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Newsletter of the Histotechnology Society of NSW

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Editorial

Well, I'm back!!!

You will be happy to know that Linda had a healthy, and I must say very cheeky, baby boy late last year and both are doing really well, though Linda has wondered what the hell happened to her ordered life! So Linda will be pre-occupied for the next 12 months being on Maternity leave. Somehow you ended up with me as the editor again. Tough bickies!!

Hopefully you find this issue interesting and worthwhile. Zika virus takes up Safety corner, Specific Gravity for those who need some Back to Basics now and then, a review on S100 immunohistochemistry and finally information on Embolisation pigments.

We have an update on the recent tissue identification workshop written by Momoko Sakaki as well as the Children's Hospital at Westmead attempt at celebrating Histotechnologists Day. There are also updates on up-coming workshops and our joint State meeting with Queensland.

Finally, a big thank you to our companies who continue to support our Society. Without their money we would be out of pocket and in the dark on many new developments in Histopathology.

Tony Henwood,
Acting Editor
tony.henwood@health.nsw.gov.au

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Chairman's Report

A lot has been happening since our last Histograph.

Workshops and meetings.

- In November we held a "MacroPath" practical workshop supported by ABACUS ALS together with a "Microscopy photos using Smartphones" supported by Trajan Scientific and Medical. The workshop was fully subscribed with a lot of interest in these products and methods.
- Our Christmas Scientific talk and dinner was held at North Ryde RSL. The Christmas scientific talk was presented by Ian Whitchurch on "Medieval Astrology and Health". Over 40 people attended. These are great nights, an interesting talk with nice food and a good chance to catch up with friends and make new ones.
- In February we held a "Tissue Recognition practical Workshop" at Sydney Institute of TAFE in Ultimo. This will be our new home for workshops due to the closure of the Histology component at Granville TAFE. The course was fully subscribed with 26 participants. The course was prepared and present by Leah Simmons [a presenter at TAFE and committee member]. Leah did an excellent presentation and presented each participant with a "Tissue Recognition Photobook" which Leah had prepared. The booklet was supported by our Society. Feedback has been very positive and we are looking at repeating it again next year. There is a separate report in this Histograph.
- This year's popular "Cut-Up" workshops which are held in Canberra and presented by Penny Whippy and Anne Prins, are fully subscribed. These are held in April and September.

Work continues on upgrading our Website. This is now much improved and with the integration of "PayPal" a lot easier to make payments for memberships and workshops. Visit our Website regularly as it is an important means of communication between yourselves and our Society.

A lot of preparation has been going into our joint 2016 Conference in Port Macquarie with the Queensland Histotechnology Group. The program is almost complete with some interesting speakers and topics. Make a note in your Diary for the weekend 30th September to 2nd of October. This will be a great weekend for learning and exchanging information and ideas with fellow delegates. There is a separate flyer with additional information in this Histograph.

Lots still to happen this year,

Cheers,

Trevor Hinwood.
Chairperson.
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Embolisation Pigment

Embolisation is a nonsurgical, minimally invasive procedure performed by interventional radiologists and interventional neuroradiologists. It involves the selective occlusion of blood vessels by purposely introducing emboli, in other words deliberately blocking a blood vessel. The treatment is used to slow or stop blood supply thus reducing the size of a tumour. It has been successfully used in kidney lesions, liver lesions, typically hepatocellular carcinoma (HCC), uterine fibroids and vascular malformations. These artificial emboli often consist of medical grade 'glue', special tiny coils or sand like particles.

Embolization microspheres are occlusive agents composed of both resorbable and non-resorbable materials, including polyvinyl alcohol, chitosan-coated alginate, degradable starch, aromatic oil gelatin, ethyl cellulose, albumin, dextran, glass, wax, silicone, polystyrene and polyacrylates (Murakata et al 2006).

A disadvantage in using microspheres for embolization is the reported potential to pass through a lesion to the pulmonary circulation. It is therefore possible that pathologists will encounter embolization microspheres in locations remote from the intended site of intra-arterial placement (Murakata et al 2006).

