Welcome to the 13th edition of New Zealand Laboratory Medicine Research Review. Highlights include assessment of an illumigene assay for group A streptococcus, an efficient TaqMan array card for investigating acute febrile illness, a Provisional Clinical Opinion on extended RAS gene mutation testing in metastatic colorectal carcinoma by the American Society of Clinical Oncology, minimum tissue requirements for complex molecular assays, and whole genome sequencing of N. gonorrhoeae direct from clinical samples.

Research Review is tent! The first ever issues of Research Review were delivered to inboxes in February 2006. Fast forward ten years and we now publish 48 regular reviews to which there are over 160,000 subscriptions. We’re grateful to each and every one of you for your support and are looking forward to even bigger and better things over the coming years.

We hope you find this issue interesting and look forward to hearing your comments.

Kind regards,
Dr Collette Bromhead
collettebromhead@researchreview.co.nz

Comparison of illumigene group A streptococcus assay with culture of throat swabs from children with sore throats in the New Zealand school-based Rheumatic Fever Prevention Program

Authors: Upton A, et al.

Summary: The New Zealand Rheumatic Fever Prevention Programme relies on prompt diagnosis and treatment of group A streptococcal (GAS) pharyngitis, but is hindered by the turnaround time of culture. In this study, 757 throat swab (Copan ESwab) specimens collected from schoolchildren self-identifying with a sore throat were tested by both routine culture and the illumigene GAS assay using loop-mediated isothermal amplification. An alternative molecular assay was employed to resolve discrepant results. The illumigene assay, using culture on blood agar as the “gold standard” and following discrepancy analysis, achieved the following respective performance results: sensitivity, 82% and 87%; specificity, 93% and 98%; positive predictive value, 61% and 88%; and negative predictive value, 97% and 97%. The authors concluded that the illumigene assay did not perform as well as previously reported, but that its improved sensitivity and rapid turnaround time compared with culture are appealing.

Comment: For those microbiologists who have been buried in an avalanche of throat swabs since the inception of the New Zealand Rheumatic Fever Prevention Programme, this paper by Upton et al. may represent something of a relief. Their laboratory-based study assessed the performance of the illumigene GAS assay on throat swabs from symptomatic children in the school-based component of the Rheumatic Fever Prevention Programme. The significant advantage that the illumigene assay offers in addition to enhanced sensitivity is a considerably shorter turnaround time, enabling same-day reporting of results and antibiotic prescription. The hands-on time in the lab is greatly reduced compared to culture and the compact illumigene instrument can be placed in any laboratory, without requiring special facilities for molecular testing. The illumigene GAS assay produced some false positives due to cross-reactivity with group C and G streptococcus, but false-positive results in the context of the Rheumatic Fever Prevention Programme are less concerning as in all likelihood treatment is being given for GAS pharyngeal colonisation as well as infection, which may reduce the rate of carriage and transmission of group A streptococcus in the community.


Abstract

For more information, please go to http://www.medsafe.govt.nz
Development of a TaqMan array card for acute-febrile-illness outbreak investigation and surveillance of emerging pathogens, including Ebola virus

Authors: Liu J, et al.

Summary: The aetiological agent in acute febrile illness (AFI) often remains unidentified. The authors of this study assessed a real-time PCR-based TaqMan array card able to yield test results for six to eight samples within 2.5 h and simultaneously detect 26 AFI-associated organisms, including 15 viruses, eight bacteria and three protozoa. In order to ensure extraction and amplification efficiency, two extrinsic controls (phocine herpesvirus 1 and bacteriophage MS2) were included. Analytical validation was performed on spiked specimens for linearity, intra-assay precision, inter-assay precision, limit of detection, and specificity. A total of 1,050 clinical blood samples were tested. Compared to individual real-time PCR assays, the TaqMan array card demonstrated an overall sensitivity of 88% (278/315; 95% CI 84–92%) and specificity of 99% (5,261/5,326; 95% CI 98–99%).

