Histograph December 2003

Editorial

On behalf of the Histotechnology Group of NSW Committee, I would like to wish you all a Merry Christmas and a Happy New Year. We would like to especially thank our Company advertisers for their tremendous support throughout the year and hope they will again look kindly on us in 2004.

2004 will be a great year with our first National Scientific Meeting in March.

The December issue of Histograph contains a discussion on Wooden Cut-up Boards, What Glove to wear as well as several abstracts from the literature. A short article from Stylewise is also included.

The Diastase PAS stain using saliva must be the most disgusting technique in Pathology! (If you do not think so, please tell me what is). An Amylase solution with a long shelf life is presented in the Stains Section that is easy and quick to use.

A reminder that if there is anything in Histograph you disagree with or have some comments on, please ring (0298453306) or email me (anthonyh@chw.edu.au) and I will include it in the next issue.

Have a Good Christmas and a Happy New Year.

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The Wood Chopping Board

Many Histopathology Laboratories continue to use wooden cut-up boards. One advantage over plastic, glass or steel boards seems to be that there is less damage to knives and scalpels. This results in prolonged blade life. One major concern is the perceived difficulty in adequately cleaning and disinfecting wooden boards.

Several years ago Dr. Dean Cliver and his associates at the University of Wisconsin-Madison, USA, presented their work on the safety of plastic and wooden cutting boards. Their research was first intended to develop means of disinfecting wooden cutting surfaces, so that they would be almost as safe as plastics. Their safety concern was that bacteria such as Escherichia coli O157:H7 (commonly known as E-coli) and Salmonella, which might contaminate a work surface when raw meat was being prepared, ought not remain on the surface to contaminate other foods that might be eaten without further cooking. They soon found that disease bacteria such as these were not recoverable from wooden surfaces in a short time after they were applied, unless very large numbers were used. New plastic surfaces allowed the bacteria to persist, but were easily cleaned and disinfected. However, wooden boards that had been used and had many knife cuts acted almost the same as new wood, whereas plastic surfaces that were knife-scarred were impossible to clean and disinfect manually, especially when food residues such as chicken fat were present.

Although the bacteria that had disappeared from the wood surfaces were found alive inside the wood for some time after application, they evidently did not multiply, and they gradually died. They could be detected only by splitting or gouging the wood or by forcing water completely through from one surface to the other. If a sharp knife is used to cut into the work surfaces after used plastic or wood had been contaminated with bacteria and cleaned manually, more bacteria were recovered from a used plastic surface than from a used wood surface. "Manual cleaning", in their experiments, had been done with a sponge, hot tap water, and liquid dishwashing detergent. Mechanical cleaning with a dishwashing machine could be done successfully with plastic surfaces (even if knife-scarred) and wooden boards especially made for this. Wooden boards, but not plastics, that were small enough to fit into a microwave oven could be disinfected rapidly, but care had to be used to prevent overheating. Work surfaces that had been cleaned could be disinfected with bleach (sodium hypochlorite) solutions. This disinfecting was reliable only if cleaning had been done successfully.

A case-control study of sporadic salmonellosis had been done in California and included cutting boards among many risk factors assessed (Kass et al 1992.). It revealed that those using wooden cutting boards in their home kitchens were less than half as likely on average to contract salmonellosis (odds ratio 0.42, 95% confidence interval 0.22-0.81). Those using synthetic (plastic or glass) cutting boards were about twice as likely on average to contract salmonellosis (O.R. 1.99, C.I. 1.03-3.85) and the effect of cleaning the board regularly after preparing meat on it was not statistically significant (O.R. 1.20, C.I. 0.54-2.68).

Dr. Dean Cliver and his co-workers have concluded that wooden cutting boards are not a hazard to human health, but plastic cutting boards may be. As yet the natural antibiotic has not been discovered. What are your thoughts?

References:

http://www.peter.hemsley.btinternet.co.uk/CDB/Technical/Bacteria/bacteria.html

Kass, P.H., et al., (1992) "Disease determinants of sporadic salmonellosis in four northern California counties: a case control study of older children and adults" Ann. Epidemiol. 2:683-696.

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Abstracts from the Literature

Disappearance of Meconium Pigment in Placental Specimens on Exposure to Light

Jacquelyn L. Morhaime, MD, Kay Park, MD, Kurt Benirschke, MD, and Rebecca N. Baergen, MD

Archives of Pathology and Laboratory Medicine: Vol. 127, No. 6, pp. 711–714, 2003

Context: Meconium discharge has been associated with fetal distress and poor neonatal outcome; thus, its presence is of clinical importance.