Polyvinyl alcohol (PVA)

Polyvinyl alcohol (PVA) was introduced as an embolic agent in 1971. Microscopically PVA particles appear as sharp, flaky spicules, coloured pale pink-blue in sections stained with H&E and black in those stained with Verhoeff's van Gieson for elastic fibres (Germano et al 1992). They may also appear as irregular faintly blue-staining finely fibrillar material (Walley et al 1993).

Germano et al (1992) noted the presence of several refractile foreign particles. There is a consensus that these particles are cotton fibres and talc powder, which commonly contaminate most contrast-material solutions.

N-butyl cyanoacrylate (NBCA)

NBCA is a fast-polymerising liquid adhesive that polymerises on contact with saline solutions or blood (Lundie et al 1985). The angionecrosis after NBCA is attributed to the exothermic reaction that occurs when NBCA polymerises and the release of formaldehyde during the breakdown process of cyanoacrylates (Gruber et al 1996, Natarajan et al 2009). Microscopically it is unstained with H&E but is refractile. Lundie et al (1985) described it as "translucent, serpiginous material within vessel lumens". A good deal of the material apparently survives processing (Lundie et al 1985) and can then be stained with Oil Red O in paraffin sections (Lundie et al 1985, Natarajan et al 2009).

EmboGold

Murakata et al (2006) described the histochemical characteristics of microspheres composed of trisacryl-co-polymer crosslinked with gelatin. The material stained positively with Sirius red and mucicarmine, variably with Masson's trichrome stain and Movat pentachrome, and did not stain centrally with periodic acid Schiff with diastase. Infrared spectrophotometric analysis of the material from all three cases

demonstrated the spectrum of acrylic polyamide plastic.

Murakata et al (2006) noted that acrylic polyamide plastic embolization particles may resemble helminths but noted the spheres lack internal structures. The remote possibility of observing helminths lacking visible internal structures may arise in areas of necrosis in which a worm has degenerated to an almost unrecognizable degree. However, in the cases Murakata et al (2006) examined, the material was surrounded by fibrosis rather than necrosis, and although folded and showing shatter 'Venetian blind effect' artefact, the particles remained relatively well preserved, as might be expected for a non-resorbable material.

Onyx Liquid Embolic Agent

Onyx is composed of ethylene-vinyl alcohol copolymer, dimethyl sulfoxide (DMSO), and tantalum. Micronized tantalum powder is added for radiopacity (Van Rooij et al 2007). The most likely cause of angionecrosis after Onyx embolization is the solvent DMSO, which dissolves and suspends the embolic agent. It is well known that DMSO is angiotoxic and that its effect is dependent on the volume of DMSO injected and its contact time with the endothelium (Natarajan et al 2009).

Microscopically, Onyx appears as black granules of various sizes, similar in appearance to India Ink as is used in margin marking (see figure). The black carbon-like appearance is possibly due to the presence of the radiopaque micronized tantalum powder (Lundie et al 1985, Walley et al 1993).

Conclusion

Foreign materials seen by light microscopy will not always occupy exactly the same plane of focus as the tissue, because foreign materials are often difficult to cut, "chatter," or fracture, and do not adhere well to slides. The material may even be "dragged" by the microtome blade and displaced on the slide well away from where it was originally lodged in the tissue. Care must be taken not to dismiss as artefactual such foreign materials. On the other hand, artefactually introduced materials must not be "overdiagnosed," nor should foreign- appearing materials, like pigment, artefactually produced in the processing or staining of tissues. A refractive index different from tissue may make foreign materials stand out from the tissue. In some cases, different abilities to take up various dyes that are components of routine or special stains may aid in foreign material demonstration. Birefringence and dichroism on polarization microscopy rely on the anisotropic and crystalline nature of some foreign materials and may aid in their differentiation (Walley et al 1993).

Often we are required to assist in the identification of foreign material in tissue sections. Walley et al (1993) have published an atlas like article depicting the histology and special stain appearances of 130 foreign materials which has been found to be very useful.

