Histograph

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Newsletter of the Histotechnology Group of NSW

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Editorial

Hi everyone,

My name is Linda Prasad and I am the editorial assistant. I am working in the Children's Hospital at Westmead as a Scientific Officer in the Histopathology department. I am currently doing my Masters in Medical Pathology on a part time basis. Tony Henwood and I put this issue together. I tried to make it pretty interactive. Thanks to everyone for all the articles sent to me for this issue. Hope you all enjoy reading it and get you all to have some histo fun with the quizzes and crossword.

The answers will be in the next issue of Histograph.

Anyone that has any interesting articles or would like to share anything to the histo world please do email me on lindag3@chw.edu.au. Thank you for welcoming me into the committee. I hope you all enjoy reading my first issue.

Linda Prasad
Assistant Editor
Lindag3@chw.edu.au

Yoga Class at Toukley meeting 9

Chairman's Report

Another year has quickly passed and we are heading into our AGM and elections. Although a number of our committee members have indicated they will be renominating we would welcome "New Blood" to provide new ideas. Nomination forms can be found on our website. A stable and unified committee is also important as we head into hosting the next National Conference in Sydney in November 2011.

On Wednesday the 23rd of June an evening meeting was held at the "Garvin Institute". The educational seminar on "Biomarkers in Pancreatic Cancer" was presented by Emily Colvin from the Cancer Research Program, a very informative evening on diagnosis, treatment and research of Pancreatic Cancer. Some 20 people managed to make it through difficult weather and road conditions. The evening was sponsored by the "Australian Pancreatic Cancer Genome Initiative" and refreshments by Dako. We would like to thank the "Garvin Institute", Alice Boulghourjian, Amber Johns and the sponsors for an excellent evening.

Planning for the 5th National Histotechnology Conference is well underway. Currently we are working on speakers, workshops and costings. We would welcome suggestions on topics and potential speakers.

Thank you to the outgoing committee for your support, enthusiasm, encouragement and ideas through out the past year.

A reminder that membership fees are now due.

Cheers,

Trevor Hinwood.
Chairperson.
Histotechnology Group of NSW.

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NZ Histology Conferences and Workshops 2010

Hi Histo colleagues. Robyn Rae, the NZ Histology SIG convenor, has asked me to give you all an update of what is happening in the NZ Histo world in 2010.

In September we have a full day programme of Histology at the NZ Institute annual general meeting and Conference at the Bay of Islands on Thursday the 26th of August.
This is the first time in recent years that we have had a significant number of Histology presentations at this conference. It is also good that they are on the same day.

Two of the presenters are from the UK. They will give cut up for Scientists presentations. Gordon McNair from the UK is a Chief Med Lab Scientist, lectures on cut up at the University of Ulster and is a Council member of the Institute of Biomedical Scientists. Carol Turnbull leads the cut up section of a large UK Hospital.

Both presenters will also lead a workshop on cut up for Med Lab Scientists at Lab Plus, Auckland City Hospital on Saturday the 21st of August. Details of this will be coming up soon on the NZIMLS website. This is a workshop designed to kick start a qualification in cut up for Scientists. A similar qualification has been available in the UK for the past eight years.

Also at the Bay of Islands Sonya Prasad from the RCPA will present on Quality Assurance. Natasha Caldwell and Tania Smith will also present on Post Graduate education and automated immuno machines. There is also space for proffered papers. If you would like to present please contact me. By sheer coincidence the Conference dinner is on Thursday night so a great opportunity to network. The venue is the Copthorne hotel, Bay of Islands.

The Annual NZ Histo SIG is booked at the War Memorial Conference centre. Napier. This will be a one day SIG and the date is the 20th of November. Please start thinking about presentations. Send them on to me.

All in all we are going to have a busy year.

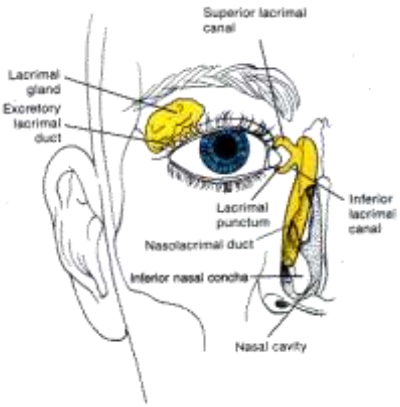
1. Workshop on cut up for Medical Laboratory Scientists. LabPlus. Auckland. 8.30am-4.00pm Sat 21st of August. Details available on NZIMLS website. Numbers will be limited.
2. Histology presentations at the NZIMLS Conference Bay of Islands. Thursday 26th of August. Details available on NZIMLS website.
3. Histology SIG. War memorial Conference centre, Napier. Saturday the 20th of November. Details to be announced soon on NZIMLS website.

Have fun,

Joe McDermott.

Here's Some Questions to get you Thinking. I'm sure you will be able to answer them all.