Objective: Loss of meconium pigment in histologic sections from light exposure has been described. We sought to confirm this finding and to measure this loss quantitatively.

Design: Sections of umbilical cord, fetal membranes, and fetal surface from 11 grossly meconium-stained placentas were processed swiftly to minimize light exposure. Two serial sections from each block were cut and stained; one set was reviewed immediately, and the other was exposed to 8 hours of direct fluorescent lighting. Each site and exposure was scored for pigment intensity (0, no staining; 1, weak expression; and 2, moderate/strong expression) and number of meconium-laden macrophages per 10 high-power fields (HPF). Results were compared on the same specimen using the X2 and the paired-samples t test.

Results: The maximum meconium macrophage count was 13.2/10 HPF in the unexposed sections versus 6.1/10 HPF in the exposed sections ($P < .001$). Unexposed sections varied from 1+ to 2+ intensity, while exposed sections were all 1+ or negative ($P < .001$).

Conclusion: Exposure to fluorescent laboratory lights for 8 hours resulted in a significant loss in the intensity and number of identifiable meconium macrophages in histologic sections. These findings have important implications in the handling of placental specimens, and we recommend that care be taken to minimize exposure to laboratory lights during processing.

Improved Methods for Staining Fibrin

Charles J. Churukian

The J Histotechnol 26:127, 2003

Two methods used to demonstrate fibrin are the Fraser- Lendrum and Mallory's phosphotungstic acid-haematoxylin. (PTAH). One problem with the Fraser-Lendrum is that it requires the use of saturated alcoholic picric acid. To prepare this solution, a considerable amount of picric acid is required, which is an explosive. Federal and

state regulations prohibit the storage of this chemical in the laboratory unless it is stored under water. We found that saturated aqueous picric acid could be substituted for the saturated alcoholic solution. Other changes were made to simplify the method. Mallory's method for fibrin requires treatment of tissue sections with potassium permanganate and oxalic prior to staining them with phosphotungstic acid-hematoxylin. We found that better staining results are obtained if the tissues are also treated with Zenker's fixative. However, Zenker's fixative contains mercuric chloride, a hazardous chemical that many laboratories have discontinued using. We found at zinc acetate can be substituted for mercuric chloride in preparing Zenker's fixatives with excellent staining results when used in the Fraser-Lendrum and Mallory methods.

Necropsy practice after the "organ retention scandal": requests, performance, and tissue retention

J L Burton and J C E Underwood

Journal of Clinical Pathology 2003;56:537-541

Aims: After the so called "organ retention scandal" in the UK this study set out to assess the impact on death certification and hospital (consent) necropsies, including the post-mortem retention of tissues and organs.

Methods: Data were prospectively gathered over a one-year period for all deaths occurring at the Royal Hallamshire Hospital, Sheffield, UK to determine the frequencies with which death certificates were completed and necropsies were requested. The seniority of the clinician undertaking these duties was recorded. Pathologists were asked to record the extent of every necropsy during the study period. The type and planned uses of tissues retained were recorded.

Results: Death certificates were issued for 88.5% of the 966 deaths for which clinicians completed proformas. Of these, 88.9% were issued by preregistration and senior house officers. Consent was sought for a necropsy in 6.2% of cases (usually by non-consultant staff) and was granted in 43.4% of these. The overall, medicolegal, and hospital necropsy rates were 13.4%, 9.9%, and 3.5%, respectively. Tissues were retained from 55.4% of necropsies for diagnostic purposes, although sampling does not appear to be systematic.

Conclusions: Death certification and seeking consent for a necropsy are frequently delegated to junior clinical staff. This may explain the low standard of death certification reported by others and the low necropsy rate. The decline in the necropsy rate and the low rate of sampling for histological examination highlight the decline of the hospital necropsy and the lack of a systematic approach to tissue sampling.