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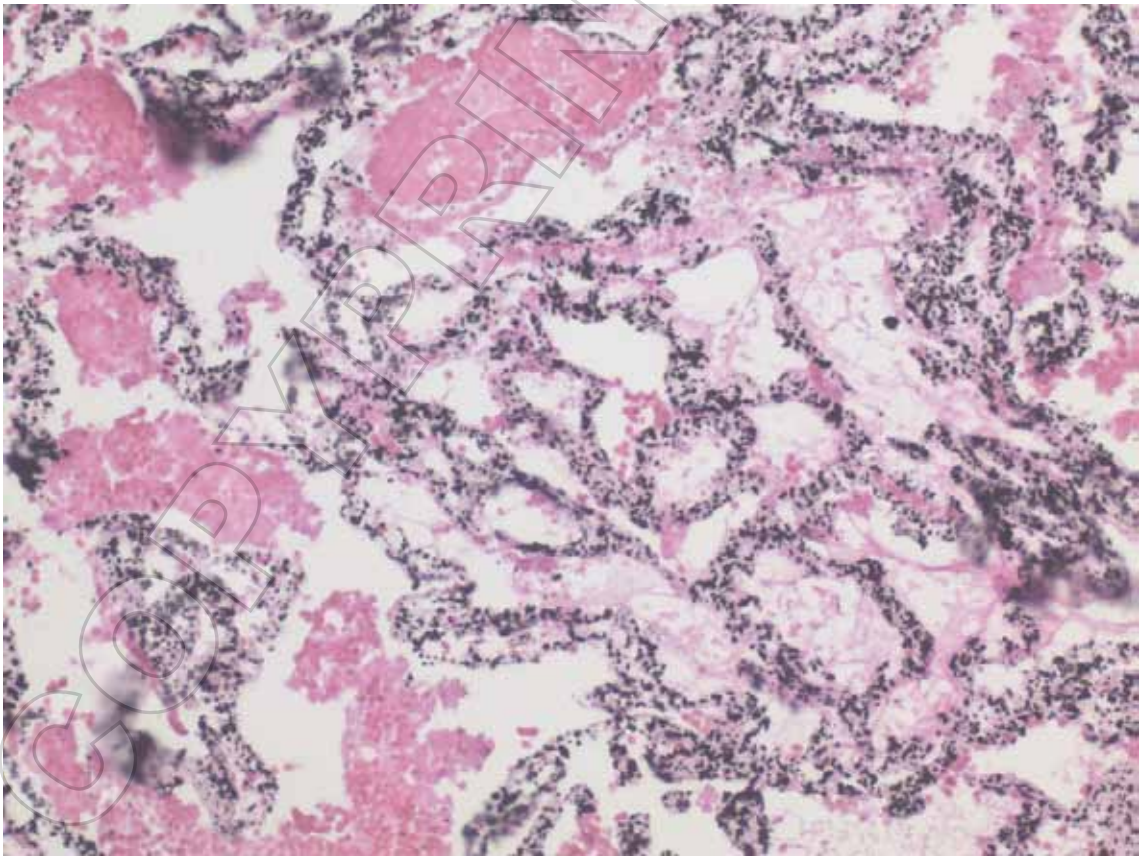

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Tissue Recognition Workshop

Momoko Sakaki, The Children's Hospital at Westmead

On Saturday 29th March, 2016, the Histotechnology Society held its first workshop of the year at Ultimo TAFE. The subject was Tissue Recognition – a skill most histologists can agree is vital in a histology laboratory. It was presented by Leah Simmons, teacher at Ultimo TAFE. 24 bleary-eyed participants staggered into registrations, wondering why they signed up to do a workshop on a Saturday. But by the end of the day, everyone was pleased to have gained new skills, contributed to their professional development and taken home nifty little 40-page tissue recognition photobook.

The day commenced with a presentation by Leah, who described her method of tissue recognition. It involved looking at the tissue both macroscopically and microscopically on the slide and asking a series of questions to narrow down what kind of tissue it is.
In the second part of the workshop, participants were given the opportunity to apply their newly learnt tissue recognition skills with guided instruction. The tissue recognition photo book prepared by Leah was a handy reference to help participants pinpoint particular features of the tissue. The slides were also placed up on the TV screens and the structures to look for were shown and explained, allowing students to compare with what they were seeing in their section.