HAVE FUN



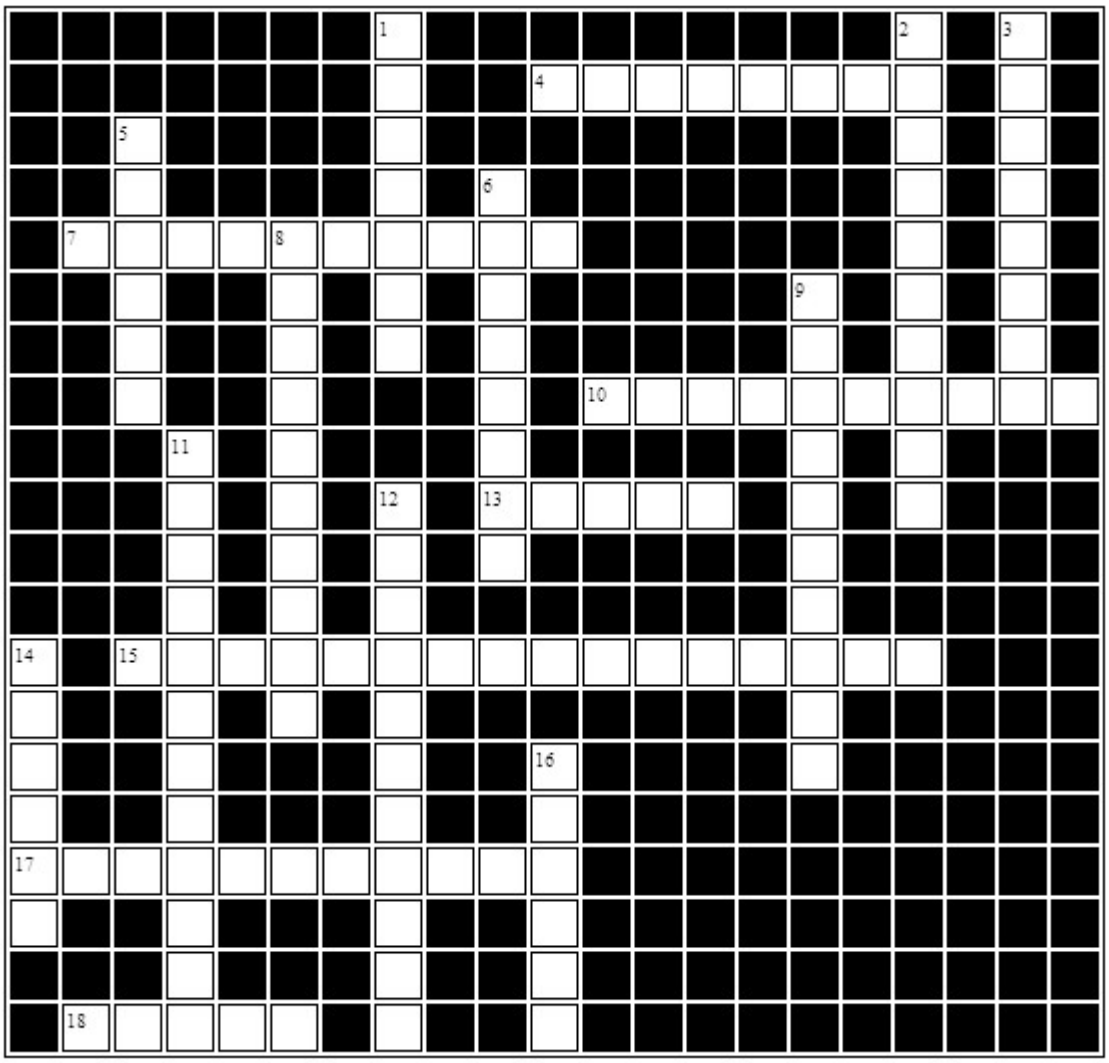
Anatomy Questions

1. Located between the capsule and medulla of the kidney.
2. Islets of Langerhans are found here.
3. Parathyroid glands help regulate this mineral.
4. These dramatically increase the surface area of the intestine.
5. The pineal gland is here.
6. The most common molecule in the body.
7. Which is most superficial: dermis, epidermis or hypodermis?
8. This lung has 3 lobes.
9. This organ plays a key role in T cell differentiation.
10. The white of the eye is called this.

Epithelium

Linda Prasad

Classification of epithelial tissues



Across

4. A..... membrane that separates the epithelium from the connective tissue
7. Main purpose of stratification in epithelial tissue is for increased .....
10. Non motile projections that cover absorptive cells
13. Glandular epithelium forming rounded masses of epithelial cells
15. What type of epithelium gives the misleading impression that the epithelium is stratified
17. Epithelium lining the vascular system
18. Motile structures found in the uterine tubes, uterus and conducting tubes of the respiratory system

Down

1. Epithelial found on the palm of the hands
2. If the cells are arranged in two or more layers, the epithelium is .....
3. Epithelial cells which have the same height, width and depth
5. Membrane that lines the peritoneal, pericardial and pleural cavities
6. Epithelial cells that are taller than they are wider
8. Consists of sheets of cells that line the cavities and surfaces of structures through out the body
9. Another name for transitonal epithelium
11. Microvilli, which function to increase surface area, are most likely to be found in ..... epithelium
12. Another name for simple squamous epithelium
14. Mucus secreting cells
16. Epithelium that is one cell thick

Report on Central Coast Scientific Meeting April 2010

17-18 April 2010

On behalf of the NSW Histogroup committee and members who attended the 2 day meeting at Toukley RSL, I would like to say thank you to Marg McLauchlan for organising a very interesting and well run weekend. I enjoyed myself immensely and Toukley put on its best weather so we had a bright sunny day and a lovely evening in which to relax and enjoy good food, wine and company.

The guest speakers were all very clear in their messages to us and gave us valuable insights into the importance of histology in their particular fields, or showed us how to 'escape' from our daily job and get more out of life.

The first speaker was Dr John Newton, a plastic and reconstruction surgeon, whose talk was *"Being a Part of Patient Care"*. Dr Newton's philosophy is that the specimen is part of the patient and he talked about the surgeon's needs, the patient's needs and how histopathology is vitally important in making decisions about the best course of treatment for a patient. Giving clear and accurate reports is instrumental in improving the person's outcome.

The second speaker was Dr Phil Woodford, Senior Staff Specialist Anatomical Pathology, John Hunter Hospital. His talk was *"A Pot Pourri of Skin Infections"*. Dr Woodford talked about exotic and not so exotic cases, illustrating the responses of skin to infection and, in answer to a question, he gave us his fascinating opinion on spider bites and patient responses to them.

The third speaker was Marg McLauchlan, formerly Pathology Assistant, Nextpath Newcastle. Her talk was *"Voice Recognition Technology and Histology Reports"*. Nextpath began using computer voice recognition for macroscopic reporting in 2007. This presentation explained how this works in a busy laboratory and gave an overview of the journey from the initial difficulties through the 'hiccups' to the advantages reaped. Her conclusion, "the pain is worth the gain".

The fourth speaker was Associate Professor Gary Hoffman, Consultant Maxillofacial Surgeon and Newcastle Member of the Multi-Disciplinary Team Head and Neck Surgery. Prof Hoffman's talk was *"Oral Mucosal/Head and Neck Surgery"*. This presentation provided a brief understanding of the aetio-pathogenesis and management aspects of oral/oropharyngeal head and neck malignancy, by taking us through the four steps to dissection- Aetiology; Biology; Clinical presentation and cervical lymph nodes; Dissection of the neck.

