerizes the Paraformaldehyde into formaldehyde and formalin solution.
- Adding the Formaldehyde 37/12 to pre-prepared and cold buffer solution did produce some reduction in turbidity, but could take weeks, if it did at all.
- Write up in method manual as approved method.

### Note:

1. Both suppliers, sourced their material from the same manufacturer, ICI Deer Park, to their specification. These specifications can be found in any of their early hard copy catalogues.
2. Formaldehyde 37/7 refers a 37% solution of formaldehyde dissolved in water with 7% Methanol added as a stabilizer to inhibit Paraformaldehyde formation (Not very effective in cold weather or southern latitudes).
3. Formaldehyde 37/12 is as the above but uses 12% Methanol as the stabilizer and is much more effective but not absolute.
4. Both solutions often referred to incorrectly as 40% Formalin.
5. 10% Formalin prepared as above actually yields a 3.7% solution of Formaldehyde, this can be corrected for rather simply, but is considered acceptable by many operators. Commercial suppliers of pre-made product should be able to provide a Certificate of Analysis for any batch indicating the concentration. A Certificate of Assurance is not the same as it is confirmation of the presence of the analyte. Certificate of Analysis is preferred for a single analyte, which is what you are really interested in!

From the personal journal of Author

**Mike Rentsch, Stain Tech**

Edited by Leah Simmons, NSW Health Pathology & Bill Sinai, HTSNSW Life Member

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## Improving patient outcome in HER2 Dual ISH breast cancer

### Trajan Series 2 adhesive microscope slides

#### Challenge

A microscope slide that provides superior adhesion and optical clarity for Human Epidermal Growth Factor Receptor 2 (HER2) in dual in situ hybridisation (ISH) analysis.

#### Solution

Product	Trajan Series 2 adhesive microscope slides
Tissue samples	Breast tissue
Application	IHC and Dual ISH
Site	Melbourne Pathology, Melbourne, Victoria, Australia

#### Introduction

In 2020, more than 2.3 million people worldwide were diagnosed with breast cancer. Approximately 1 in 5 breast cancers are HER2 positive.

HER2 is a protein that promotes aggressive growth of cancer cells in some breast cancers. HER2 status has both prognostic implications and is predictive of patient response to its targeted treatment.

Dual ISH combines silver enhanced in situ hybridisation (SISH) and chromogenic in situ hybridisation (CISH), offering an alternative to the traditional method of fluorescence in situ hybridisation (FISH) for determination of HER2 breast cancer, using conventional brightfield microscopy.

#### Summary

Trajan Series 2 adhesive microscope slides assist the Dual ISH analysis of HER2 breast cancers, providing enhanced tissue adhesion and eliminating background staining.

Trajan Series 2 adhesive microscope slides are manufactured with a specialized adhesion coating that improves adherence of tissue samples whilst reducing background staining.

The slides are made of fine glass with superior flatness and transmittance with minimal intrinsic fluorescence. Significantly reducing the need for

rework, Trajan Series 2 adhesive slides improve laboratory workflow and turnaround time of diagnostic results.

#### Company

Sonic Healthcare is one of the world's leading providers of medical diagnostics.

Melbourne Pathology, part of Sonic Healthcare, is a leading provider of pathology services in Victoria, Australia. With 100 years of commitment to diagnostic excellence, Melbourne Pathology provides comprehensive pathology services to more than two million patients each year.

Melbourne Pathology performs HER2 Dual ISH analysis as part of their extensive Immunohistochemistry (IHC) service.\*

*\*Melbourne Pathology uses the VENTANA HER2 Dual ISH DNA Probe Cocktail Assay from Roche Diagnostics for determining HER2 gene status. The VENTANA HER2 Dual ISH DNA Probe Cocktail Assay was approved for clinical use by the FDA in 2020.*

#### Performance

Melbourne Pathology uses Trajan Series 2 adhesive microscope slides for all of their IHC work, as well as their Dual ISH and CISH analysis. The slides have enhanced the laboratory workflow through reducing the amount of tissue sample rework required, improving the turnaround time for patient results.

“Since using Trajan Series 2 microscope slides, we have found a huge improvement; tissue adherence to the slides has greatly improved. Flow of reagents on the slides is also excellent, resulting in even staining across the slide. Both of these have resulted in a reduction of repeated stains. We have seen strong adherence of tissue sections to the slides.”

*Maria Flynn, Senior Scientist - IHC, Anatomical Pathology.*

In addition, Melbourne Pathology also observed zero background staining, even when performing Dual ISH and CISH analysis (Figures 1 and 2).

