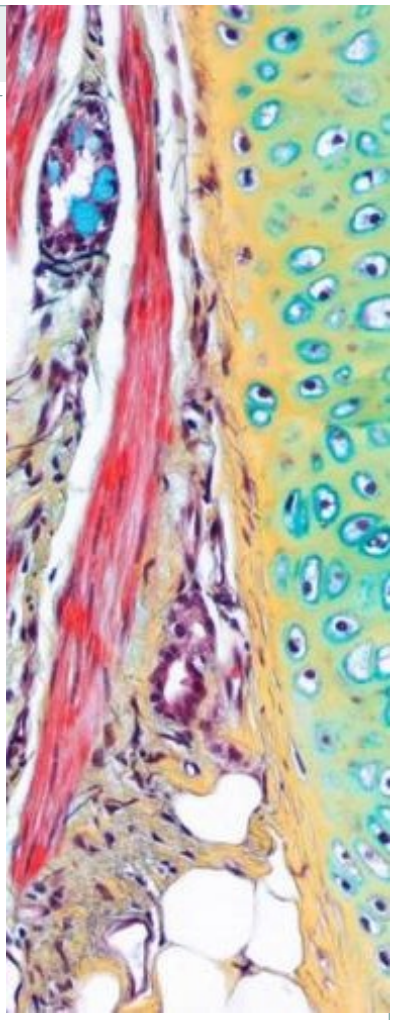
www.histonsw.org.au

ISSUE 3
December 2020

Newsletter of The Histotechnology Society of NSW

HISTOGRAPH



Editorial

Hi and welcome to the last issue of the Histograph for 2020. Hope all our member's are staying safe and well during this COVID-19 pandemic. Tony Henwood's Technical Note on Coronavirus Disinfection in Histopathology labs has been well received and has had over 55,000 views in 4 months. A big congratulation to him for his recent international prestigious award , the Jules Elias Excellence in Immunohistochemistry from the National Society of Histotechnology, Maryland USA.

In this issue Tony Henwood has submitted 2 articles which histologists would find very useful. Histology teaching and research depend on quality stained sections. With time slides start to fade even with the best preservations. The article "Causes of Stain Fading" should help trouble shoot fading issues that many Histologists come across.

The second article is "Alcohol Shortages in Histopathology during Covid-19 Pandemic" which details the alternatives to alcohol in the Histology laboratory and the potentials of alcohol recycling.

The committee would like to wish everyone a Merry Christmas and a Fantastic 2021.

Linda Prasad

MILESTONE
HELPING PATIENTS

ZERO FORMALIN EXPOSURE

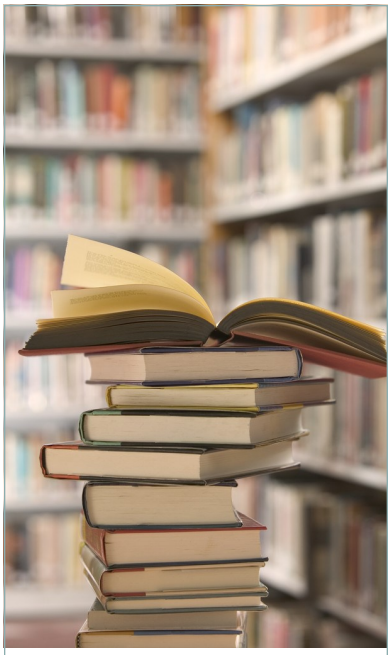
UltraSAFE

Automated Formalin Dispensing System for Biospecimens

abacus dx

Distributed by Abacus dx

1800 222 287 | info@abacusdx.com | www.abacusdx.com

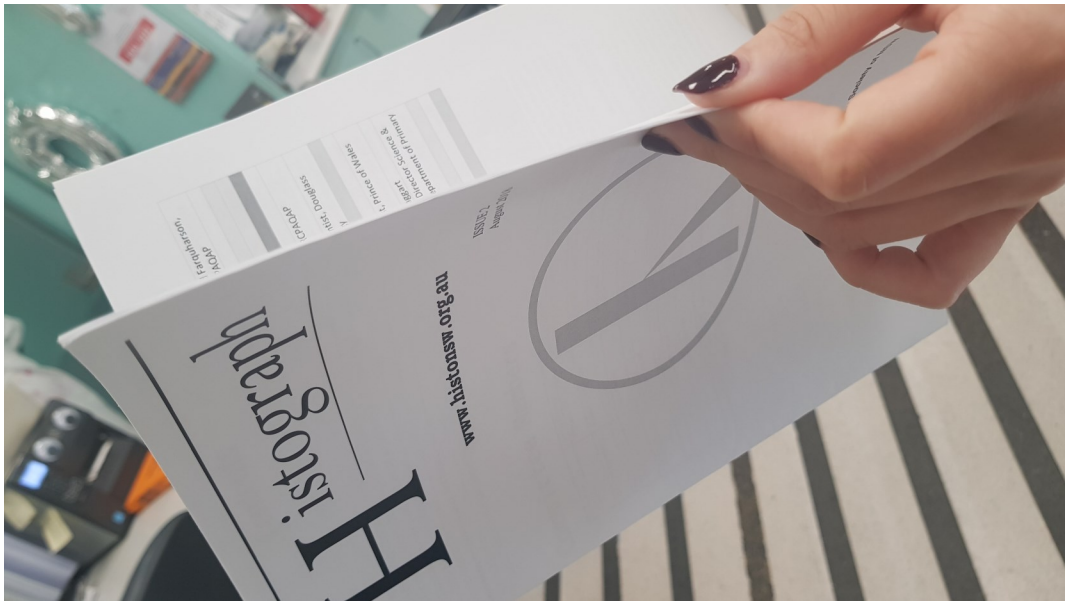


Histotechnology Society of NSW COMMITTEE MEMBERS are volunteers who work tirelessly to promote histotechnology and provide educational opportunities for continuing professional development. Thank you team for the GREAT JOB