The fifth speaker was Steve McLauchlan, Trainer and Video Producer who spoke on *"How to avoid Occupational Overuse Syndrome"* otherwise known as RSI. Steve has produced several OHS training films for the health industry and is aware of the widespread potential for health workers to develop RSI. This presentation examined why it happens and what can be done to reduce the occurrence.

The sixth speaker was Bill Sinai, former Laboratory Manager Anatomical Pathology Westmead Hospital. *"Life After Pathology"* sounds wonderful and very busy as Bill showed us his retirement through slides of his golf, family, golf, trips and golf, and yes he did mention golf.

SUNDAY

The seventh speaker was Dr Eugene Leong, Breast Cancer Research Fellow ANZ BCGT. His talk was on *"Breast Cancer Research Clinical Trials"*. Dr Leong first gave us an overview of the ANZ BCTG and its role in breast cancer research in Australia/New Zealand, then some background information about classification of breast cancer before proceeding to discuss several currently open clinical trials in which the ANZ BCTG is involved, including any relevant pathology issues. Finally he gave us review of the interaction and collaboration between the trials group and the pathology laboratories.

I will mention here the web site for the ANZ BCTG as there is a preventative trial at the moment that is relevant to a lot of women I know. They are still enrolling women as the trial number of 6000 has not been reached yet. The web site is www.anzbctg.org. The trial is IBIS II.

The eighth and last speaker was Dr Jon Gani General and Abdominal Surgeon. His talk was *"Rapport and Communication"* and the two cases he presented highlighted the need for good communication between pathologist and surgeon.

Also on Sunday, we had a quiz, presented by Bill Sinai and NOT all on histology, our general knowledge was tested and sadly found wanting in some cases. The winners were the *'The Roses'* group (see Photo) with Tony Chow as the thorn among the Liverpool roses. There was also a Lucky Door prize won by Pat Skalkos.

The last mention goes to Susan Cook, a yoga-stretch and relaxation instructor, who gave us two sessions of yoga, stretching and relaxation. It was a marvellous way to end the two days and I think introducing a yoga session into our work day would lower RSI and improve our overall wellbeing.

Thank you and Cheers,

Julie Bilkey.

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Yoga class at Toukley meeting

Winners at Toukley meeting

What is a Tissue Microarray (TMA)?

Nicole Mackie, Daniel Catchpoole, Albert Chetcuti and Tony Henwood
The Tumour Bank , The Children's Hospital at Westmead, NSW.

Tissue microarrays are produced by a method of re-locating tissue from many conventional histological paraffin blocks to a single block so multiple patients or blocks can be seen on the same slide.
This is done by using a needle to biopsy the area of interest from the conventional block and placing the core into an array on a recipient block in a grid like pattern.

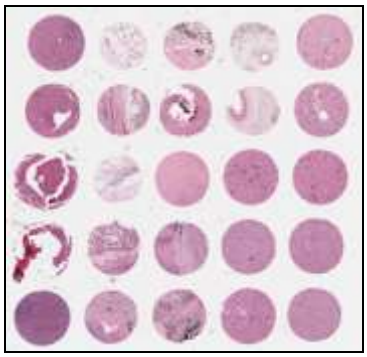

Figure 1 H&E 1mm cores tissue microarray

The method was originally described in 1987 by Wan, Fortuna and Furmanski in the Journal of Immunological Methods. They published a modification of Battifora's "sausage" block technique whereby tissue cores were placed in specific spatially fixed positions in a block.

In 1998, Kononen and colleagues in the lab of Ollie Kallioniemi invented a mechanism for examining several histologic sections at one time by arraying them in a paraffin block (Kononen et al, 1998). These tissue microarrays are assembled by taking core needle "biopsies" of pre-existing paraffin-embedded tissues and re-embedding them in an arrayed "master" block.

The Tissue Arrayer
A tissue arrayer is an instrument that enables a user to construct tissue microarrays. The punches are sharp and care should be taken to prevent injuries.
The tissue arrayer is used to extract 0.6mm, 1mm or 1.5mm diameter core samples from specimens and deposit them into a blank wax block.

Figure 3 Beecher Manual Tissue Arrayer (Beecher Instruments, USA)

Applications for research in the Histopathology laboratory
In this way, tissue from hundreds of specimens can be represented on a single paraffin block that can be analysed using a variety of techniques, including immuno-histochemistry and in situ hybridization (Bubendorf et al, 1999; Kononen et al, 1998; Moch et al, 1999; Mucci et al, 2000; Perrone etal, 2000; Schraml et al, 1999).

In contrast to traditional techniques, which require the processing and staining of hundreds of slides, microarray technology enables the study of an entire cohort of cases by analysing just one (or a few) master slide(s). Microarray analysis has the added advantage that all specimens are stained at one time using identical conditions. Furthermore, it markedly reduces the amount of archival tissue required for a particular study, thus preserving ample remaining tissue for other research or diagnostic needs (Mills et al, 1995).

Making the Tissue Array
Sampling the correct site from donor blocks is critically important for constructing tissue arrays. A fresh H&E stained slide should be obtained from each block and used as a guide to select the regions for sampling. The H&E slide should be circled or otherwise mark the sampling site on the corresponding H&E slide before starting the arraying process.
(Manual Tissue Arrayer MTA-1 Beecher Instruments Instruction Manual)

A blank paraffin block is the recipient for the tissue samples. Prepare the block by melting paraplast wax and dispensing it into a deep eyeball size mould. Place

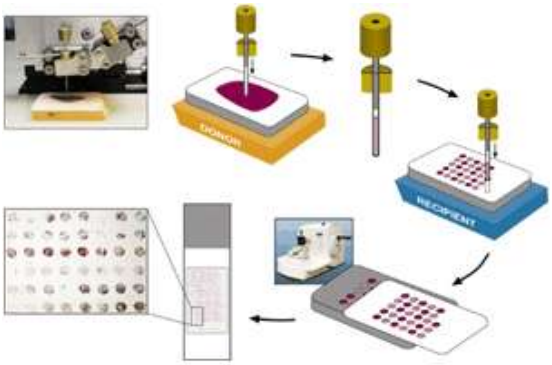

Figure 1

Figure 2. An example of a tissue microarray and its construction. The arrays are assembled by taking core needle "biopsies" from specific locations in pre-existing paraffin-embedded tissue blocks and re-embedding them in an arrayed "master" block, using techniques and an apparatus developed by Konenen et al. In this way, tissue from 600 specimens can be represented in a single paraffin block.

a cassette on top of the melted paraffin until the wax is cooled and the mould is ready to be removed. Check block for any holes or cracks that may have arisen during the block preparation.

