Laboratory Medicine Research Review™

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Issue 1 – 2013

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Abbreviations used in this issue
BMTB = bone marrow trephine biopsy
CTCs = circulating tumour cells
EGFR = Epidermal growth factor receptor
FFPE = formalin-fixed and paraffin-embedded
HAV = hepatitis A virus
HER2 = human epidermal growth factor receptor 2
Ig = immunoglobulin
IHC = immunohistochemistry
ISH = *in situ* hybridization

Welcome to the first edition of New Zealand Laboratory Medicine Research Review.

This new Review is a unique NZ publication bringing you some of the most important laboratory medicine research published around the world. Each issue will contain an independent selection of papers chosen by the medical specialists based at Canterbury Health Laboratories, with commentaries on what is important and how it can potentially impact current practice. The Review also provides website links to the abstracts or fully published papers where available, so you can make your own judgements. This and future issues will cover various specialist areas including cancer genotype testing, DNA profiling, infectious disease screening, antigen testing, cell counts, and toxin detection. If you have colleagues or friends who would like to receive our publication, feel free to forward this issue to them or send us their contact email and we will send them a copy of the next issue. We hope you find this first edition stimulating reading and we welcome any comments or feedback.

Kind regards,

Dr Chris Tofield
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A specific antidote for dabigatran: functional and structural characterization

Authors: Schiele F, et al.

Summary: The anticoagulant dabigatran, a direct thrombin inhibitor, is widely used for the prevention of stroke in patients with atrial fibrillation. In relation to the inherent risk of bleeding with anticoagulation therapy, the authors present the first report of a specific antidote for dabigatran, known as aDabi-Fab. The binding affinity of dabigatran for aDabi-Fab is ~350 times greater than that for thrombin. Furthermore, aDabi-Fab does not bind to known thrombin substrates and has no coagulation or platelet aggregation activity. In an *in vivo* rat model of anticoagulation, aDabi-Fab was found to rapidly reverse the anticoagulant activity of dabigatran.

Comment (SM): At present, major haemorrhage in a dabigatran patient could see a bleary-eyed haematologist scrabbling for the novoSeven and a spare dialysis machine. Whilst guideline-approved, such treatment is expensive and unlikely to be completely effective (Br J Haematol. 2013;160:35-46.). The development of a specific antidote for dabigatran might soon be cause for celebration. aDabi-Fab attaches to dabigatran's thrombin-binding site, immediately reversing anticoagulation. Despite structural homology with thrombin, aDabi-Fab does not appear to activate any part of the clotting cascade. If the promise shown in animal studies translates into safe treatment for humans, demand for aDabi-Fab will increase along with dabigatran prescription. Scottish clinicians who remember the 1980s will no doubt rechristen this drug "Fandabidozi"… provided that it is of course.

Dr Sean MacPherson (BSc[Hons], MBChB[Hons], FRCP[Glasg], FRCPath), Consultant Haematologist.

Reference: Blood. 2013 Mar 8. [Epub ahead of print].

http://bloodjournal.hematologylibrary.org/content/early/2013/03/08/blood-2012-11-468207.abstract

For more information, please go to http://www.medsafe.govt.nz

Assessing HER2 amplification in breast cancer

Authors: Bilous M, et al.

Summary: As part of the approval of subsidized trastuzumab therapy for HER2-positive breast cancer in Australia in 2006, the Australian government mandated *in situ* hybridization (ISH) testing, instead of immunohistochemistry (IHC) testing, for HER2 in all newly diagnosed breast cancer patients. The authors reviewed the subsequent results from this first regulated, Australia-wide HER2 ISH testing programme, involving 26 laboratories and 53,402 tests over a 4-year period. The rate of HER2 positive tests for primary cancers fell from 23.8% to 14.6%.