Comment: The slow recognition of Ebola in Guinea was largely due to delays in laboratory diagnosis and the inability to perform molecular testing in the region. This paper details the extensive development of a multiplex TaqMan array card (Life Technologies) for real-time PCR detection of 15 viruses (Ebola, Marburg, West Nile, Yellow fever etc.), eight bacteria (Bartonella spp., Brucella spp., Leptospira spp., Salmonella spp., etc.) and three protozoa (Plasmodium etc.) that cause AFI in Africa. Using spiked as well as clinical specimens, the card had an overall sensitivity of 88% and 99% specificity compared to individual real-time PCR assays, although high burden infections such as Ebola showed 100% sensitivity and specificity. Given that there may be multiple agents contributing to fever (45% of Ebola-infected samples were co-infected with Plasmodium), this card is efficient and with its ability to test six to eight samples within 2.5 h will be a useful tool for its intended use in outbreak investigation and AFI surveillance in Africa.


Abstract

Independent commentary by Dr Collette Bromhead, Massey University

Collette obtained her PhD in 2004 and is a registered Medical Laboratory Scientist with 20 years experience in molecular diagnostics for infectious diseases. She was recently appointed as a Senior Lecturer in Molecular Microbiology in the College of Health at Massey University. As well as academic teaching and research, she maintains roles with IANZ and the National Cervical Screening Program, latterly as part of the Technical Reference Group advising the ministry on the implementation of HPV Primary Screening for Cervical Cancer. She has been a representative on the NZ Institute of Medical Laboratory Scientists Council and maintains her contributions to the profession as deputy editor of the NZIMLS Journal.

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Prospective validation of a 21-gene expression assay in breast cancer

Authors: Sparano JA, et al.

Summary: The authors prospectively evaluated the prognostic value of gene-expression assays in 10,253 women with hormone-receptor-positive, human epidermal growth factor receptor type 2 (HER2)-negative, axillary node-negative breast cancers with tumours of 1.1-5.0 cm in the greatest dimension (or 0.6-1.0 cm in the greatest dimension and intermediate- or high-tumour grade) who met established guidelines for the consideration of adjuvant chemotherapy on the basis of clinicopathologic features. Using a reverse-transcriptase PCR assay of 21 genes on paraffin-embedded tumour tissue, a risk of breast cancer recurrence score was calculated. On a scale of 0-100, a recurrence score of 0-10 was considered indicative of a very low risk of recurrence. A total of 1,626 women (15.9%) with a recurrence score in this range were assigned to receive endocrine therapy without chemotherapy. At 5 years, these patients had a rate of invasive disease-free survival of 93.8% (95% CI 92.4-94.9), a rate of freedom from recurrence of breast cancer at a distant site of 99.3% (95% CI 98.7-99.6), a rate of freedom from recurrence of breast cancer at a distant or local-regional site of 98.7% (95% CI 97.9-99.2) and a rate of overall survival of 98.0% (95% CI 97.1-98.6).

Comment: This substantial 5-year study followed a cohort of women with hormone-receptor-positive, HER2-negative, axillary node-negative breast cancers treated only with endocrine therapy. The women included in the study had a low risk of recurrence (0-10 score) as judged by a 21 gene reverse-transcriptase PCR panel (Oncotype DX) and thus were treated with endocrine therapy alone without chemotherapy. Recurrence events were uncommon regardless of histologic grade and were not significantly affected by younger age at diagnosis. This prospectively conducted study supports the use of the 21-gene assay to spare the use of chemotherapy in patients who otherwise would be recommended to receive it on the basis of clinicopathologic features.


Abstract

A comparative study between smartphone-based microscopy and conventional light microscopy in 1021 dermatopathology specimens

Authors: Jahan-Tigh RR, et al.

Summary: This prospective, blinded study assessed the diagnostic performance of a simple smartphone microscope constructed with a 3 mm ball lens by evaluating 1021 referred, consecutive dermatopathology specimens from the community at a single university hospital. Compared to conventional light microscopy, the performance characteristics of the smartphone platform were calculated for the diagnosis of melanoma, non-melanoma skin cancers, and other miscellaneous conditions. The sensitivity and specificity of smartphone microscopy for basal cell carcinoma (n=136) were 95.6% and 98.1%, respectively, and for squamous cell carcinoma (n=94) were 89.4% and 97.3%, respectively. The lowest sensitivity (60%) was reported for melanoma (n=15), although melanoma that the smartphone microscopist called a solar lentigo and two squamous and inflammatory conditions. Among the more alarming discrepant results was a tape in the diagnosis of dermatopathology specimens. It produced a new discipline of smartphone-based microscopy and conventional light microscopy in dermatopathology.