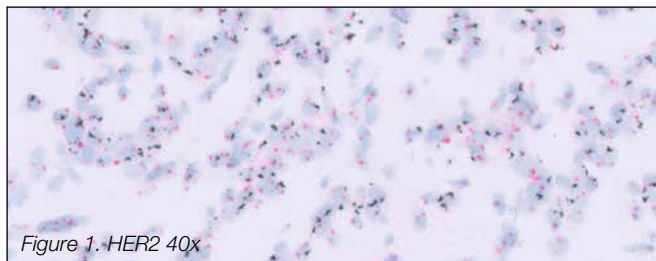


Figure 1. HER2 40x

## Conclusion

“Slide selection is critical to the diagnostic process. The ageing population and prevalence of chronic disease means laboratories are busier than ever. They cannot afford to rework tissue due to poor adhesion or excessive background staining. Melbourne Pathology, and many other laboratories around the world, have demonstrated the value of using Trajan’s Series 2 slides to protect precious tissue and give the pathologist the best possible presentation – the first time.”

*Renee Orlandi, Pathology General Manager – Trajan*

Trajan’s Series 2 adhesive microscope slides improve tissue adherence to the slide, enhancing the diagnostic evaluation. This leads to a reduction in false negative error rates and improved turnaround times to get the right diagnostic result to the treating clinician.

The impact is enhanced patient care, quicker treatment and ultimately better patient outcomes.

The laboratory benefit is demonstrated by the reduced need for laboratory staff to repeat tests constantly causing undue stress and productivity losses. Rather than have laboratory staff wasting precious time trouble shooting and reworking tissue samples, they can focus on high value laboratory work.

## Trajan Series 2 adhesive microscope slides for IHC staining

- Robust tissue adhesion of formalin fixed, paraffin embedded tissue for immunohistochemistry and in-situ hybridisation specimen.
- Minimal background staining.
- Faster turnaround on test results for patients.

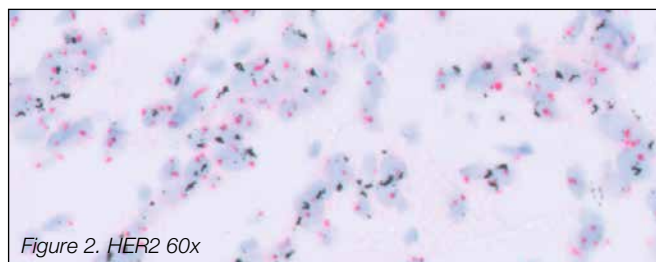


Figure 2. HER2 60x

## Acknowledgments

Maria Flynn, Senior Scientist - IHC, Anatomical Pathology, 103 Victoria Pde Collingwood VIC 3066, Australia.

Trajan proudly supports the National Breast Cancer Foundation in their fight to achieve zero deaths from breast cancer by 2030.



## Information and support

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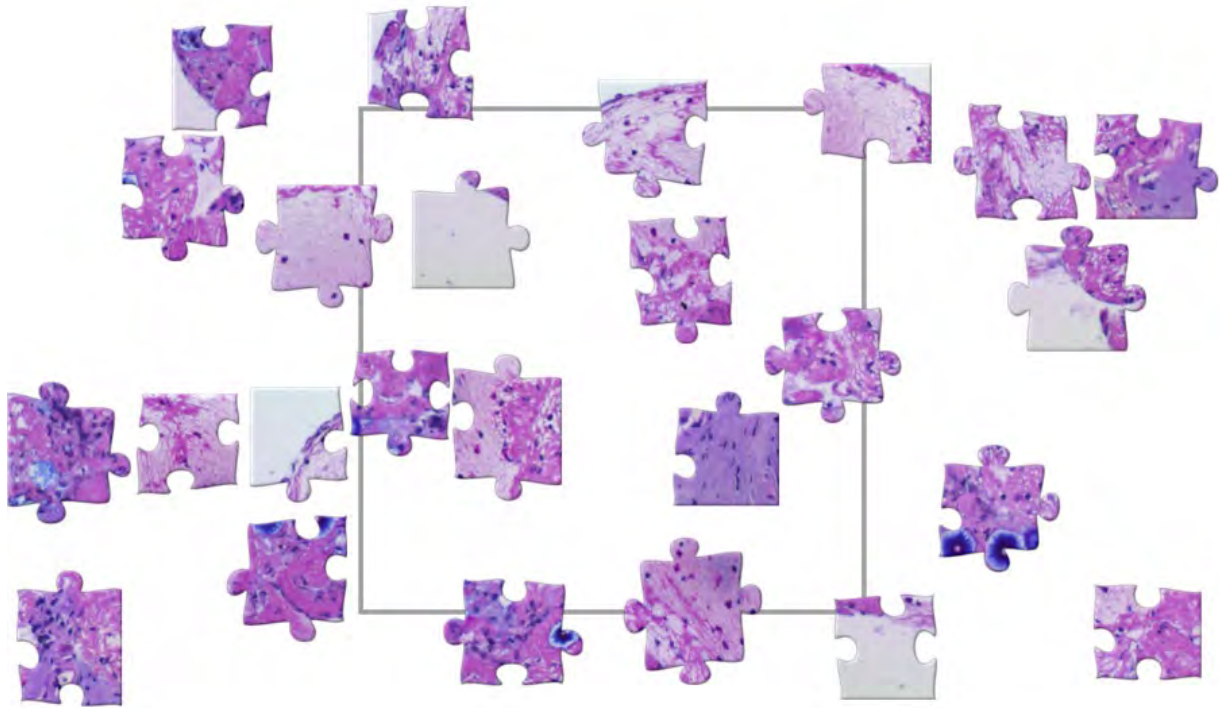
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## Puzzle: Piece it together