Removal of Mercuric Chloride Deposits From B5-Fixed Tissue Will Affect the Performance of Immunoperoxidase Staining of Selected Antibodies

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Applied Immunohistochemistry & Molecular Morphology 2003; 11(1):92-95

B5 clearing is a step used before immunoperoxidase staining to remove the precipitated mercuric chloride deposits caused by B5 fixation of tissue. In the B5 clearing procedure, the slides are treated with Lugol's iodine and 5% sodium thiosulfate before antigen retrieval and the application of the primary antibody. The goal of this project was to study the effect of the B5 clearing protocol on immunoperoxidase staining on paraffin-embedded tissue, which has not been previously reported in a series of antibodies. We evaluated 75 antibodies using the 2-step clearing protocol and performed paired immunoperoxidase staining on the Ventana ES instrument, with and without the clearing protocol. We found that among 75 antibodies studied, 3 (CD5, CD30, and synaptophysin) showed total obliteration of reactivity, and 3 (ALK, Ulex, and GFAP) showed partial reduction of the staining compared with the controls. Pathologists must be aware of the possible false-negative staining effect caused by the routinely used B5 clearing protocol. Control tissues must receive the same clearing protocol (i.e., placed on case slides) to ensure detection of this effect.

HOPE Fixation of Cytospin Preparations of Human Cells for In Situ Hybridization and Immunocytochemistry

Oliver Umland, Artur J. Ulmer, Ekkehard Vollmer, and Torsten Goldmann

J Histochem Cytochem 51:977–980, 2003

In primary or cultured cells, in situ hybridisation (ISH) or immunocytochemistry (ICC) is often performed on tissue that has been fixed by paraformaldehyde or Carnoy's. Recently we reported an optimised HOPE (HEPES–glutamic acid buffer-mediated organic solvent protection effect) fixation protocol for ISH targeting mRNA in lung tissues. We have now examined whether HOPE fixation could also be used on in vitro cultured cells for targeting mRNA by ISH or proteins by ICC on cytospin preparations. Using the myeloid stem cell line KG-1a as a model system, we showed that HOPE fixation can be applied for ISH and ICC on cultured cells. HOPE can be used with cells and tissues and with a broad spectrum of immunohistocytochemical and molecular techniques.

A Glove, A Glove, My Kingdom for a Glove.

Often wondered what safety gloves you should wear in a particular laboratory situation? The following table from the USA Department of Energy might be of use:

Chemical and Liquid Resistant Gloves are made from rubber (latex, nitrile, or butyl) or a synthetic composition such as neoprene. Frequently used gloves are described below:

- Butyl Rubber Gloves provide protection from nitric acid, sulphuric acid, hydrofluoric acid, red fuming nitric acid, rocket fuels, and peroxide. These gloves have a high impermeability to gases, chemicals, and water vapour, and resistance to oxidation and ozone attack. They have high abrasion resistance and remain flexible at low temperatures.

- Natural Latex or Rubber Gloves provide protection from most water solutions of acids, alkalis, salts, and ketones. They are also resistant to abrasions occurring in sandblasting, grinding, and polishing. These gloves have excellent wearing qualities, pliability, and comfort and are a good general-purpose glove.

- Neoprene Gloves provide good protection from hydraulic fluids, gasoline, alcohols, organic acids, and alkalis. They have good pliability and finger dexterity, high density and tensile strength, plus high fear resistance.

- Nitrile Rubber Gloves provide protection from chlorinated solvents (trichloroethylene, perchloroethylene). They are intended for jobs requiring dexterity and sensitivity, yet they stand up under mechanical use even after prolonged exposure to substances that cause other glove materials to deteriorate. They also resist abrasion, puncturing, snagging, and tearing.

Gloves chemical resistance selection chart

Chemical	Neoprene gloves	Latex or rubber gloves	Butyl gloves	Nitrile latex gloves
Acetaldehyde	VG	G	VG	G
Acetic acid	VG	VG	VG	VG
Acetone	G	VG	VG	P
Ammonium hydroxide	VG	VG	VG	VG
Amyl acetate	F	P	F	P
Aniline	G	F	F	P
Benzaldehyde	F	F	G	G
Benzene	P	P	P	F
Butyl acetate	G	F	F	P
Butyl alcohol	VG	VG	VG	VG
Carbon tetrachloride	F	P	P	G
Chlorobenzene	F	P	F	P
Chloroform	G	P	P	E
Chromic acid (50%)	F	P	F	F
Citric acid (10%)	VG	VG	VG	VG
Dibutyl phthalate	G	P	G	G
Dimethylformamide	F	F	G	G
Epoxy resins, dry	VG	VG	VG	VG
Ethyl acetate	G	F	G	F
Ethyl alcohol	VG	VG	VG	VG
Ethyl ether	VG	G	VG	G
Ethylene dichloride	F	P	F	P
Ethylene glycol	VG	VG	VG	VG
Formaldehyde	VG	VG	VG	VG
Formic acid	VG	VG	VG	VG
Glycerin	VG	VG	VG	VG
Hexane	F	P	P	G
Hydrochloric acid	VG	G	G	G
Hydrogen peroxide (30%)	G	G	G	G
Hydroquinone	G	G	G	F
Linseed oil	VG	P	F	VG
Methanol	VG	VG	VG	VG
Methyl Methacrylate	G	G	VG	F
Nitric acid	G	F	F	F
Oxalic acid	VG	VG	VG	VG
Phenol	VG	F	G	F
Potassium hydroxide	VG	VG	VG	VG
Propyl alcohol	VG	VG	VG	VG
Sodium hydroxide	VG	VG	VG	VG
Sulphuric acid	G	G	G	G
Tetrahydrofuran	P	F	F	F
Toluene	F	P	P	F