COMMITTEE MEMBERS

Position	Name	Organisation
Chairman	Trevor Hinwood	Hin Sci Pty Ltd
Vice-Chairman	Bill Sinai	Life Member
Secretary	Bharathi Cheerala	Sonic Healthcare Limited
Assistant Secretary	Katherine Wells-Reed	Douglass Hanly Moir Pathology
Treasurer	Fredrick Reader	Parramatta Mission
Industry Representative	Mark Mullin	Leica Biosystems
Committee Member	Dianne Reader	Pathology North
Committee Member	Leah Simmons	TAFE NSW
Committee Member	Ewen Sutherland	Epredia
Committee Member	Richard Farquharson	Douglass Hanly Moir Pathology
Committee Member	Tamara Sztynda	UTS
Student Representative	Adrian Ureta	UTS
Student Representative	Andrew Da Silva	TAFE Ultimo

Sub-committee (Media & Newsletter)		
Position	**Name**	**Organisation**
Editor- Histograph	Linda Prasad	Children's Hospital, Westmead
Sub-committee Member	Momoko Sakaki	Children's Hospital, Westmead



Chairman's Report

With the continuation of COVID-19 it has meant more changes to our 2020 plans. Our committee meetings are being held by Zoom and we are currently finalising the purchase of a Microsoft NFP (registration of Office 365 and Azure Tenant) package. More workshops have been converted to Webinar's and our Annual General meeting has been postponed and transferred to the 26th of March 2021. The NSW Department of Fair Trading is allowing 2020 Annual General Meetings to be held in 2021. This hopefully will enable us to consider a face to face meeting, arrange a presentation for members and give us time to prepare some constitutional changes the committee is proposing.

We have held three additional Webinars to complete our 2020 program. A trade Webinar on Digital Histology in August which covered developments by companies in the digital histology area such as slide scanners, image formats, compatibility of software as well as location and storage requirements. Webinars on Skin Pathology were split into two sessions in October and November examining conditions from inflammation to wounds, keloids, warts and malignancies. Our webinars have proved to be popular with attendees coming from around Australia and overseas. We are in the process of planning Webinars for 2021 and maybe some workshops.

With the cancelation of the planned Joint National Conference with IAP next June, NSW will still be proceeding with the next National Conference in Sydney without the involvement of IAP. A potential time frame is late 2021 or early 2022 depending on the situation with COVID-19 next year.

Following discussions with Anne Prins and Penny Whippy, we have decided to close the Canberra sub group. Two ladies in Townsville (Townsville Base Hospital, James Cook University), have been working with Anne on holding future Cut Up workshops. The Canberra bank account has been closed and money transferred to the NSW Society's Credit Union. It has been agreed the funds will be held in a separate account to fund an ongoing bursary for a student to attend a Conference. Anne and Penny will be involved in the selection process which still needs to be worked out. Support for students is an important part of our Society's operation.

It has been a challenging year although we have still been able to hold a number of Webinars and our regular committee meetings. We would like to thank Dr Tamara Sztynda for the incredible time and effort she put into planning and conducting this year's Webinars with the help and assistance of Sergio Joshua and others. Thank you also to the University of Technology, Sydney for allowing the use of their facilities and accommodating the change from practical workshops to Webinars.

On behalf of our Committee we wish you all the best for the Xmas break and 2021. Stay safe and we look forward to providing an interesting program next year.

Cheers,

Trevor Hinwood

Chairperson

Designed for performance
Optimised for comfort

Deliver high quality routine results comfortably and efficiently with the Epredia™ HM525 NX Cryostat, a high performance routine cryostat with intuitive software and touchscreen for simple, efficient operation. The HM525 NX Cryostat combines the proven operation and reliability of the HM525 cryostat and the new ergonomics and intuitive interface of the Epredia™ NX series cryostats.

Exceptional sectioning quality and reliability
Reliable stepper motor technology delivers reproducible section thickness

User friendly touch screen interface
Icon driven interface allows intuitive operation

Enhanced ergonomics
Light-touch handwheel requires minimal force to operate

Operator safety
Mechanical handwheel lock completely immobilises the specimen head

27 cryobar storage positions including 4 quick freeze positions
Allows for greater workflow flexibility and improved specimen management

UV disinfection

EPRN956651EC70
HM525NX Cryostat with UV Disinfection and Knife Holder

Epredia is powered by:
Menzel-Gläser | Richard-Allan Scientific | Microm | Shandon

epredia
Enhancing precision
cancer diagnostics

Bio-Strategy PTY Limited
T 1800 00 84 53 | sales.au@bio-strategy.com
www.bio-strategy.com | shop.bio-strategy.com

ALCOHOL SHORTAGES IN HISTOPATHOLOGY DURING THE COVID-19 PANDEMIC:

ETHANOL-FREE STAINING AND ETHANOL RECYCLING

Anthony F. Henwood [a,b]

[a]Histopathology Department, The Children's Hospital at Westmead, Sydney, Australia;

[b]School of Medicine, University of Western Sydney, Sydney, Australia

There has been increased demand for alcohol-based disinfectants, especially hand sanitisers, during the current Covid-19 epidemic. Since Histopathology departments are significant consumers of alcohol, concern has been raised that shortage of supply will directly affect the ability of these departments to process tissue samples for diagnosis. Alcohols are mainly used in Histopathology for processing tissues through to paraffin wax, for tissue fixation (mainly specimens for cytology) and tissue staining. There are several alcohol-free alternate processes that can be used to alleviate this situation and these well presented.