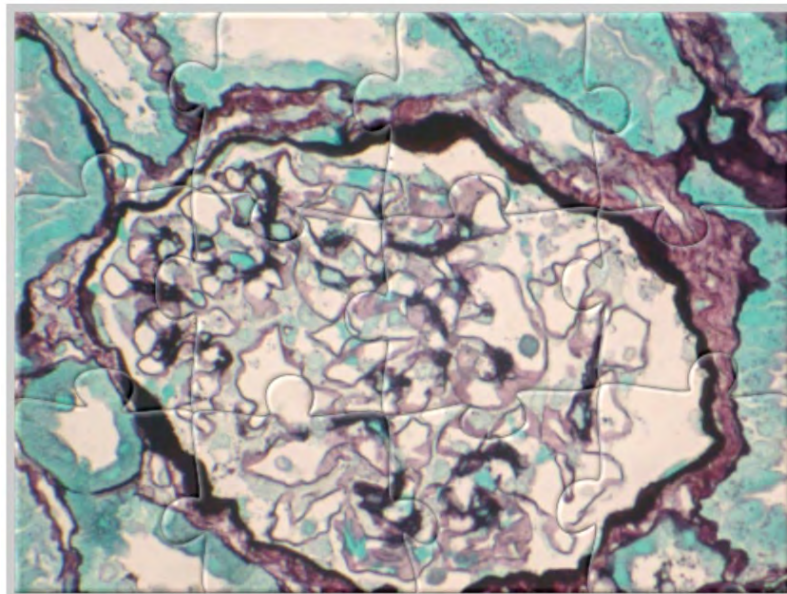


Click the puzzle or [link](#) to attempt the digital puzzle.

How many pieces need to be in place before you can identify the stain, tissue and organism?

Answers in next edition.