The last part of the day involved self-directed learning using the Histology Slide Set from the TAFE. It gave people a chance look at tissues they might have not seen before or ask questions about any aspects of tissue recognition they were unsure about. Participants also had time to play with the MiPlatform, a smartphone adapter for microscopes developed by Trajan Scientific and Medical. It allows the user see the field of view on their screen as well as to quickly take focused, quality microscope photographs.

Thank you to Leah for organising and presenting an informative and valuable workshop. Thank you also to Bill Sinai, Trevor Hinwood, Chris Carrodus, Sandy Pitchfork and Cristina Antonio for helping set up for and assisting during the workshop. Thank you to the Histotechnology Society and Built for Learning for sponsoring the printing of the photobooks. Special thanks to Alex Anderson from Trajan Scientific and Medical for providing morning tea and lunch as well as demonstrating the MiPlatform.

Back to Basics - Specific Gravity

Specific Gravity compares the density of a substance with water.

Density is defined as the weight of an object divided by the volume of that object. For example, 1g of feathers takes up more room than 1g of water.

The higher the density, the tighter the particles are packed inside the substance
At higher temperature the substance becomes less dense.

So, let's examine this further:
Water (H_2O), which has a Molecular Weight (MW) of 18 and has a lot of molecules in 1 cubic metre. Ethanol (CH_3CH_2OH) has a MW of 46. This is 2.56 times heavier than the MW 18 of Water. So $1m^3$ of Ethanol should be heavier than $1m^3$ of Water. But Ethanol molecules are spaced further apart than Water molecules and thus for an equal volume, Ethanol is lighter than water.

	Specific Gravity
Water	1
Sea Water	1.03
Ethanol	0.79
Xylene	0.87
30% Formaldehyde	1.08

Water molecules have both a positively and a negatively charged side. The positive part of one water molecule will be attracted to the negative side of a different molecule. These two molecules will be pulled closer together. So, more water molecules can be packed into a given volume. Ethanol, on the other hand, has only one negatively charged side. The negative side on one ethanol molecule will repel the negative side of a second ethanol molecule. The ethanol molecules will therefore try to move as far away from each other as possible. So there will be fewer "spaced out" ethanol molecules within a given area, when compared to "tightly packed" water molecules. Ethanol is less dense, water is denser.
Specific Gravity (sg) is the ratio of density of a substance to the density of water at a specified temperature. It is common to use the density of water at 4^oC as reference - at this point the density of water is at the highest - 1000 kg/m^3 (ie water always has a density of 1 gram per millilitre). Chemicals are either lighter (less dense) than water and have a sg less than "1", or are heavier (more dense) than water and have a sg greater than "1".

Archimedes principle

More than twenty-two centuries ago, the Greek mathematician, physicist, and inventor Archimedes (c. 287-212 BC) received orders from the king of his hometown—Syracuse, a Greek colony in Sicily—to weigh the gold in the royal crown. According to legend, it was while bathing that Archimedes discovered the principle that is today named after him. He was so excited, legend maintains, that he jumped out of his bath and ran naked through the streets of Syracuse shouting "Eureka!" (I have found it).

What Archimedes had discovered was, in short, the reason why ships float: because the buoyant, or lifting, force of an object immersed in fluid is equal to the weight of the fluid displaced by the object.
Think of a ship:

- If it is intact and filled with air, the average density of metal and air is less than the water it displaces, so the boat will float.
- If the ship has cargo or people in it, replacing some of the air, the overall density will be greater, and the ship will sink lower into the water.
- If there is a hole in the ship and water replaces the air, the average density of the air, water and metal is now greater than the water alone. The boat will sink, totally.