Make sure that the block surface is flat and parallel to the underside of the cassette, by facing off or trimming the block surface on a rotary microtome taking care not to have any scores or nicks in the block.

Cores are deposited in a grid pattern to form a 'tissue microarray' Dependent on the size of the cores up to 1000 patient samples can be put into the one recipient block. Place blocks under a low wattage lamp before coring making them softer and less likely to crack and easier to punch.

Before sectioning the array block the surface must be smoothed and levelled. Annealing or warming up of the paraffin promotes adherence of the tissue biopsies (cores) to the walls of the array block and makes the wax flexible to handle.
This can be done in a slide oven at 60 degrees at 10 minute intervals four times until the block looks sweaty and feels like blue tac. Let the block cool over night before sectioning.

Tissue microarray blocks are cut into many ultra-thin slices between 4 - 8µm. One recipient TMA block can produce between 100-500 sections, dependent on the depth of the donor cores.

It is advisable to limit the time the section is floated out on the waterbath to prevent excess stretching of the cores.
Sections are fixed to superfrost plus glass microscope slides.

Heat slides at 60 degrees for 20 minutes then perform stain required.

Figure 4 Archival Blocks from histopathology stores.

Figure 5 Match H&E slide with block and mark area of interest to be cored.

In collaboration with the Histopathology Department within the Children's Hospital at Westmead, the Tumour Bank has manufactured custom tissue microarrays (TMA), containing tissue cores from specific paediatric cancers. The Tumour Bank has the expertise to prepare specific cancer TMA's upon request.

For any further information please contact The Tumour Bank at The Children's Hospital at Westmead
Ph: 02 9845 3028
Email:tumourb@chw.edu.au
Locked Bag 4001
Westmead NSW 2145

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1. Battifora, H. (1986) The multitumor (sausage) tissue block: Novel method for immuno-histochemical antibody testing. *Lab. Invest.* **55,** 244–248.

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EBV Update

The Epstein-Barr virus (EBV) was originally described in 1964 by Epstein, Achong and Barr in cells cultured from a lymphoma identified by Dennis Burkitt in Ugandan children. In 1968, Werner Henle and his wife Gertrude identified EBV as the cause of infectious mononucleosis. After its recognition, the virus was constantly detected in neoplastic cells of endemic Burkitt's lymphoma and undifferentiated nasopharyngeal carcinoma, raising the question of an aetiological role for the virus in the development of these tumours[1].

EBV is a ubiquitous human herpes virus that causes infectious mononucleosis and is strongly implicated in the pathogenesis of several human malignancies. The cellular receptor for EBV is the CD21 molecule, which is expressed in both lymphoid and epithelial cells. B lymphocytes are the usual site of latent infection, whereas epithelial cells usually become productively infected. In these latently infected cells, the EBV genome is maintained as a multicopy episome, but no virus is produced. These cells express a restricted subset of viral genes: 6 nuclear proteins, 2 membrane proteins, and 2 classes of RNA polymerase III transcripts. Immunologically competent hosts control latent EBV infection through HLA-restricted cytotoxic effectors that recognize a number of the latent-phase proteins as antigens[2].

EBV associated post-transplant lymphoproliferative disease (PTLD) is an important complication in transplant patients. The risk of developing PTLD is variable depending, among others, on the nature of the transplanted organ and on the level of iatrogenic immunosuppression. In general, patients undergoing primary EBV infection after transplantation and those treated with anti-T cell reagents are particularly at risk. The incidence of PTLD is higher in paediatric patients than in adults. PTLDs are usually of B cell lineage, and include a spectrum of disorders ranging from polyclonal polymorphic lymphoproliferations to monoclonal non-Hodgkin's lymphoma. The prognosis of PTLD remains poor and treatment is controversial[3].

Engel et al[4] studied the incidence of EBV in childhood Hodgkins lymphoma and found that patients whose lymphomas that had demonstrable EBV had a better survival than those that did not. Conversely it has been shown that there is a poor survival of EBV-positive HL in elderly and immunosuppressed patients.

Becker et al[2] studied whether EBV infection of renal proximal tubule cells played any role in the initiation and/or progression of the inflammatory and injury pattern seen in primary interstitial nephritis. Using ISH, they demonstrated that EBV but not cytomegalovirus or adenovirus, was detected primarily in renal proximal tubule cells. Furthermore, the CD21 antigen, which serves as the receptor for EBV in B lymphocytes, was detected by immunocytochemistry primarily on proximal tubule cells and was markedly up-regulated in the EBV-infected tissue. They suggest that EBV infection of renal proximal tubular cells may participate in evoking a cellular immune response that results in a damaged renal interstitium.

Tsutsumi[5] was able to demonstrate CD15, and EBV RNA (using ISH) in paraffin sections of Hodgkin's lymphoma collected 170 years ago by Dr Thomas Hodgkin. It is amazing that the antigenicity of CD15 and nuclear RNA EBER-1 survives in fixed tissues over a long, long time.