Alcohols used in histopathology are usually denatured. Denatured alcohol, also called methylated spirit (methylated spirits in Australia and New Zealand) or denatured rectified spirit, is ethanol that has additives to make it poisonous, bad tasting, foul smelling or nauseating, to discourage recreational consumption. In some cases, it is also dyed. Pyridine, methanol, or both can be added to make denatured alcohol poisonous, and denatonium can be added to make it bitter (1). "Reagent Alcohol," comprised of approximately 90% ethanol, 5% methanol and 5% isopropanol, is one example of a denatured ethanol (13). This blend can be labelled as "Reagent Alcohol." Alcohols labelled as "denatured ethanol" use other chemical denaturants (e.g., gasoline, ammonia, pine tar) that can possibly cause excess drying of tissue samples. They should only be used after validation (2).

Alcohol-free Tissue Processing

Methods for xylene-free histological tissue processing have appeared in the literature and most replace xylene (and sometimes alcohol and xylene) with isopropanol (3-6). Unfortunately, during the Covid-19 Pandemic, a maintainable supply of isopropanol is also an issue.

Alcohol-free Section Dewaxing

The second area of substantial alcohol use is in histochemical staining. Sections need to be de-waxed (usually xylene) and rehydrated via alcohols prior to staining. After staining, sections are usually dehydrated via graded alcohols, cleared in xylene (or similar hydrocarbon) and coverslipped with a permanent mountant. In recent years, there has been an increased utilisation in heated detergent de-waxing. Validations of this method

There is no need for rehydration with graded alcohols since the detergent is water-based, and excess detergent and melted wax is removed with hot water prior to staining. Commercially, this innovative technology has been used in the Roche Ventana Special Stains and Immunohistochemistry platforms where immunohistochemical demonstration of close to 200 antigens and 16 special stains (AFB, Alcian blue pH2.5, Congo red for amyloid, Gram and Steiner to name a few) can be achieved.

Alcohol-free Coverslipping

Following staining, there are a few options for coverslipping without dehydrating in graded alcohols and clearing in xylene. The majority of these involve rinsing the slides in water and drying the slides prior to coverslipping with the preferred mountant. There are several issues with air-drying that need to be appreciated. Firstly, incomplete drying can cause small water droplets, microscopically resembling "grey sand", to appear (9). Renee Buesa (9) recommends that sections must be washed thoroughly in distilled water, shaken and placed in a convection oven 5–10 min at 60° C until they are completely dry. A second issue occurs if H&E stained sections are allowed to dry at a higher temperature or for a longer than recommended time. In these instances, nuclei will appear smudged, lacking chromatin detail.

There are several modifications to the routine H&E stain that can be utilised to reduce the use of alcohol. Since acid-alcohol (e.g. 1% hydrochloric acid in 70% alcohol) is often used to differentiate the regressive haematoxylins used in many protocols, we have found that 1% aqueous hydrochloric acid or 1-3% acetic acid works quite well. It is believed that the alcohol aids in the dissolution of haematoxylin precipitates that can occur with aged haematoxylin solutions. This can be reduced by regular filtering of the haematoxylin solution. Many eosin solutions used in staining are alcohol-based. Buffered aqueous eosin solutions work equally well and cannot be easily differentiated from routine alcoholic solutions. Two modified eosin solutions are given in table 1 (10,11). The tinctorial properties of the buffered eosin-phloxine solution can be varied by changing the pH of the solution. Figure 1 compares H&E staining using alcoholic eosin-phloxine with that using aqueous eosin-erythrosine (Figure 2).

Figure1: Kidney Alcoholic Eosin—Phloxine x 40

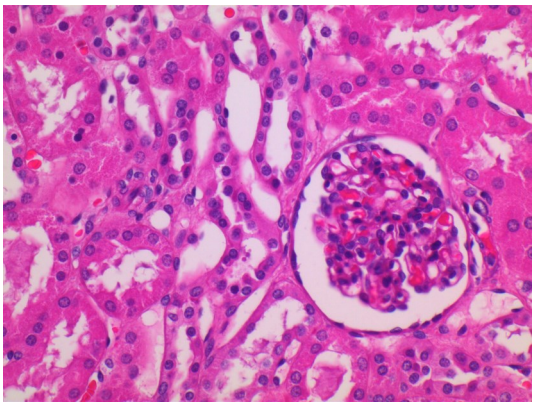
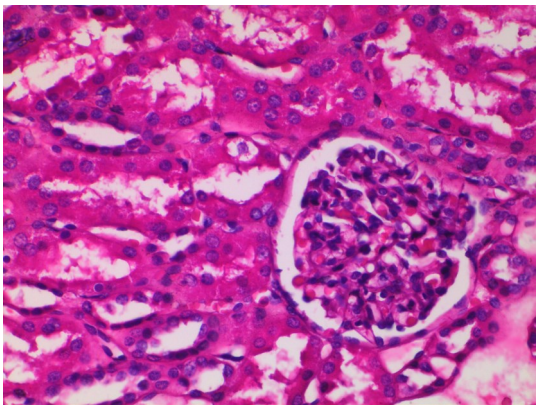


Figure2: Kidney Erythrosin x 40



Some histochemical techniques, including Van Gieson and Phosphotungstic acid-hematoxylin (PTAH), are impaired by immersion in water. Slides stained by these methods are immersed quickly in acetone before drying in the oven (9). It needs to be appreciated that often the choice of rinsing and dehydration method can depend on the choice of counterstain used. Many other stains use a Picric acid-based counterstain, such as Van Gieson. Acetone is recommended as the rinsing solution. There are a few stains that still require ethanol for dehydration since alternative dehydrants do not seem to be available. These include the modified Brown-Hopp's Gram stain for bacteria and modified Gomori stain for Intestinal Microsporidiosis. Table 2 presents a guide to effective dehydration of a range of histochemical stains. These non-alcoholic dehydration techniques appear to work quite well but further assessment will be needed.