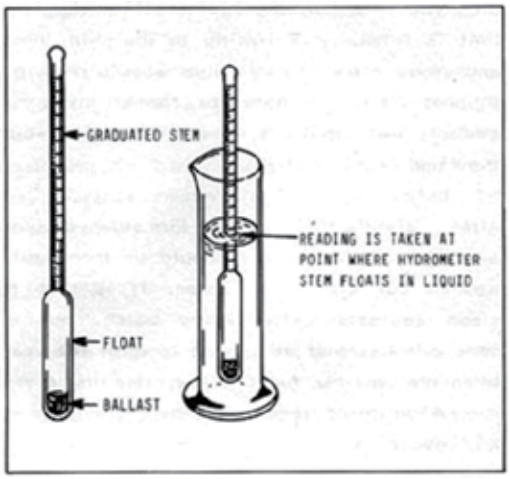
So how can we measure specific gravity? There are three common methods:

Using a Volumetric Pipette and an Analytical Balance

1. Weigh a container that will take just over 10 ml to 4 decimal places.
2. Using a volumetric pipette, place 10 ml of the solution into the container.
3. Weigh it again to 4 decimal places.
4. Subtract the weight of the empty container from the weight with the solution to get the weight of 10 ml solution.
5. Divide the weight of the solutionl by 10 (move 1 decimal place to the left).
6. This will give you the specific gravity of the solution.

Using a Hydrometer

A hydrometer is an instrument that measures the specific gravity (relative density) of liquids—the ratio of the density of the liquid to the density of water.

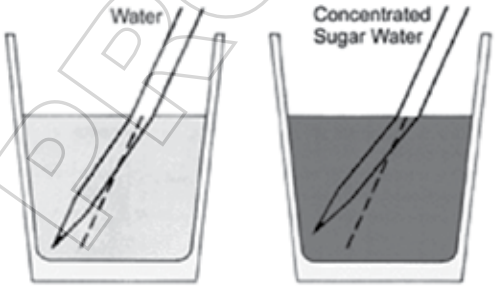


Operation of the hydrometer is based on Archimedes' principle that a solid suspended in a fluid is buoyed by a force equal to the weight of the fluid displaced by the submerged part of the suspended solid
A hydrometer is usually made of glass, and consists of a cylindrical stem and a bulb weighted with mercury or lead shot to make it float upright. The liquid to test is poured into a tall container, often a graduated cylinder, and the hydrometer is gently lowered into the liquid until it floats freely. The point at which the surface of the liquid touches the stem of the hydrometer correlates to specific gravity. Hydrometers usually contain a scale inside the stem, so that the person using it can read specific gravity. A variety of scales exist for different contexts.
In low-density liquids such as kerosene, gasoline, and alcohol, the hydrometer sinks deeper, and in high-density liquids such as brine, milk, and acids it doesn't sink as far.

Using a Refractometer

A second method of measuring specific gravity is to use a Refractometer.
The speed of light in a vacuum is always the same, but when light moves through any other medium it travels more slowly since it is constantly being absorbed and reemitted by the atoms in the material. The ratio of the speed of light in a vacuum to the speed of light in another substance is defined as the index of refraction (refractive index) for the substance. Whenever light changes speed as it crosses a boundary from one medium into another its direction of travel also changes, i.e., it is refracted.

The refractometer operates on the principle that, as the concentration of a solution increases, its refractive index changes proportionately.
Specific gravity, whether measured using a Hydrometer or a Refractometer has been used in wine making (to measure alcohols and sugars) and salinity measurements (in tropical fish aquariums). Specific gravity is important for determining how well a person or animal's kidney is working. The job of the kidney is to filter unwanted molecules out of the blood and into the urine. In order to keep you from getting totally dehydrated, it also has to be able to make the urine very concentrated (so you don't lose too much water). The more concentrated the urine is, the higher its specific gravity.



Increased urine specific gravity	Decreased urine specific gravity
Loss of body fluids (dehydration)	Damage to kidney tubule cells (renal tubular necrosis)
Diarrhea that causes dehydration	Diabetes insipidus
Heart failure	Drinking too much fluid
Sugar (glucose) in the urine	Kidney failure
Syndrome of inappropriate antidiuretic hormone secretion (SIADH)	

Since incorrect preparation of Parenteral Nutrition solution can lead to patient death, Chang et al (2008) used the refractive index as a quality assurance tool to monitor the preparation of these solutions.
Simos et al (2011) used a Digital Refractometer to measure formaldehyde concentrations in fixative solutions.
A Hydrometer is often used by tissue processors to measure the alcohol concentration of the first alcohol in order to initiate a dump and rotate of processing reagents when this alcohol concentration falls below 45%.