### Answers from previous edition:



**Stain:** Grocott's Methenamine Silver

**Tissue:** Kidney

**Structure:** Glomerulus

Did not attempt the puzzle – here is the [link](#) again



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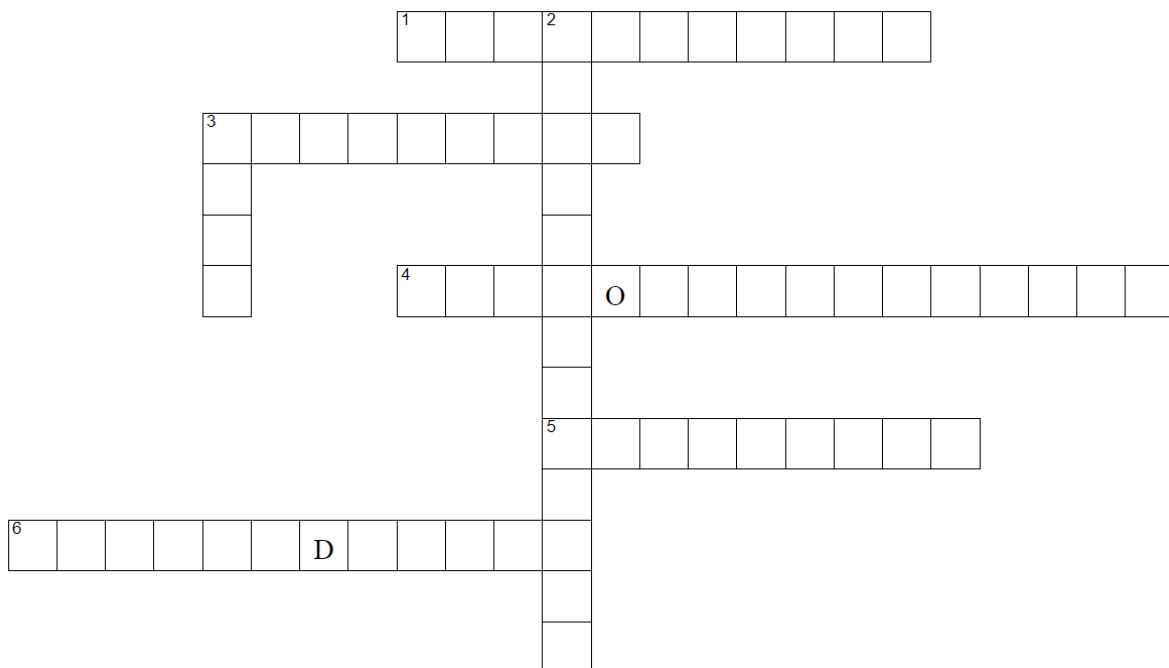
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## Crossword: Know your techniques



### Across:

1. Name a tissue structure or substance reaction which stains with silver without an external reducer.
3. Oxidation of Hydroxyl groups with Periodic Acid creates which group?
4. How is endogenous peroxidase blocked in IHC staining?
5. What is the most common mordant for hematoxylin?
6. Which chemical is the most commonly used in histology as a fixative?

### Down:

2. Bonding between dye and tissue is most commonly \_\_\_\_\_
3. At pH 2.5 what type of mucins will stain with Alcian blue?

Answers on page 36



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- **New slide drawer design** mitigates fluid ingress and egress resulting in continuous and highly reliable drawer performance.
- **New retractable work surface** gives technicians additional space to work while at the instrument.

**THIS PRODUCT IS NOT AVAILABLE FOR PURCHASE BY THE GENERAL PUBLIC.  
ALWAYS READ THE LABEL AND FOLLOW THE DIRECTIONS FOR USE.**

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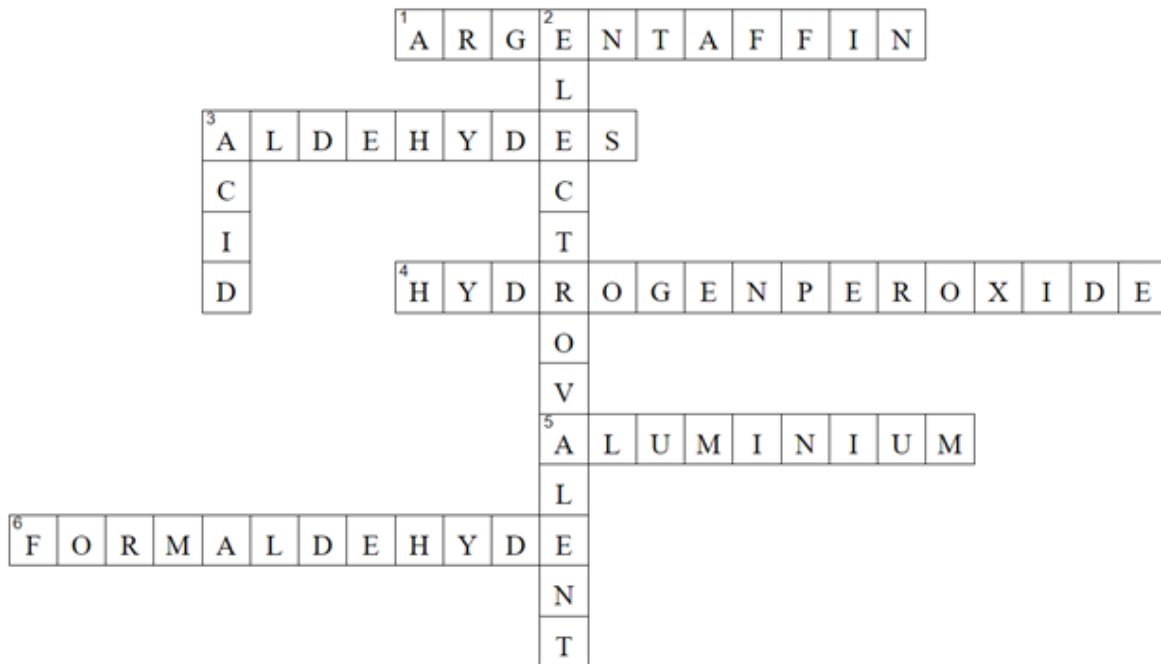
Roche Diagnostics Australia Pty Limited 2 Julius Avenue North Ryde NSW 2113