In-Laboratory Alcohol Recycling

One of the most effective disinfectants for coronavirus is 62-71% ethanol (12). There is a growing shortage of disinfectant ethanol due to the spread of SARS-CoV-2. Histopathology laboratories are often fortunate to have access to commercial solvent recyclers such as those available from B/R Instruments and CBG Biotech. These are commonly used to recycle xylene but can be reprogrammed to recycle contaminated ethanol back to about 95%. Unfortunately, the distillation of alcohol is difficult since alcohol is an azeotrope. An azeotrope is a mixture of at least two different liquids that as it boils, has the same composition in the vapour phase as the liquid phase. These azeotropes can either have a higher boiling point than either of the components or they can have a lower boiling point. In the case of ethanol, for example, pure water boils at 100°C; pure ethanol boils at 78.3°C; but a mixture of 95.63% ethanol and 4.37% water (by weight) boils at a lower temperature,78.2°C. Therefore by conventional distillation, ethanol can only be recycled to about 95% purity (13). Alcohol is also sensitive to contamination by xylene or xylene substitutes. Alcohol which contains even small amounts of xylene or xylene substitutes cannot be completely purified by recycling, because these compounds cannot be completely removed. The mixture of water, alcohol and xylene, for example, forms a complicated azeotropic mixture with each of the three isomers (ortho, meta and para) of xylene (13).

It is important that traces of xylene are not allowed to contaminate the alcohol to be recycled. To test for the presence of xylene in the recycled ethanol, take 5ml of recycled ethanol and add 5ml distilled water drop by drop, noting the appearance of any white turbidity indicating the presence of contaminating xylene (13).

Since surface disinfection with 62–71%(v/v) ethanol is recommended for the inactivation of coronavirus (12), the recycled alcohol can be used for this purpose. The recycled alcohol will need to be appropriately diluted for use. It is accepted that the concentration of the recycled alcohol, should be 95%(v/v) or greater. This can be verified by measuring the specific gravity of the recycled alcohol. Specific gravity is defined as mass divided by volume. There are several methods available for determining specific gravity. The most convenient is to weigh 10ml of recycled alcohol and then divide the weight by 10. This will give you an approximate specific gravity. More accurate methods involve the use of a hydrometer or a refractometer (14). Table 3 gives the specific gravity for various concentrations of alcohol.

It needs to be remembered that ethanol-water mixtures are non-ideal solutions, thus when ethanol and water is mixed, they are attracted to each other and thus the final volume contracts. It is important that when preparing 100ml of a 70%(v/v) ethanol solution that 30ml of water is added to 70ml of ethanol and not to take 70ml ethanol and make it up to 100ml with water, otherwise, you will end up with approximately a 68% (v/v) ethanol solution (14).

It has been suggested that recycled alcohol could be used for hand sanitiser. This may not be appropriate since stringent regulations exist for solutions (for example soaps, lotions and cosmetics) that are destined for human or animal use (15, 16).

CONCLUSION

During the Covid-19 pandemic, ethanol shortages can occur. Histopathology is a significant consumer of ethanol and several alternate procedures have been presented to reduce its usage. Heated detergent dewaxing and non-alcoholic dehydration prior to coverslipping not only minimises xylene exposure but also reduces ethanol use. Recycled ethanol from tissue processing can be used as a disinfectant for surfaces.

Table 1

Buffered Eosin – Phloxine (9)	**Eosin – Erythrosin (10)**
Solution 1 Glacial acetic acid - 5.75 ml Distilled water - 1000 ml **Solution 2** Sodium acetate - 8.2 gm Distilled water - 1000 ml Mix 295ml of Solution 1 with 705ml of Solution 2. Add 5 gm of Eosin Y (CI 45380) and 0.5 gm Phloxine B (CI 45410). Add 2 crystals of thymol to prevent mould growth. Final pH is 4.85. Stock solution has a shelf life of several months.	Eosin Y (CI 45380) 5g Erythrosin B (CI 45430) 5g Sodium Hydrogen Carbonate 1.25g Magnesium Sulphate 10g Distilled water 500ml Dissolve sodium hydrogen carbonate and magnesium sulphate in water. Add Eosin Y and Erythrosin B and mix.
Staining time 2 minutes	Staining time 1 minutes