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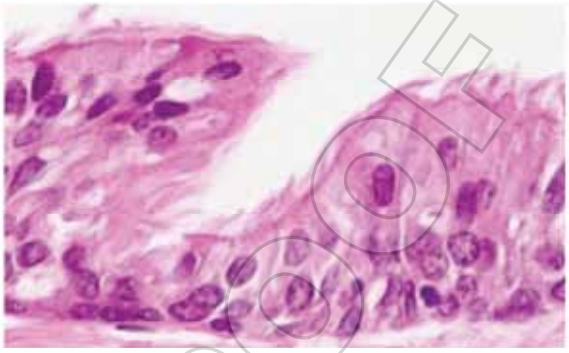

Sept 30th – Oct 2nd 2016

Tides of Change

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Members: $350 Non-members: $400

Student members: $100 Student Non-members: $150

Fee for partner/guest attending dinner: $100

Workshop Prices: (Limited places, registration closes 30th June 2016)

Workshop 1 – IHC Validation by Tony Henwood, CHW, NSW

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Zika Virus- Histopathology Concerns

Zika virus is an emerging arthropod-borne virus (arbovirus) belonging to the family Flaviviridae and genus Flavivirus. ZIKA VIRUS was first isolated from a monkey in the Zika forest of Uganda in 1947 (Musso et al 2014). Other arboviruses include West Nile Virus, Dengue virus and chikungunya virus (Nhan & Musso 2015). Zika virus is has an indistinguishable presentation from dengue infection. Outbreaks of Zika, dengue and Chikungunya have increased in frequency in recent years, particularly in the Pacific (Keighley et al 2015).

Zika virus is transmitted by the bite of infected mosquitoes, and has been isolated from several Aedes mosquito species, notably Aedes aegypti, which is widespread in the tropics and subtropics, and Aedes albopictus, which is established in many parts of Europe, especially in Mediterranean countries. In French Polynesia, Aedes polynesiensis is also suspected to contribute to Zika virus transmission (Musso et al 2014). In Australia the main culprit is Aedes aegypti which lives in tropical regions, including North Queensland. This is the same mosquito that transmits dengue and yellow fever. Aedes aegypti is not present in New Zealand (RCPA 2016).

In the majority of cases, Zika fever is a self-limited disease. The most frequent reported symptoms (over 60% during the French Polynesia outbreak) are mild fever, fatigue, cutaneous rash, arthralgia-myalgia and conjunctivitis. Other reported symptoms are headache, malaise, dizziness, oedema of the extremities, retro orbital pain, anorexia, photophobia, gastro intestinal disorders, sore throat, cough, aphtous ulcers, back pain, sweating and lymphadenopathies. None of these symptoms are specific and Zika fever can be misdiagnosed with other bacterial and viral infections, especially with other arboviruses in endemic areas (Nhan & Musso 2015).

During the French Polynesia outbreak, severe neurological complications of Zika fever were described; the incidence of Guillain-Barré syndrome was 20-fold higher than usually observed (Nhan & Musso 2015). Guillain-Barré syndrome is a rare disease that occurs when the immune system damages nerve cells.

Zika virus is suspected – but so far unproven - associated with an apparent rise in detected cases of microcephaly and other neurological disorders in newborn babies in Brazil. Similar birth defects were reported in French Polynesia in 2014, and the World Health Organization has now declared these as extraordinary events that constitute a Public Health Emergency of International Concern. Microcephaly is a rare condition where a baby is born with a small head, or the head stops growing after it is born. These babies can suffer convulsions and may develop physical and learning disabilities when they are older (RCPA 2016).

The CDC recommends that if a pregnancy results in a fetal loss in a woman with history of travel to an area of Zika virus transmission with symptoms consistent with Zika virus disease during or within 2 weeks of travel or findings of fetal microcephaly, Zika virus RT-PCR and immunohistochemical staining should be performed on fetal tissues, including umbilical cord and placenta (Petersen et al 2016).