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Histo History

Arthur Bolles Lee (1900) "The Microtomtst's VADE-MECUM - A Handbook of the Methods of Microscopic Anatomy" fifth ed June.p73

"Formaldehyde is the chemical name of the gaseous compound HCOH, obtained by the oxidation of methyl-alcohol. "Formalin" is the commercial name given by Schering & Co. to a 40 per cent solution of this substance in water. "Formol" is the commercial name given to the same solution by Meister, Lucius & Bruning…… As I have before pointed out (Anat. Anz., xi, 8, 1895, p. 255), the already extensive literature which treats of the anatomical uses of formaldehyde is much confused by inaccurate use of these terms; many writers use them indiscriminately. It is frequently impossible to discover from the statements of an author whether he means such or such a percentage of formaldehyde, or such or such a percentage of the commercial 40 per cent, solution employed by him, the one being of course two and a half times stronger than the other. All that can be said is that the majority of authors seem to quote in percentages of the commercial solutions. I think it must be admitted that the proper way of stating the strengths of these solutions is either to state them in terms of formaldehyde, and say so, or to say "formol, or formalin, diluted with so many volumes of water". The present confusion is most inconvenient."

"If tissues be left in alcohol for only a few days before further preparation, the injurious effects of a sojourn in alcohol will perhaps not be very disagreeably evident. But it is otherwise if they are put away in it for many weeks or months before the final preparation is carried out. The dehydrating action of the alcohol being continuously prolonged, the minute structure of tissues is sometimes considerably altered by it; they become over-hard and shrink, and become brittle, and their capacity for taking stains well becomes seriously diminished."

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Are Formalin Substitutes Germicidal?

It is believed that formalin given time will kill any microorganisms that are present in tissue and that formalin will inactivate tuberculosis.

Vardaxis et al (J Clin Pathol 1997;50:429-433) were quite rightly concerned with the disinfection of bacterial endospores. Endospores can survive the most adverse chemical and physical environments and can cause such diseases as tetanus, anthrax, gas gangrene and botulism.

They used autoclave spore containing test strips and fixed them in various fixatives such as 10% neutral buffered formalin, ethanol in various concentrations (70% & 50%) and two commercial non-formalin fixatives (Kryofix & Spuitfix). Fixation times varied from 24 hours to 14 days.

They found that 10% formalin killed all microbes within 24 hours, where as only one non-formalin fixative (Spuitfix) killed the spores and this was only after 7 days fixation. Microwave fixation also did not kill the spores.

These workers did note that spore strips do not behave like tissue and that tissue may have a protective effect on pathogens.

Cleary et al (J Clin Pathol 2005;58:22-25) studied the antimicrobial effects of UMFix, an alcohol based tissue fixative, on various microorganisms. The UMFix solution was compared with 10% neutral buffered formalin.

After a short exposure, UMFix rapidly killed vegetative bacteria, yeasts, moulds, and viruses. Bacterial spores were resistant to killing by UMFix. All organisms were killed by the 10% neutral buffered formalin preparation. They concluded that UMFix was microbicidal for vegetative bacteria, yeasts, and aspergillus species after a short exposure, although it was not active against spore forming bacillus species. The methanol content of the fixative was responsible for the killing effect of this fixative. No killing was seen when polyethylene glycol was used alone.

Kappel et al (HUM PATHOL 27:1361--1364, 1996) attempted to grow TB from formalin fixed lung tissue that had previously been shown to be positive by sputum culture. They were unable to culture TB from these tissues.

Gerston et al (HUM PATHOL 35:571-575.2004) in South Africa analysed 138 formalin fixed lungs with histological evidence of AFB and were able to culture TB from 12 of these cases (one of these cases had been fixed for 80 days before being tested).

Gerston et al suggest that there is a risk of contracting tuberculosis from tissue that has been fixed in formalin, if aerosols or accidental inoculation should occur.

Trimming and sectioning wax blocks are of concern but no studies have been done yet.

Of concern to histotechnolgists are:

1. Tissue with Inflammation-induced Encapsulation may protect bugs from formalin.
2. Formalin dilutes as it penetrates tissue.
3. Formalin substitutes may not be germicidal.

QUIZ -TRUE or FALSE

Ref. Choice Health Reader

1. Circadian rhythm has an effect on the effectiveness of cancer treatments

2. Eating carrots will protect your eyes against the glare of computer screens

3. *Indigo naturalis* is a red plant-based powder used to treat psoriasis

4. "French paradox" is a term referring to the French enjoying regular

5. Consumption of red wine and experiencing low rates of heart disease

6. Diverticular disease is a disorder effecting one-third of the American population over 60

7. Eating meat, fish and dairy products and drinking alcohol could protect the brain from shrinkage in old age

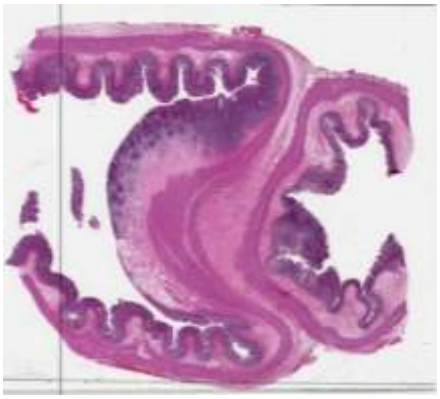
8. Cardiorespiratory fitness can reduce the risk of dying of cancer associated with being overweight

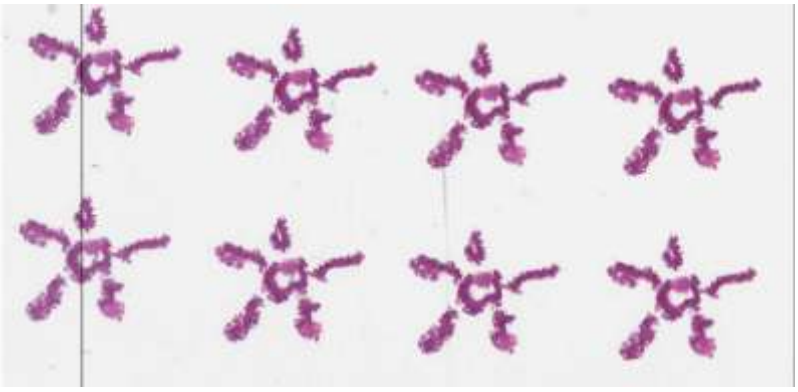
9. Human breast milk is a sterile food source for babies