Comment (GH): In 2006 the Australian government approved subsidized trastuzumab (Herceptin®) therapy for HER2 overexpressing early breast cancers only on positive results using ISH (uses DNA probes to determine HER2 gene copy number) rather than IHC (uses anti-HER2 antibodies to detect cell surface protein expression levels). As a consequence, Australian laboratories established a nationwide program where ALL early breast cancers received ISH testing. Twenty six laboratories were involved and 53,402 tests were undertaken. During the introduction of this new ISH test, the HER2 positivity rates fell from 23.8% to 14.6%. The authors felt that ISH testing offered a higher level of accuracy, objectivity and reproducibility. Certainly the 14.6% HER2 positivity rate is more in line with internationally expected levels of approximately 15%, and would suggest that a proportion of the IHC positive patients would have been ISH negative – i.e., false positives. ISH seems to have provided a better testing platform, also saving unnecessary expenditure on a costly therapy that is unlikely to be effective in the ISH negative group. The current testing algorithm undertaken in New Zealand is IHC scored (0 to 3+) with 0 or 1+ regarded as negative, 3+ as positive and 2+ equivocal. These equivocal samples then require ISH testing, with subsidized trastuzumab available for those with a 3+ IHC result or positive ISH result. Extrapolating from the Australian study to New Zealand, with approximately 2,800 cases annually, this would equate to 252 patients who would be considered to have false positive IHC results. This would translate in saving the health budget several millions of dollars, far exceeding the cost of introducing ISH testing for all early breast cancers. There appears to be an emerging principle that more accurate testing, whilst apparently costing more, saves health resources by more correctly identifying patients likely to respond to expensive targeted therapies. The New Zealand health system needs to take note of this and apply this model as more costly targeted therapies come into routine use.

Dr. Gavin Harris (BMedSci, BMBS, FRCPath), Medical Director Anatomical Pathology, Canterbury Health Laboratories.

Reference: Breast Cancer Res Treat. 2012;134:617-24.
http://link.springer.com/article/10.1007%2Fs10549-012-2093-6

Assessment of a fully automated high-throughput DNA extraction method from formalin-fixed, paraffin-embedded tissue for *KRAS*, and *BRAF* somatic mutation analysis

Authors: van Eijk R, et al.

Summary: There is a growing need for high quality and sensitive DNA analysis of small amounts of formalin-fixed and paraffin-embedded (FFPE) tissue samples to guide the treatment of cancer. Faster turnaround times are also in demand. This study assessed a fully automated DNA/RNA isolation system in comparison with a routine, manual pathology method for the detection of hotspot mutations in *KRAS* and *BRAF* genes from both tissue cores and micro-dissection samples using hydrolysis probe assays. High-quality DNA, from both small FFPE tissue cores and micro-dissection samples, was isolated with the automated system, with 50% less hands-on time and significantly reduced turnaround times compared with classic, manual methods. Less that 50% of micro-dissection tissue was sufficient with the automated system.

Comment (PG): DNA sequence analysis of tissue samples is now in routine use for the selection of cancer patients for treatment with targeted therapies. In New Zealand this already includes *BRAF*, *EGFR* and *KRAS* gene analysis for patients with melanoma, lung and colon cancers. Timely and accurate analysis is obviously critical but these analyses are limited by difficulties of extracting DNA from very small samples containing a mixture of cancer and normal cells, often after degradation due to formalin fixation and paraffin embedding. Availability of suitable material often limits the required DNA analysis. Reliable, automated, methods for this extraction are beginning to become available and will find rapid application in the laboratory, allowing these analyses to become a routine component of cancer care.

Prof. Peter George (MBBS, BSc, FRCPA), Clinical Director & Medical Director Biochemistry, Canterbury Health Laboratories.

Reference: Exp Mol Pathol. 2013;94(1):121-5.
http://www.sciencedirect.com/science/article/pii/S0014480012000986

Circulating tumour cells, their role in metastasis and their clinical utility in lung cancer

Authors: O'Flaherty JD, et al.