Table 2 Suitable alcohol replacements for Histochemical Stains

Technique	Dehydrant	Notes
Periodic Acid Schiffs	Air-dry after water rinse	
Van Gieson (and variants)	Acetone	Water removes yellow
Orcein for Elastic fibres	Air-dry after water rinse	
Modified Orcein for Hepatitis B Surface Antigen, Copper Associated Protein and Sulphated Mucins	Air-dry after water rinse	
Phosphotungstic Acid Hematoxylin Method	Acetone	
Silver Reticulin Methods	Air-dry after water rinse	
Gomori's One-step Trichrome	Air-dry after water rinse	
PASM (eg Jones) for Basement Membranes	Air-dry after water rinse	
Masson's Trichrome	Air-dry after a quick water rinse	Extended water rinsing can remove the green collagen staining
Verhoeff's Elastic Stain	Acetone	Ethanol, unfortunately, is still needed to remove tissue bound iodine (after ferric chloride differentiation)
Lendrum's MSB for Fibrin	Air-dry after water rinse	
Alcian Blue pH 2.5 and pH 1	Air-dry after water rinse	
Colloidal Iron for acidic mucins	Air-dry after water rinse	
Perl's Stain for hemosiderin	Air-dry after water rinse	
Fouchet's Stain for Bilirubin	Acetone	
Rhodanine Technique for Copper	Air-dry after water rinse	
Von Kossa's Method for Calcium	Air-dry after water rinse	
Schmorl's Reaction	Air-dry after water rinse	
Gram-Twort	Air-dry after water rinse	
Warthin Starry for Spirochetes	Air-dry after water rinse	

Table 3. Specific Gravities for a selection of water-ethanol mixtures

Ethanol % by Volume	Ethanol % by	Specific Gravity (g/ml at 20°C)
62%	54.1%	0.9046
65%	57.1%	0.8977
70%	62.4%	0.8856
80%	73.5%	0.8593
90%	85.7%	0.8293
95%	92.4%	0.8115
100%	100%	0.7893

Author declares no conflict of interest.

References

1. Wikipedia "Denatured Alcohol" https://en.wikipedia.org/wiki/Denatured_alcohol viewed 30/3/2020.

2. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. In Histopathology 2014 (pp. 31-43). Humana Press, New York, NY.

3. Andre GG, Wenger JB, Rebolloso D, Arrington JB, Mehm WJ. Evaluation of clearing and infiltration mixtures (CIMs) as xylene substitutes for tissue processing. Journal of Histotechnology. 1994 Jun 1;17(2):137-42.

4. Falkeholm L, Grant CA, Magnusson A, Möller E. Xylene-free method for histological preparation: a multicentre evaluation. Laboratory investigation. 2001 Sep;81(9):1213-21.

5. Buesa RJ, Peshkov MV. Histology without xylene. Annals of diagnostic pathology. 2009 Aug 1;13(4):246-56.

6. Metgud R, Astekar MS, Soni A, Naik S, Vanishree M. Conventional xylene and xylene-free methods for routine histopathological preparation of tissue sections. Biotechnic & Histochemistry. 2013 Jul 1;88(5):235-41.

7. Henwood AF. The application of heated detergent dewaxing and rehydration to immunohistochemistry. Biotechnic & Histochemistry. 2012 Jan 1;87(1):46-50.

8. Henwood AF, Prasad L, Bourke VM. The application of heated detergent dewaxing and rehydration to techniques for the demonstration of fungi: a comparison to routine xylene-alcohol dewaxing. Journal of Histotechnology. 2013 Jun 1;36(2):45-50.

9. Buesa RJ. Coverslipping without xylene. Biotechnic & histochemistry: official publication of the Biological Stain Commission. 2010 Aug;85(4):269-70.

10. Castro MD. A hematoxylin-eosin phloxine stain for tissues embedded in glycol methacrylate. Journal of Histotechnology. 1985 Mar 1;8(1):23-4.

11. Henwood AF. Hematoxylin and eosin staining of mucins of the gastrointestinal tract. Journal of Histotechnology. 2017 Jan 2;40(1):21-4.

12. Henwood AF. Coronavirus disinfection in histopathology. Journal of Histotechnology. 2020 Mar 2:1-3.

13. Hawkes J, Kumar MSA. Recycling Histopathology Solvents: A Funding Source for Plastination. Journal of Plastination. 2014 26(2):16-20.

14. Henwood, A. A survival guide for laboratory professionals. Scotts Valley, CA, USA, Amazon Create Space Independent Publishing Platform, 2019. Chapter 18 Disinfection, pp 149-155, Chapter 3 Mixing Things, Chapter 9 Specific Gravity