Non-vector borne Zika transmission through sexual intercourse and perinatal transmission has been reported. Given that transfusion-related Zika virus transmission is a potential risk, molecular screening was implemented in French Polynesia for blood donors during the outbreak: 2.8% of blood donors, who were asymptomatic at the time of donation, tested positive for acute Zika virus infection (Musso et al 2014).

From CDC, Division of Vector-Borne Diseases: Zika and dengue viruses are classified as biological safety level (BSL) 2 pathogens. Until the association between Zika virus infection and congenital microcephaly is better understood, pregnancy should be considered a significant factor in risk assessment for individuals working with Zika virus, and the involvement of pregnant workers in studies with Zika virus should be minimized.

Zika virus is an enveloped virus. Enveloped viruses are viruses that possess an envelope or outer coating that is composed of a lipid layer (fat-like substance that is water insoluble). The envelope is needed to aid in attachment of the virus to the host cell. Loss of the envelope results in loss of infectivity which makes it relatively fragile and also easy to kill by disinfectantants. Since Zika is an enveloped virus, products with proven efficacy against non-enveloped viruses would be expected to be effective. Wood and Payne (1998) found that chloroxylenol and cetrimide/chlorhexidine were effective in inactivating the enveloped viruses: herpes simplex virus type 1 and human immunodeficiency virus type 1. Zika virus is killed by potassium permanganate, ether, and temperatures >60°C, but it is not effectively neutralized with 10% ethanol (Hayes 2009).

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S100 Immunohistochemistry – A Review

S100 has been used for over 20 years in routine immunohistochemistry (Nakajima et al 1982, Henwood 1992). In fact it was originally used without retrieval (since retrieval **had no**t been invented yet (Shi et al 1991)).

S100, named for its solubility in 100% ammonium sulphate, was identified as a "brain-specific" protein in the 1960s and early 1970s by Dr Blake W. Moore. The clinical diagnostic utility of immunohistochemistry directed at S100 protein (a polyclonal antibody directed at several members of the S100 Ca2+-binding protein family) was first rigorously described in 1982. These works also described a multitude of non-neural crest–derived tissues including fat, cartilage, and dendritic cells, which showed apparent S100 expression. S100 remains the most commonly used immunohistochemical stain to identify tumours of neural crest origin (Ng et al 2015).

The S100 family of proteins consists of 17 members, the majority of which are located in the epidermal differentiation complex on human chromosome 1q21 and function as calcium binding proteins in various signal transduction pathways. These proteins are frequently expressed in normal epidermis and in states such as psoriasis and cutaneous malignancies. The S100A6 member of this family is found in neurons, astrocytes, fibroblasts, and epithelial cells among others. S100A6 has been used in the diagnosis of various melanocytic lesions, including Spitz nevi and spindle cell melanoma (West et al 2014).

The most common antiserum to S100 used by histopathology laboratories is the rabbit antiserum against S100 protein (polyclonal), which reacts strongly to not only S100B but also very weakly to S100A1 and A6 (Nonaka et al 2008, Karamchandani et al 2012).

Apart from melanocytes, Langerhans and Schwann cells also strongly express S100 protein (Robson et al 2001). S100B is also found in glial cells, adipocytes, and chondrocytes (Karamchandani et al 2012).

Polyclonal verses Monoclonal S100

Recently monoclonal antibodies to S100 have become available. Sheffield et al (2002) compared Microphthalmia transcription factor, Melan-A, tyrosinase, HMB45 and a monoclonal antibody to S100 and found S100 to be not as sensitive as other markers. The paper is quite confusing in that it in the Methods they state that they used: "S-100 protein (clone S-100, Ventana Medical Systems)", whereas in the Discussion they state "In the present study, S-100 protein, which was a polyclonal antibody,…". This is an example of poor reporting in the literature. Ventana carries two S100 antibodies, a polyclonal and a monoclonal, so we cannot be certain of which antibody the authors used!