Summary: Reliable and reproducible methods of detecting circulating tumour cells (CTCs) have only recently been developed, sparking much interest in their potential as a cancer biomarker and as a means of studying the process of metastasis. Although there are challenges to overcome, CTC counts have been used as a prognostic guide in patients with metastatic disease and as a surrogate marker for therapeutic response. CTC assays also offer the exciting possibility of molecular and genetic characterization representative of a primary tumour by acting as a "liquid biopsy" – it has already been shown to be possible to detect HER2-, KRAS- and EGFR-mutation status in breast, colon and lung cancer CTCs, respectively.

Comment (AM): Cancer is a leading cause of death in New Zealand and most cancer deaths are related to metastases, which follows from circulation of tumour cells in the blood. For over a century it has been possible to detect circulating tumour cells (CTCs) at autopsy in those who have died from advanced cancers. However, even in advanced disease, these CTCs are "rare", and it is only recently that techniques have been developed that can reliably detect CTCs in patients with earlier stage cancers, particularly with metastatic carcinomas of the lung, breast, prostate and colon. This review paper outlines how detection and analysis of CTCs are likely to have clinical utility in the near future, "*perhaps most importantly as a means of obtaining a non-invasive "liquid biopsy" of representative tumour tissue*". The paper also points out that only a small proportion of CTCs is capable of forming a metastasis. Once in the bloodstream, some CTCs aggregate with platelets and this appears to increase their chances of forming a metastatic deposit. CTCs covered by platelets are hidden from immune surveillance, receive platelet-derived growth factors and may also more easily attach to endothelial cells and move out of vessels in distant sites. However, other characteristics of the subpopulations of CTCs that are capable of forming metastases are still to be identified. While there is still much to be discovered about CTCs, the authors of the paper point out that, given what is already known, "*Antiplatelet therapy, targeting CTC-endothelium interactions or inhibiting the process of EMT may all be worthwhile future endeavours*".

Dr. Andrew Miller (MBChB, DipObst, FRCPA), Anatomical Pathologist, Canterbury Health Laboratories.

Reference: Lung Cancer. 2012;76:19-25.
http://www.lungcancerjournal.info/article/S0169-5002%2811%2900533-2/abstract

DNA methylome profiling using neonatal dried blood spot samples

Authors: Hollegaard MV, et al.

Summary: Methylation of cytosine residues occurs within CpG dinucleotide repeat "islands" located in the 5' promoter region of many, especially housekeeping genes. This epigenetic process profoundly affects gene expression and has been implicated in numerous diseases including malignancies. This proof-of-principle study aimed to evaluate if realistic quantities of material extracted from dried blood spots at birth can be used for reliable methylome profiling. Two volunteer siblings provided reference venous blood and blood spots, the latter stored for 3 years. The neonatal blood spots from these individuals were retrieved from the Danish Newborn Screening Biobank and DNA from each of the 3 sources eluted, bisulphite treated and labelled for methylation. The percentage methylation of 27,578 sites, detected by array, were compared. This revealed that only 50 sites (19 hyper- and 31 hypomethylated) in individual A and 51 sites (35 hyper- and 16 hypomethylated) in individual B exhibited significant differential methylation from birth to young adulthood. The subjects shared 5 hyper- and 10 hypomethylated sites. One gene only has been previously associated with changes in methylation due to ageing.

Comment (RM): Newborn bloodspots in Denmark are stored permanently at -20°C whereas in New Zealand a recent Cabinet decision has allowed the storage of bloodspots permanently, but this is at room temperature. Further, this group were able to research this under an umbrella clause in the Danish regulations allowing research into "new methods of screening" without ethics approval. Here, Ethics consent is necessary for nearly all bloodspot uses, and approval from the National Screening Unit of the Ministry of Health is required to ensure best use of a precious resource. The study group above provided evidence that the small changes seen were biological rather than artefactual (we are not told if the sibling patients were the same gender) and conclude that use of stored sample is a viable operation and that remarkably few changes are noted from birth to young adulthood.

Dr Richard MacKay (FRACP, FRCPA), Clinical Biochemist, Canterbury Health Laboratories.