15. https://www.fda.gov/cosmetics/cosmetics-laws-regulations/cosmetics-us-law

16. https://www.tga.gov.au/hand-sanitisers-information-consumers

Advancing Cancer Diagnostics
Improving Lives

HistoCore SPECTRA Workstation
Routine and Special Staining

Efficiency • Flexibility • Confidence

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

LeicaBiosystems.com

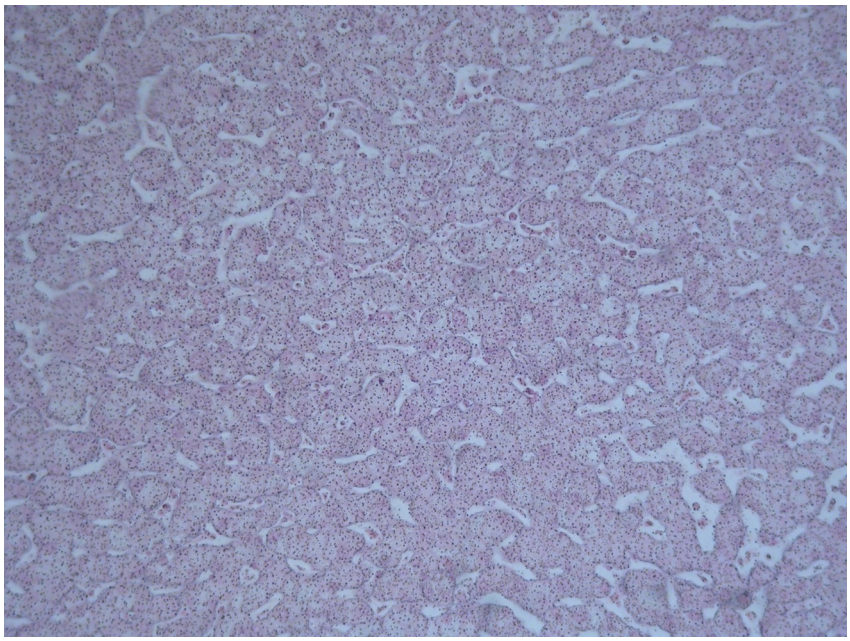
181208 Rev A 9/2018

TEST AND TEACH FROM LAST ISSUE

Linda Prasad, Histopathology, the Children's Hospital at Westmead, Sydney.

ANSWERS

1. **What is the tissue? Liver**
2. **What is the stain? Retic**
3. **Why is this? Read below for answer**



Reticulin – Too much Ammonia

The Reticulin Silver stain is a histochemical technique with a complex mechanism. Although there are many modified versions, the basic chemical principal is still the same. The crucial steps involve using an oxidiser, mordant, silver impregnation and a reducer.

Reticular fibers are difficult to demonstrate with routine H&E and resist binding to dyes due to its small fiber size. These fibres will stain best with metallic impregnation techniques because reticular fibers are argyrophilic (silver loving).

Reticular fibres have low natural affinity for silver ions so, they must be pre-treated with potassium permanganate or periodic acid to enhance staining while the sensitising agent (uranyl nitrate/dilute silver nitrate) initially binds to the tissue component of interest. The silver is readily able to precipitate as metallic silver from the ammonical silver solution (diamine silver). The ammonical silver solution consist of a strong base (ammonium hydroxide) added to an aqueous silver nitrate solution at pH 5.0, addition

of base immediately raises the pH of silver nitrate solution to PH 9.5, producing a brown mucky precipitate of silver hydroxide. Ammonium hydroxide is then added to the solution to produce gradual clearing of the precipitate to form a silver diamine complex. Finally a small amount of ammonium hydroxide is added to raise the pH to 12. Formalin, a reducing agent, causes deposition of silver in the form of metal. Any excess silver in the unprecipitated state is removed by treating with Gold chloride, providing stability, contrast and clarity.

In the test and teach slide rather than seeing the distinct well defined linear black reticular fibers you will see that the silver has deposited as small black punctate dots and looks very granular. If too much ammonia is added there is a great loss of sensitivity. Also if old uranyl nitrate or ammonium hydroxide was used it would cause a granular pattern.

REFERENCES

Brown, R. W. (2009). Histologic preparations: common problems and their solutions. College of American Pathologists.

Horobin, R. W. & Bancroft, J. D. (1998). Troubleshooting histology stains. Churchill Livingstone.

http://www.ihcworld.com/_protocols/special_stains/gordon_sweet's_ellis.htm

Causes of Stain Fading

Tony Henwood, Histopathology, the Children's Hospital at Westmead

Stained histological sections can be required for review years after initial coverslipping. It is hoped that the staining will be of the same quality as when first coverslipped. Some of the properties of a good mountant are that it be colourless, be quick drying and permanent, does not shrink or crystallise over time, is not prone to air bubble formation whilst drying, should have a refractive index close to 1.5, and is neutral to and does not contribute to the fading of stains (Henwood 1994).

Dyes are known to be either "fast" (resistant to fading) or "fugitive" (fade rapidly when exposed to light or other factors) (Puchtler et al 1989).

Several dye classes have notoriously poor light fastness (triarylmethyls, acridines, xanthenes, and most cationic dyes). Many sulfonated azo dyes possess fair to good light fastness. Since around 1900, new azo dyes with excellent fastness properties have been synthesized, e.g., the Levanol (Supranol) Fast and Sirius Supra series. Levanol fast cyanine 5RN proved invaluable for demonstration of myosins in myoendothelial cells of classical myoepithelium, and in epithelial cells of various organs. Levanol fast cyanine 5RN is also a very sensitive reagent for visualization of early lesions of myocardium; alterations of myofibrils. Sirius red F3BA and Sirius supra red 4BLA can be substituted for acid fuchsin (known to fade over time) in Van Gieson's stain (Puchtler et al 1989).

In histotechnology, dyes are exposed not only to reducing or oxidizing sites in tissues, but also to chemicals used in staining procedures and mounting media. Fading of dyes is accelerated in the presence of certain compounds (Sodium hydroxide, sodium carbonate, oxalic acid, potassium dichromate, phenols, gelatine, glycerol, starch, and gum). Considering the reported bleaching of Methylene Blue by gelatine, contact with gelatine can be expected to shorten the useful life of some stains. Deleterious effects of gelatine may be potentiated by addition of glycerol or potassium dichromate, compounds known to accelerate fading. Since protons (H^+) can reduce various dyes, thorough washing of stained sections in running tap water to remove acids will delay fading for months or years, as with the cresyl fast violet stain for bacteria and fungi. Similarly, traces of alkali, as in differentiating fluids, should also be removed in order to prolong the useful lifetime of stains. (Puchtler et al 1989).