Trichloroethylene	F	F	P	G
Xylene	P	P	P	F

Legend:

VG = Very Good G = Good F = Fair P = Poor (not recommended)

Reference:

http://tis.eh.doe.gov /docs/osh_tr/ch5c.html (Last modified: Monday September 28 1998)

Using the principles of PLAIN ENGLISH

Australian Style is a biannual print newsletter published by the Style Council Centre at Macquarie University. To add your name to the mailing list, please contact: Australian Style, c/- Government Services and Information Environment Division, National Office for the Information Economy, GPO Box 390, Canberra ACT 2601 or by email: subscribe.stylewise@noie.gov.au

Here are some common problems encountered when reading bureaucratic documents:

- long sentences
- passive voice
- weak verbs
- superfluous words
- jargon and acronyms
- numerous defined terms
- abstract words
- unnecessary detail.

Don't ban the passive voice —use it sparingly

Although Commonwealth style doesn't advocate using the passive voice it may make sense when it's not important for the reader to know the person or thing performing the action.

But, only use the passive voice when you have a very good reason for doing so.
If in doubt, choose the active voice.

Positive writing

Positive sentences are shorter and easier to understand than their negative counterparts. Your sentences will be shorter and easier to understand if you replace a negative phrase with a single word that means the same. Positivism on a gloomy day invigorates the reader.

For example:

not able	unable
not certain	uncertain
not unlike	alike
does not have	lacks
not many	few
not often	rarely

Feeling invigorated?

http://www.noie.gov.au/publications/NOIE/info_access_network/stylewise_vol6-3.pdf
http://www.shlrc.mq.edu.au/style/articles.html

A Stain to Try

Diastase Removal Of Glycogen

Principle:

The enzyme solution is applied to one of two sections of the tissue (preferably consecutive sections) and then both are stained by the PAS method. The presence and relative amount of glycogen in the sections can be determined by examining the extent of loss of staining in the enzyme treated section as compared with the untreated section.

This amylase reagent has a long shelf life and uses an incubation time of 10 minutes at room temperature. It is suitable for formalin and Brazil's fixed paraffin sections as well as air-dried and ethanol fixed frozen sections.

Controls: Liver containing glycogen

Reagents:

1. Amylase Reagent
   Warning: Harmful, contains azide – see MSDS

Alpha Amylase from Bacillus Subtilis (Fluka Cat No 10070)	1g
Oxoid PBS Tablets (Cat No BR14a)	1 tablet
Distilled water	100ml
Sodium Azide	0.1g

   This solution, once prepared is stored at 4^{o}C when not in use. A recycled antibody dropper bottle (often used in commercial immunoperoxidase kits) is useful for storage and application.

2. PAS Reagents (see PAS Stain)
   Warning: Suspected Carcinogen – see MSDS

Procedure:

1. De-wax and hydrate paraffin sections, hydrate frozen sections.
2. For amylase digestion, place slides on a rack, cover sections with amylase solution and incubate for 10 minutes at room temperature.
3. Wash slides well in water.
4. Place slides in 1% periodic acid 10 minutes.
5. Wash slides well in water.
6. Rinse slides in distilled water.
7. Place in Schiff's reagent 10 minutes.
8. Rinse slides in distilled water and then wash slides in tap water 3 minutes.
9. Counterstain slides with haematoxylin, differentiate and blue.
10. Dehydrate, clear and mount.

Results:

- Glycogen is extracted and so loss of PAS positive staining will occur in the enzyme treated section.

- Mucopolysaccharides are not extracted and so staining will be the same in both sections.

Reference:

V-M. Mangan, V. Farago, M. Kelly, and A. F. Henwood (2002) " An Amylase Reagent with a Long Shelf Life for the Removal of Glycogen from Tissue Sections" J Histotechnol. 25(3): 153.