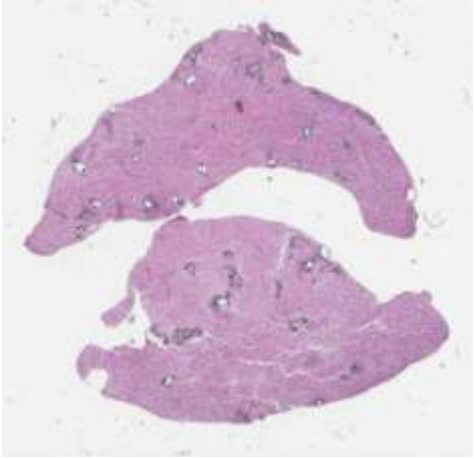
10. It is no longer necessary for you to see your GP to get a medical certificate; your pharmacist can do instead

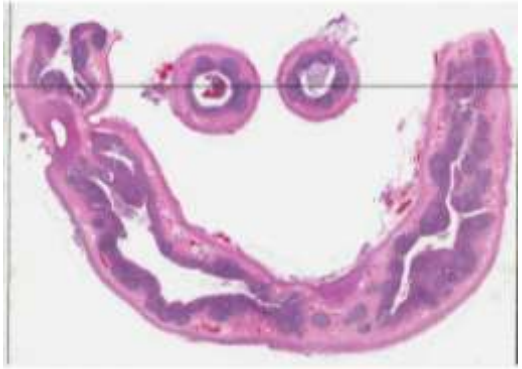
11. Smokers are more likely to have a restless night then non smokers

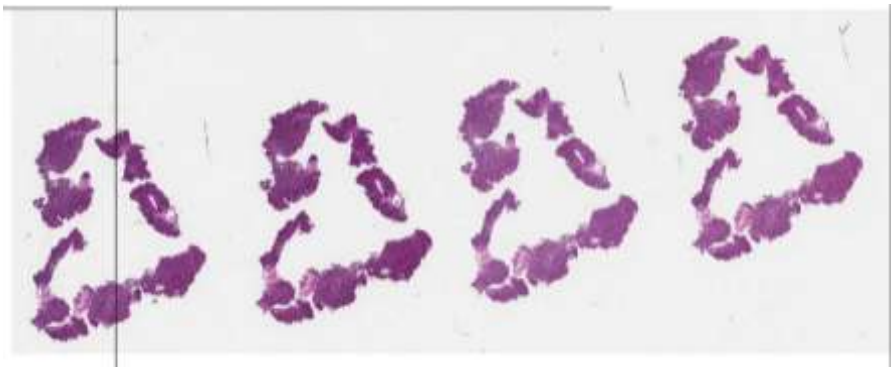
Histo Artwork







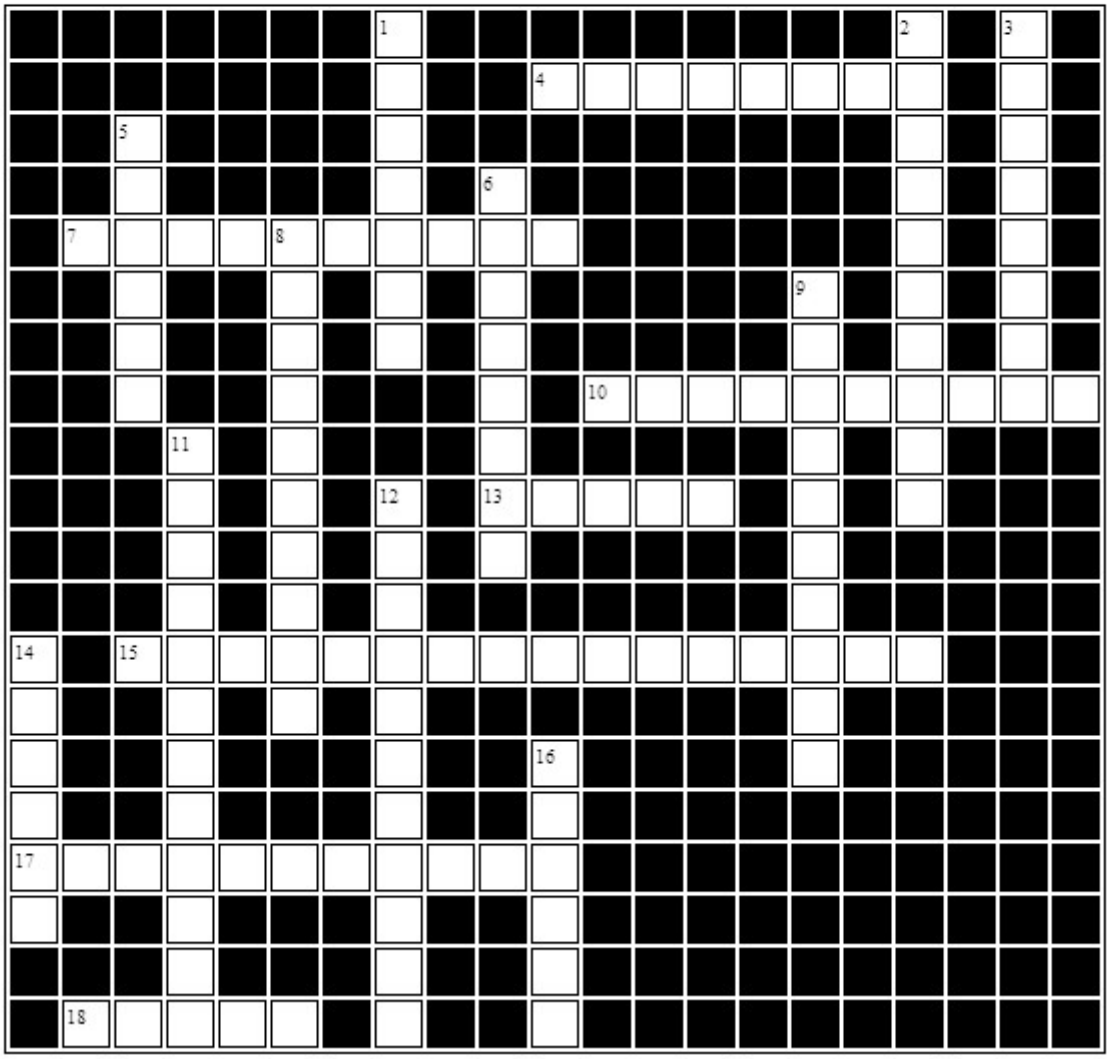




Epithelium

Linda Prasad

Classification of epithelial tissues



Across

4. A..... membrane that separates the epithelium from the connective tissue
7. Main purpose of stratification in epithelial tissue is for increased .....
10. Non motile projections that cover absorptive cells
13. Glandular epithelium forming rounded masses of epithelial cells
15. What type of epithelium gives the misleading impression that the epithelium is stratified
17. Epithelium lining the vascular system
18. Motile structures found in the uterine tubes, uterus and conducting tubes of the respiratory system