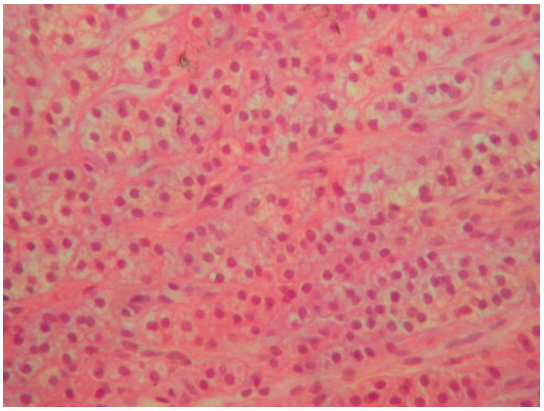
Barr (1970) after testing 22 commercial mountants confirmed that bleaching will occur in any mountant media. Dewse and Potter (1975) showed that fading of the Feulgen stain occurred regardless of the mountant media used or whether slides were stored in the dark or in darkness. Henwood (1994) showed that one commercial mountant (Eukitt) caused the complete fading of Warthin-Starry stained sections within 24 hours. The degree of fading of H&E stained sections over time varied with the mountant with some causing complete loss of hematoxylin staining.

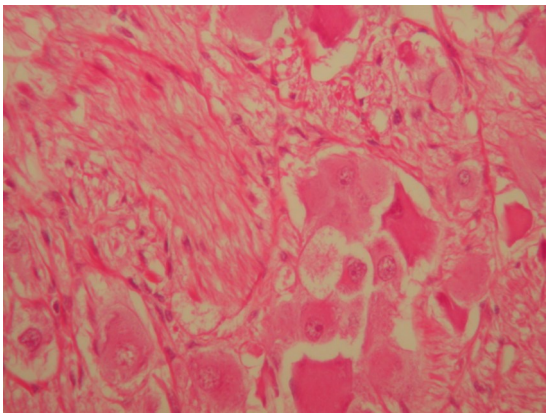
Farshid et al (2010) discovered a similar rapid fading of silver in situ hybridisation (SISH) signals for HER2 when mounting with DePex. The manufacturer of the SISH kit lists several mounting media as being contraindicated for use with SISH since oxidation, fading or disappearance of the SISH signal may occur with their use. These products included EUKITT and Entellan.

Ravikumar et al (2014) noted that sections mounted in Canada balsam tend to yellow with age, and as it becomes increasingly acidic over time, cationic dyes are poorly preserved and the Prussian blue product of the Perls' reaction for iron is bleached.

Lillie et al (1953) suggests that fading appears to be related to the amount of free carboxylic acid in the resin. The rate of fading varied depending on the mountant used. There may be other contributing factors that need to be considered. Sections may be acidic after staining and low pH may continue through dehydration, clearing and mounting. Poor quality xylene (containing sulphur) has been suggested to contribute to fading due to the acidic nature of such solvents (Humphrey & Pittman 1977). Xylene substitutes based on citrus oils may be acidic thereby contributing to fading. Inadvertent exposure of slides to direct sunlight has been shown to fade stains, though some mountants are more protective than others (Barr 1970, Henwood 1994).

Attached are two examples of stain fading of the H&E of two old cases of neuroblastoma.





References

Barr, W. T. (1970). Effects of sunlight on stained sections mounted in various media. Stain technology, 45(1), 9-14.

Dewse, C. D., & Potter, C. G. (1975). Influence of light and mounting medium on the fading of Feulgen stain. Stain Technology, 50 (5), 301-306.

Farshid, G., Davies, R., Arora, N., & Moffat, D. F. (2010). Some mounting media can lead to rapid fading of SISH signals in breast cancer. Pathology, 42(5), 481-483.

Henwood, A. F. (1994). Commercial Mountants: Assessment of Durability and Fading. Tissue Talk May p4-5.

Lillie, R. D., Zirkle, C., Dempsey, E. W., & Greco, J. P. (1953). Final report of the committee on histologic mounting media. Stain technology, 28(2), 57-80.

Puchtler, H., McGowan, A. L., & Meloan, S. N. (1989). Why do some stains fade? Part II. Effects of dye structure, molecular orbital systems, metals, substrates, moisture, and chemical reactions. Journal of Histotechnology, 12(2), 129-134.

Ravikumar, S., Surekha, R., & Thavarajah, R. (2014). Mounting media: An overview. Journal of Dr. NTR University of Health Sciences, 3(5), 1.

Fellow awarded

Faculty of Science Founding Fellow Tony Henwood is pictured with his National Society for Histotechnology award described below

Jules Elias Excellence in IHC Award, Sponsored by Dr. Richard Cartun: Tony Henwood

Tony Henwood, Principal Scientist at the Westmead Children's Hospital in Sydney, Australia, is the author of over 50 publications, and is currently serving on the Editorial Board for the *Journal of Histotechnology*. He is receiving the Jules Elias Award for his extensive research in IHC, but he also received an additional special recognition this year for his publication of his JOH article, Coronavirus Disinfection in Histopathology, in February 2020 at the onset of the COVID-19 pandemic. This timely publication has been viewed over 58,500 times and has 29 citations in the few months since its publication. It serves as an essential guide for histotechs handling COVID-19 positive specimens and autopsies.

MERRY CHRISTMAS FROM LABBY

This year has been a very difficult and uncertain year for everyone. Labby has been very sad that he hasn't been able to have as many lovely visitors to the lab or see all his wonderful friends at workshops and talks.

Hopefully next year will be better!