Reference: Mol Genet Metab. 2013;108:225-31.
http://www.sciencedirect.com/science/article/pii/S1096719213000383

Estimation of cell density to aid in assessment of percentage cells of a particular lineage or of cells expressing a specific antigen in bone marrow trephine biopsies

Authors: Al-Shieban S, et al.

Summary: In order to calculate the percentage of antigen-positive cells in bone marrow trephine biopsies (BMTBs) of varying cellularities, the authors developed a tool to assess the total cell numbers in a microscope's field of vision. Cell-counting software was used to generate precise estimates of cell densities from 179 images of BMTBs of varying cellularities, which were then validated using as independent set of BMTBs. A strong linear association was observed between marrow cellularity and cell numbers. Standardised cell densities (cells/mm^2 of bone marrow) were subsequently deduced for BMTBs of varying cellularities, which correlated strongly with actual cell numbers. Adaptation of the estimates can be made for any microscope.

Comment (SM): Estimating cellularity of a trephine is relatively easy, ditto counting the number of positive-staining cells in a high-power field. The actual percentage of positive cells is harder to determine but this is what you usually want to know. Having used a bank of trephines and proprietary software the authors provide a table which correlates cellularity with a standard cell count per high-power field. You count the numerator, the table provides the denominator and the percentage of positive cells is simple to calculate. Personally, I'm inclined to rely more on the aspirate or flow cytometry for this figure but sometimes the trephine is the only useful sample. I'll give this a go. It might make a nice, accessible smartphone app...

Dr Sean MacPherson (BSc[Hons], MBChB[Hons], FRCP[Glasg], FRCPath), Consultant Haematologist.

Reference: J Clin Pathol. 2013;66:155-9.
http://jcp.bmj.com/content/66/2/155.abstract

NZMA
New Zealand Medical Association

For more information, please go to http://www.medsafe.govt.nz

Quantitative analysis of methamphetamine in hair of children removed from clandestine laboratories – evidence of passive exposure?

Author: Bassindale T

Summary: Drugs chronically present in the systemic circulation may be incorporated into growing hair. This New Zealand study analysed routinely taken hair samples for methamphetamine and amphetamine from 52 children removed from clandestine laboratories following police intervention: 38 (73%) tested positive for methamphetamine (>0.1 ng/mg), of which 34 also tested positive for amphetamine. Analysis of hair washes indicated a low level of external contamination, suggesting that in these cases methamphetamine was incorporated into the hair via the blood stream.

Comment (PG): Monitoring of exposure to various toxic environmental compounds can be achieved by measuring the relevant compounds in biological samples. Although measurements made in blood or urine are common in occupational settings, they are not always ideal since concentrations are often very low and usually only reflect recent exposure. Tissue samples, such as hair, potentially reflect a longer period of exposure and can be collected in a (relatively) non-invasive manner. Hair samples are also often analyzed in 'fringe medicine' applications to assess nutritional status and environmental exposure to toxins. Unfortunately, hair is often grossly contaminated by the application of various hair products and may not reflect systemic exposure. This paper suggests that measurement of hair levels of methamphetamine may reflect chronic passive exposure during the illicit production of this drug. While caution should be exercised in extrapolating this finding to other exposures it suggests a method for monitoring this group

Prof. Peter George (MBBS, BSc, FRCPA), Clinical Director & Medical Director Biochemistry, Canterbury Health Laboratories.

Reference: Forensic Sci Int. 2012;219:179-82.

http://www.fsijournal.org/article/S0379-0738%2812%2900005-9/abstract

Multiple factors contribute to positive results for hepatitis A virus immunoglobulin M antibody

Authors: Alatoom A, et al.

Summary: This study reviewed all positive hepatitis A virus (HAV) IgM antibody results in the US between January 2007 and December 2010 and assessed the effect of changing ordering options. A total of 10,735 tests were performed during the study period. Of 49 positive results (from 35 patients), most tests were ordered to assess patients with liver disease, rather than clinical acute hepatitis, in an outpatient setting: 20 patients had recent and/or resolved hepatitis, 10 had another established cause of liver disease, and 4 had acute hepatitis A. There was a reduction in the total annual number of requested tests of more than 35% following the introduction of computerized physician order entry.