Down

1. Epithelial found on the palm of the hands
2. If the cells are arranged in two or more layers, the epithelium is .....
3. Epithelial cells which have the same height, width and depth
5. Membrane that lines the peritoneal, pericardial and pleural cavities
6. Epithelial cells that are taller than they are wider
8. Consists of sheets of cells that line the cavities and surfaces of structures through out the body
9. Another name for transitonal epithelium
11. Microvilli, which function to increase surface area, are most likely to be found in ..... epithelium
12. Another name for simple squamous epithelium
14. Mucus secreting cells
16. Epithelium that is one cell thick

Match the invention with a year.

1965 2500BC 1608 1839 1250

1590 1714 133BC 1938 1919

1. ball point pen
2. concrete
3. mercury thermometer
4. magnifying glass
5. telescope
6. tea bags
7. bicycle
8. microprocessor
9. compound microscope
10. ink

Stains Questions

1. Favourite nuclear stain. ……..
2. Congo red stains this. ……..
3. This should be neutralised with NaCl before discarding.……
4. A good stain for elastic fibres. ……..
5. What substance does Fouchets stain. ……
6. A good fat stain. ……
7. A stain for fungi (3 words) ……
8. Staining process for mast cells.……
9. Reticulum is stained by what stain……..
10. Stain for positive and negative organisms.……

Phaeochromocytoma

Phaeochromocytomas are tumours that arise in the adrenal medulla and chromaffin bodies and are recognised by their secretion of catecholamines (adrenaline and noradrenaline). It is often a complication of surgery for this tumour's resection that, when manipulated, it can cause a sudden rise in blood pressure.

The adrenal glands are orange-coloured endocrine glands that are located on the top of both kidneys. The adrenal glands are triangular shaped and measure about one-half inch in height and three inches in length. Each gland consists of a medulla that is surrounded by the cortex. The medulla is responsible for producing epinephrine also known as adrenaline. The adrenal cortex produces other hormones necessary for fluid and salt balance in the body such as cortisone and aldosterone. Disorders of either the cortex or the medulla can result in hypertension.

Pheochromocytoma accounts for less than 1 percent of all hypertensive cases. There is no male to female sex preference and it can occur at any age, but is most common in people between the ages of 40 and 60. Ninety percent of cases are sporadic. However, 10 percent can be linked to hereditary causes.

Immersion of thin sections of this tumour in dichromate containing fixatives colours this tumour brown. This is known as the chromaffin reaction and is presumably due to a reaction with the catecholamines. Epinephrine and norepinephrine can be readily oxidized by most potassium dichromate solutions to form a dark-brown dye. When this reaction occurs in a tumour containing the epinephrines, the resulting brown dye stains the tumour cells and the supporting structures.

As a rule a brown colour of diagnostic significance became evident in 5 to 15 minutes.
Zenker's solution was the most effective, producing a much darker colour than other dichromate solutions (3.5% potassium dichromate-in water, in 10% Formalin, and in 10% Formalin with 2% hydrochloric acid, the latter producing a weak and muddy-brown colour having a green tint).

Enterochromaffin cells of the intestine also give a positive chromaffin reaction even after formalin fixation. This is due to the presence of 5 hydroxytryptamine. It is difficult to obtain a positive reaction with adrenal medulla cells even after a short fixation time with formalin.

Phaeochromocytomas stain brown to black with ammoniacal silver and also give a positive Schmorl's reaction.

Neuroblastomas and ganglioneuromas may also produce catecholamines and so give a positive chromaffin reaction.

The differentiation of Phaeochromocytomas from anaplastic carcinomas of the adrenal cortex can be difficult. A positive chromaffin reaction and negative stain for fat (eg Oil red O), favours a medullary tumour (eg Phaeochromocytoma) whereas the converse occurs in cortical tumours. The presence of lipids is a characteristic of cortical tumours even when anaplastic.

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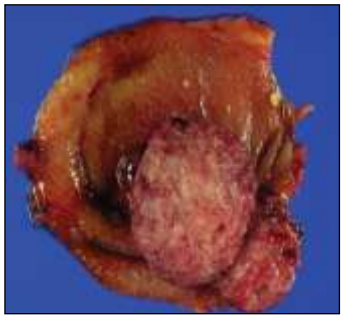
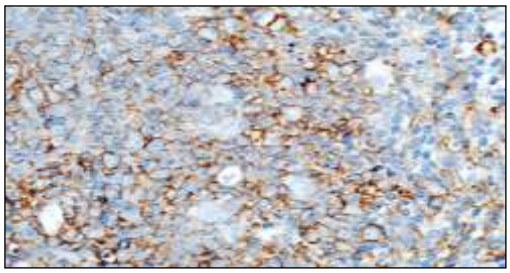
LYMPHOMAS from A - Z

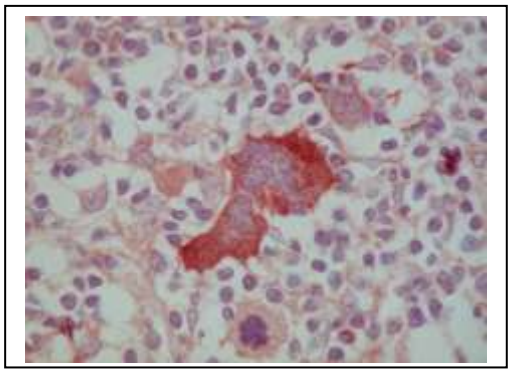


AG

Dr Ivan Burchett

Douglass Hanly Moir Pathologist
will discuss lymphomas from diagnosis
to treatment with reference to specific cases





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or nswhistogroup@bigpond.com
or fax attn: Kathy Drummond 9855 5169

I will be attending the above invitation.