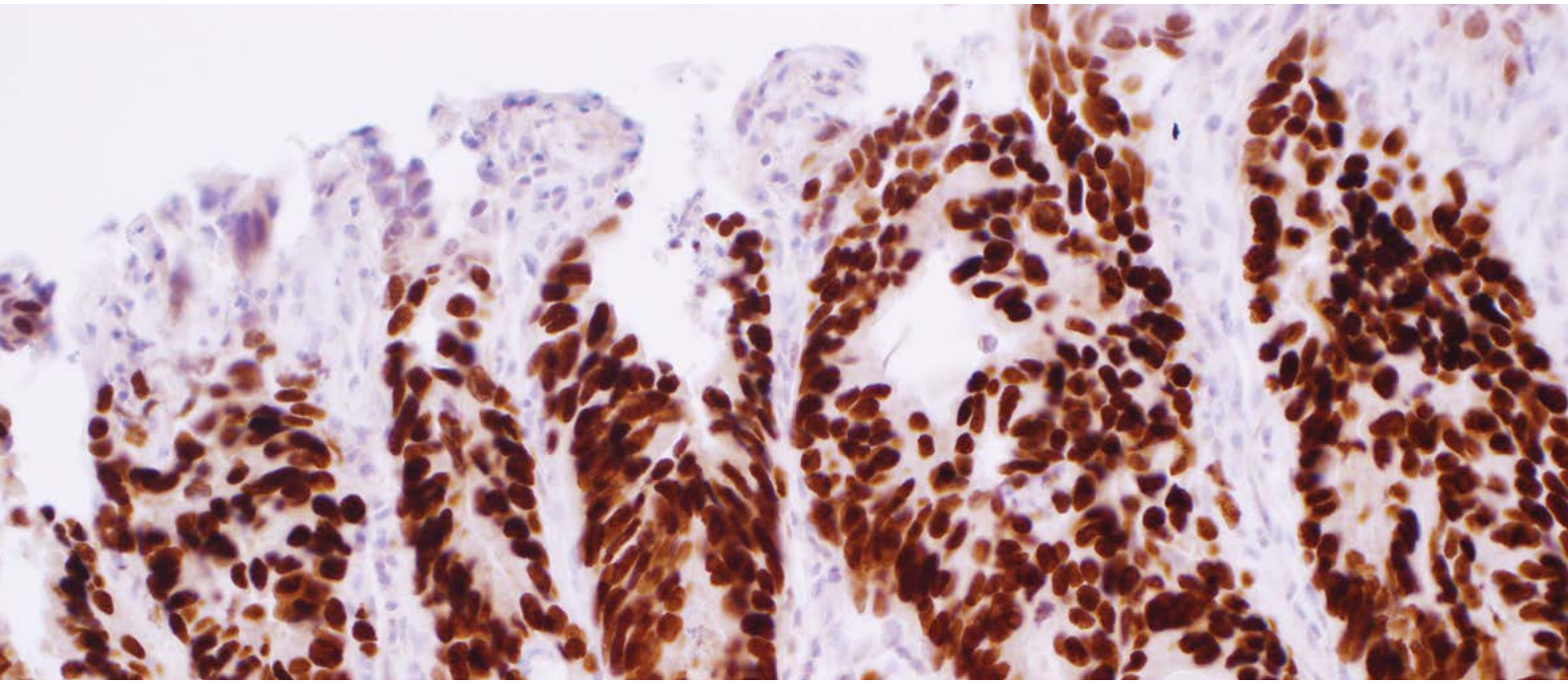
In the meantime, the Histotechnology Society of NSW is on Facebook. Please like or follow us to get updates and keep up with Labby's adventures. There is also a **Histotechnology Society of NSW Discussion Group**. Join to connect with other local Histology Professionals.

Labby wishes everyone a happy festive season and a great start to the new year.

Be safe and keep on Histo-ing!

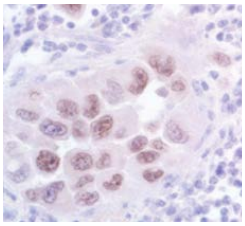
Find us on

SATB2 (EP281)
Rabbit Monoclonal Primary Antibody
Deliver diagnostic confidence

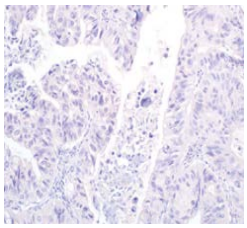


Product Name	Ordering Code	Comments
SATB2 (EP281) Rabbit Monoclonal Primary Antibody	08313415001	Colorectal

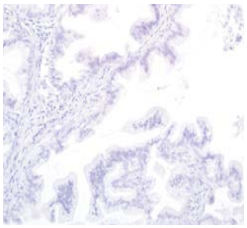
Special AT-rich sequence-binding protein 2 (SATB2) is a transcription factor involved in gene regulation. Among epithelial cells, it is only expressed in the glandular epithelium of the lower GI tract.[1] SATB2 has been reported to have 93% specificity and 100% sensitivity for colorectal carcinomas when utilised in the immunohistochemical panel with cytokeratin 7 and cytokeratin 20.[2] The sensitivity and specificity of SATB2 for colorectal cancers make it a valuable antibody for differentiating carcinomas of unknown primaries.[3]



Tumour cells of medullary carcinoma of the colon are positive for SATB2.



Tumour cells of oesophageal adenocarcinoma are negative for SATB2.



Rabbit monoclonal anti-SATB2 stains negatively for invasive mucinous adenocarcinoma of the lung.

References

1. Brocato J, et al. Carcinogenesis. 2015; 36:186-91.
2. Dragomir A, et al. Am J Clin Pathol. 2014; 141:630-8.
3. Ordonez NG. Adv Anat Pathol. 2014; 21:63-7.

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

MC-AU-00668