Tımar et al (2004) compared the rabbit polyclonal S100 antibody to the monoclonal S100B antibody and found that the monoclonal performed better than the polyclonal. Interestingly, they recorded that they used heat-induced Antigen Retrieval for these antibodies. Unfortunately they did not give details but referred to an earlier paper (Oncology, 64(4), 374-379) and when this was obtained, low and behold, the HIER method referred to an even earlier paper (The Journal of pathology, 180(1), 106-110) whose title: "Working

Experience With A New Vacuum-Accelerated Microwave Histoprocessor", seems to have little to do with immunohistochemistry. Another example of poor reporting?

Shams et al (2014) found that polyclonal anti-S100 antibody and monoclonal anti-S100B antibody are more suitable than monoclonal anti-S100A antibody for diagnostic investigations of malignant melanoma, irrespective of the histological type of melanoma.

Is S100 better than others?

There are several markers that have been used to assist in the detection of melanomas including HMB45, NKI-C3, Mart-1 and Melan A. How does S100 stack up against these later "models"?

Henwood (1992) found all melanoma positive for S100, whereas 94% were positive for HMB-45 and only 69% were positive for NKI-C3. Similar findings have been presented by Blessing et al (1998), Zubovits et al (2004) and Viray (2013).

Kucher et al (2006) compared S100 with Melan A and HMB45 in the evaluation of sentinel lymph nodes from patients with malignant melanoma. They found that all 42 lymph nodes with metastatic melanoma demonstrated S-100 reactivity where as five of these lymph nodes failed to express either HMB-45 or Melan-A.

Desmoplastic melanoma is a rare form of melanoma characterized by a dermal proliferation of spindle cells which may display only mildly atypical cytomorphology set in a prominent collagenous stroma. HMB-45 and melan-A are not expressed by the dermal spindle cell component of desmoplastic melanoma, S100 protein is used for this purpose. Although a few Desmoplastic Melanoma are negative or only focally positive the vast majority of primary desmoplastic melanomas show S100 expression in most of the spindle cells (Robson et al 2001).

Staining Pattern

Nybakken et al (2013) considered cytoplasmic S100 immunoreactivity specific whereas Nonaka et al (2008) found cytoplasmic and nuclear expression in conventional, spindle cell and desmoplastic melanomas, respectively, and the expression was predominantly diffuse. Karamchandani et al (2012) noted that nuclear staining in tumour cells was required for a positive interpretation for S100.

Effect of HIER

In 2013, the RCPA QAP distributed slides for S100 immunohistochemical assessment. One of the questions asked was whether antigen retrieval was used. 84% of participants reported that antigen retrieval was used for S100 immunohistochemistry. It is not clear from the report what the percentage of antigen retrieval used was enzymatic. The average mark for those using retrieval was 2.8, whereas it was 2.7 for those who did not. I would suggest that this is not significant.

Pileri et al (1997) found an increase in the intensity of S100 staining from 2+ to 4+ after citrate HIER

Namimatsu et al (2005) found no difference in intensity of S100 staining before and after HIER using Citraconic Anhydride.

Von Wasielewski et al (1994) compared immunostaining for a range of antigens on tissues fixed for differing time periods with and without HIER and for S100 found no difference in immunoreactivity for tissues fixed up to 42 days before processing. HIER was found to only improve tissues fixed for more than 3 years pre-processing. In conclusion, for the routinely processed tissue, fixed for the usual 24 hours to 3-

4 days (catering for weekends), it seems that there is no benefit to using HIER when using a polyclonal S100 antibody in immunohistochemistry.

So, S100 immunohistochemistry has sensitivity for melanoma close to 100% without heat induced antigen retrieval (Nakajima et al 1982, Henwood 1992, Blessing et al 1998, Zubovits et al 2004 and Viray 2013), so why would you antigen retrieve?

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On the 10th March, the Histopathology staff at the Children's Hospital at Westmead celebrated International Histotechnologist's Day with a Barbeque for Hospital Staff. Organised by Jacky Mayfield, Momoko Sakaki, Vicki-Maree Bourke and Nicole Mackie the day was memorable. Nibbles, sausages, chicken, salad AND dessert were available.

Histotechnologists wore specially printed Shirts with Histo themes, though the genital reference to an infamous rapper did not go un-noticed!