Comment (AW): It is of interest to review the range of reasons for the detection of HAV IgM in immunoassays, in particular since the introduction of an efficacious vaccine. This article ambitiously tries to cover the reasons for IgM detection and also looks for possible solutions. The article title and the publishing journal suggest an investigation of assay-related reasons for HAV IgM detection other than that of acute hepatitis. These reasons are well covered. Additionally, the authors suggest a role of decision support systems, such as electronic ordering software packages, to decrease the number of inappropriate test requests. Unfortunately, some of the conclusions presented in this article appear based on assumptions rather than evidence.

Dr. Anja Werno (MD[Germany], FRCPA), Medical Director Microbiology, Canterbury Health Laboratories.

Reference: Arch Pathol Lab Med. 2013;137:90-5.

http://www.archivesofpathology.org/doi/full/10.5858/arpa.2011-0693-OA

Rapid detection of carbapenemase-producing Enterobacteriaceae

Authors: Nordmann P, et al.

Summary: The authors developed the so-called 'Carba NP' test, involving *in vitro* hydrolysis of imipenem in isolated bacterial colonies, in order to rapidly identify carbapenemase-producing Enterobacteriaceae. They found it to be 100% sensitive and specific, rapid (<2 hours) and inexpensive compared with molecular-based techniques.

Comment (RG): This article describes a rapid, sensitive, specific and cost-effective method for the detection of carbapenemase-producing Enterobacteriaceae. Data presented show the Carba NP test (named after the first two authors) is useful in determining Class A, B and D carbapenemases. It is more evident now than at any other time that the role of the clinical microbiology laboratory is pivotal in detecting and characterizing these multiresistant organisms. It is also imperative that resistance markers are detected and reported in a timely manner. Readers should pay attention to the references sited, particularly those written by the same author(s).

Rosie Greenlees (BSc PGDip MLS), Technical Lead Bacteriology, Canterbury Health Laboratories.

Reference: Emerg Infect Dis. 2012;18:1503-7.

http://wwwnc.cdc.gov/eid/article/18/9/12-0355_article.htm

Inexpensive optical system for microarray ELISA

Authors: López-Muedano C, et al.

Summary: Antibody-based diagnostic testing can involve either rapid and inexpensive point-of-care immunochromatographic tests yielding qualitative positive or negative test outcomes, or quantitative tests using expensive laboratory instruments. There is thus a need for low-cost instruments that can offer relatively inexpensive quantitative assessment of disease markers. The authors describe a novel optical device for reading an array of colorimetric and fluorescent immunodiagnostic test results.

Comment (MS): The hardware described is an interesting approach to providing a low-cost alternative to ELISA kits and readers, making diagnostic assays available to small laboratories in developing countries. Printing antigens onto membranes is a tried and tested technology at least 10 years old and so not novel but reliable. The system is described as a multiarray, although the assay performed detected a single antigen so assay conditions could be optimised for best performance. In multiarrays, conditions are generally a compromise. Only 12 serum samples were tested showing around 90% correlation for the colorimetric assay with commercial ELISAs and 100% for the fluorescence assay. No disease controls were included and no assessment made of the clinical performance. Quality-control samples were not included with the 'kit' and no comment made about the potential interference by anti-rodent antibodies in patient serum or Rheumatoid factor. This makes it hard to asses how the quality of the assay(s) would be assured. Cheaper diagnostic equipment has the potential to make a huge difference in developing countries, provided clinical performance is assured and the diagnosed disease(s) can be treated.

Dr. Myfanwy Spellerberg (BSc[Hons], PhD), Section Head of Immunology, Canterbury Health Laboratories.

Reference: Talanta. 2012;100:405-9.

http://www.sciencedirect.com/science/article/pii/S0039914012006911