Name: _____ Contact no. _____

Number of people attending: _____
Remember NATA Accreditation requires personnel who work in laboratories to attend continuing education sessions. All meetings sponsored by the Histotechnology Group of NSW count towards these requirements.

Solvent Recycling and Liquid Waste Minimization – The Time May Be Now

*Gerald W. Camiener PH.D.**

Today's laboratories literally are being caught between the proverbial "rock and a hard place". On one hand, laboratories are facing ever increasing pressures to cut costs, to cut personnel, to make their workplaces safer, to use less space, and so on. At the same time, these same laboratories are facing ever increasing costs for solvents and waste disposal, the reduced availability of trained personnel, reduced space, and the increasing-need for compliance with a plethora of government-mandated waste-minimization programs. In short, today's laboratories are expected to do much more with much less. It really is a tough balancing act.

A recent bright spot, however, is the availability of some newer-types of solvent recyclers and liquid-waste concentrators that offer a way to simplify and solve at least some of these seemingly intractable and conflicting problems. This newer equipment takes advantage of some recent advances in electronics, computers and distillation technology. The following is an attempt to summarize some of the problems that are faced by today's laboratories, and some suggestions as to how they might be solved using this newer equipment:

1. Cost Reductions:
   a. The costs for purchasing new solvents usually can be reduced by at least 90-95 % (and in some extreme instances, by as much as 99 %) by simply recycling the solvents. The older concerns about the purities of recovered solvents, or their possible contamination with other solvents have pretty well been eliminated with the advent of the newer fractional-distillation technology. The purities of solvents usually obtained with these recyclers often exceed 99.5 %. It is also interesting to note that the purity of the recycled solvents is usually higher than the starting purities of the solvents that are being purchased.
   b. The costs for disposing of solvent wastes correspondingly are reduced by the same percentages as those obtained from purchasing new solvents (see above). Clearly, reclaimed solvents don't require disposal.
   c. Other hazardous liquid wastes that are generated in today's laboratories also are required to be collected and disposed of in a safe manner. Such wastes include radio-isotopes, metal like mercury, chromium, and arsenic (cacodylic acid buffers), infectious liquids, carcinogens like DAB and TMB, phenolic solutions, and the like. The disposal costs for these types of liquid wastes are much more expensive than solvent disposal, and in some cases, then can be as high as $2,000-$2500 per 55-gallon drum, a brutally high cost.

   The key to understanding why it is important to have a program that reduces the volumes of these hazardous liquid wastes (waste minimization) is to remember that waste disposal costs are based on volume, not concentration. With the newer concentrators, liquid waste volumes (and hence, disposal costs) are easily reduced by 90-95%, and in many cases, by as much as 99%. This reduction is equivalent, for example, to reducing a waste stream of 1 drum per week down to a level of 1-2 drums per year. The savings can be enormous.

   There is one important requirement, however, that must be observed without exception. That is the absolute need to produce a non-hazardous distillate fraction that can be disposed of easily, as for example, pouring it down a sink. Until the advent of the new concentrations, however, this was not possible. But with the new technology, for example, it's now possible to reduce the volume of a mercury -containing fixative by 30-100 times, while producing a distillate-fraction that contains mercuric iron as less than 1 part per billion, a level required by Federal regulations.

2. Personnel Reduction and Simplification:
   a. Highly trained safety personnel and lots of hours are needed for the collection of solvent wastes and other hazardous liquid-wastes: their transportation from laboratories to on-site collection point; their judicious mixing and storage at the collection point; the off-loading of these wastes to an outside carrier; the record-keeping and documentation associated with this waste disposal; and the purchasing of new solvents. All of these costs can virtually be eliminated by locating the newer-type solvent recyclers and concentrators at the same sites where the solvent and liquid wastes are being generated.
   b. Highly trained laboratory personnel are increasingly difficult to obtain and their salaries are increasing every year. Fortunately, the newer recyclers and concentrators are so automatic that they require only a few minutes time of staff time per run, and they are easily operated by relatively unskilled personnel.
3. Space Reduction:

Contrary to conventional wisdom, the installation of recyclers and concentrators in a laboratory results in having more space available, rather than less. The reasons are two-fold: First, the recyclers and concentrations themselves require only a relatively small amount of floor space – only about 5 square feet per piece of equipment; and second, by having this equipment available, almost all of the flammable storage lockers can be eliminated from the laboratory and from the central collection points.

4. Safety Considerations:
   a. General laboratory safety is increased manifold simply by not having large amounts of flammable solvents and other hazardous wastes present in the laboratory, and by not having to transport those wastes through the facility. In addition, the amount of fumes present in a laboratory also are much reduced with the newer equipment.
   b. The risk of spills is significantly reduced with recycling and liquid-waste reduction programs: (i) smaller volumes of liquids are handled in any given day; (ii) the newer equipment contains built-in spill containment devices; and (iii) the newer equipment is manufactured with unbreakable components like metals and plastics.
   c. "Safe" solvents can be used in the laboratory to replace hazardous solvents, like toluene and xylene. This is because the cost differences between the solvents are virtually eliminated with recycling programs. Cost no longer needs to be a factor in deciding whether to use safer solvents.
   d. Internal safety features on the newer types of equipment provide high levels of safety. Among the features that are routinely present are the following:
      - Explosion-proof electrical components and compartments.
      - Automatic shut-down due to over-heating and other malfunctions.
      - Pressure relief valves.
      - Listed to (i.e., meets) UL 2208 (Solvent Recycler), UL 3101 (General Laboratory Equipment), CSA (Canadian), EC (European), and CB (International) Standards.
5. Meets latest Uniform Fire Codes for Solvent Recyclers.

**Gerald W. Camiener, Ph.D., 2211 Lake Club Drive, Suite 205, Columbus, Ohio 43232, 1-800-941-9484.*
Submitted by Ewan Sutherland (Thermo – CBG)

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