Ph: +61 2 9860 2222 ABN 29 003 001 205

MC-AU-02065



## Crossword Answer Key



### Across:

1. Name a tissue structure or substance reaction which stains with silver without an external reducer.
3. Oxidation of Hydroxyl groups with Periodic Acid creates which group?
4. How is endogenous peroxidase blocked in IHC staining?
5. What is the most common mordant for hematoxylin?
6. Which chemical is the most commonly used in histology as a fixative?

### Down:

2. Bonding between dye and tissue is most commonly \_\_\_\_\_
5. At pH 2.5 what type of mucins will stain with Alcian blue?



# BOND-PRIME

## IHC & ISH STAINING SYSTEM

THE BOND-PRIME HAS BEEN PROUDLY **DESIGNED, CREATED AND MANUFACTURED** IN AUSTRALIA.

The BOND-PRIME staining system has an innovative design that has been proudly engineered and manufactured for worldwide distribution at our multi-award-winning site located in Mount Waverley, Victoria.

### OUR HISTORY

Leica Biosystems has more than 20 years of manufacturing in Australia and is recognized for Manufacturing Excellence. Our Australian site has been the recipient of numerous manufacturing and export awards.

Our dedicated cross-functional associates throughout Australia support the entire Leica Biosystems product portfolio - enabling customers to have access to a complete end-to-end solution for anatomical pathology products.



**57<sup>TH</sup>** Australian  
Export Awards  
2019 NATIONAL WINNER

Winner  
**2018** VICTORIAN  
MANUFACTURING  
Hall of Fame Awards

Advancing Cancer Diagnostics  
Improving Lives

FOR IN VITRO DIAGNOSTIC USE

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**Leica**  
B I O S Y S T E M S



# BECOME A CORPORATE MEMBER!



**HISTOTECHNOLOGY**  
SOCIETY OF NSW



## COST

\$110/financial year

## BENEFITS

- ▶ Networking & communication with experts
- ▶ Learning best practices and access to technical advice
- ▶ Access to members only educational content via the website
- ▶ All the benefits of full membership for two company delegates
- ▶ 3 Free e-Newsletters per year and other relevant information
- ▶ Marketing opportunities in the 'Histogram' newsletter workshops, conferences and other events\*
- ▶ Potential to promote educational content to members via the committee\*

*\*Terms and conditions apply  
Always refer to <https://histonsw.org.au>*

## ELIGIBILITY

Corporate membership is open to any organisation interested in Histotechnology or Histology who supports the objectives of the Histotechnology Society of NSW.

## FOR MORE INFO

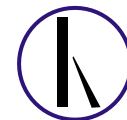
Email Elena Petrovska, Membership Officer

✉ [memberships@histonsw.org.au](mailto:memberships@histonsw.org.au)

## Scan to Join







## Committee Members

### Executive

Leah Simmons	Chairperson
Trevor Hinwood	Vice Chairperson
Kathy Wells-Reed	Secretary
Bharathi Cheerale	Assistant Secretary
Nevena Kostovska	Treasurer

### Members (alphabetical by surname)

Andrew Da Silva	
Richard Farquharson	
Mark Mullin	Industry Representative
Elena Petrovska	Membership Officer
Noelia Roman	
Bill Sinai	
Ewen Sutherland	
Adrian Ureta	
Alfred White	

### Sub-committee Member

Momoko Sakaki	Social Media Coordinator
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## Committee Meetings

Most meetings are scheduled for the first Monday of each month starting at 6 pm with some exceptions. These dates may change from time to time.

If you wish to contact the committee, make suggestions, or ask questions please feel free to contact the society's secretary [secretary@histonsw.org.au](mailto:secretary@histonsw.org.au)

May 1, 2023	Aug 7, 2023	Oct 27, 2023 (AGM)
Jun 5, 2023	Sept 4, 2023	Nov 6, 2023
Jul 3, 2023	Sept 25, 2023	Dec 4, 2023

**Thanks for reading! :-)**