Comment: This rather unimpressive paper compared the performance of a light microscope to that of a smartphone with a ball lens attached via double-sided tape in the diagnosis of dermatopathology specimens. It produced a new discipline in laboratory medicine: the “smartphone microscope”. Figures 1-3 showed the setup and comparative view of a nodular basal cell carcinoma, which through the smartphone camera lens looked indistinct and foggy. It is not surprising then that the study of 1021 specimens showed poor performance for melanomas, fungal and inflammatory conditions. Among the more alarming discrepant results was a melanoma that the smartphone microscope called a solar lentigo and two squamous cell carcinomas that were mistaken for a melanoma and a verruca, respectively, by the phone lens. Whilst the authors assert that mobile tele-dermatology can bring much needed expertise to resource poor healthcare settings, I think the technology is better used for objective tests such as PCR or biochemical measurements as has been shown in previous studies.


Abstract
Cell-free DNA as a molecular tool for monitoring disease progression and response to therapy in breast cancer patients

Authors: Liang DH, et al.

Summary: The authors performed a retrospective chart review of 100 patients with stage 4 or high-risk stage 3 breast cancer to assess genomic sequencing of cell-free DNA compared with that of tumour DNA for monitoring disease progression and response to therapy. TP53 and PIK3CA mutations and EGFR and ERBB2 amplification were the most common genomic alterations. Robust agreement between tumour DNA and cell-free DNA (Cohen’s kappa = 0.64 and 0.77, respectively) was reported for TP53 mutation and ERBB2 amplification; poor agreement (Cohen’s kappa = 0.18 and 0.33, respectively) was reported for TP53 mutation and EGFR amplification. A significant association between directional changes of TP53 and PIK3CA mutant allele frequency and response to therapy was demonstrated (p=0.002). Presence of TP53 mutation (p=0.0004) and PIK3CA mutant allele frequency (p=0.01, HR 1.074 [95% CI 1.018-1.134]) were significant predictors of progression-free survival.

Comment: This retrospective study of 100 stage 4 or high-risk stage 3 breast cancer cases attempted to identify selected cancer-specific genomic alterations from cell-free DNA as indicators of disease progression and opportunities for targeted therapy. The promise of a simple blood test to monitor breast cancer progression is attractive. However, these data are complicated to assess because of the small sample size, lack of consistency between sample collection time points and diversity of cancer specific mutations being screened for by different companies over time. The authors conclude that due to an imperfect agreement between tumour DNA genomic alternations and those found in cell-free DNA, the latter is unlikely to replace the need for tumour DNA analysis and traditional imaging studies. More work is needed.


Abstract

Extended RAS gene mutation testing in metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy: American Society of Clinical Oncology Provisional Clinical Opinion update 2015

Authors: Allegra CJ, et al.

Summary: This American Society of Clinical Oncology Provisional Clinical Opinion update gives recommendations on the use of extended RAS gene mutation testing in patients with metastatic colorectal cancer (mCRC) to detect resistance to anti-epidermal growth factor receptor (EGFR) monoclonal antibody therapy. Patients with mCRC harbouuring RAS mutations in exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146) have been found to be unlikely to benefit from EGFR antibody therapy as monotherapy or combined with chemotherapy in recent phase II and III clinical trials. Additional recent data from 11 systematic reviews with meta-analyses, two retrospective analyses, and two health technology assessments based on a systematic review include outcomes in patients with mCRC and the presence or absence of mutation in additional exons in KRAS and NRAS. The weight of current evidence indicates that anti-EGFR antibody therapy should only be considered in patients without detected mutations after such extended RAS testing. Thus, it is recommended that all patients with mCRC who are candidates for anti-EGFR antibody therapy should have their tumour tested in a Clinical Laboratory Improvement Amendments-certified laboratory for mutations in both KRAS and NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146).

Comment: As a result of their assessment of 11 systematic reviews with meta-analyses, two retrospective analyses and two health technology assessments, ASCO now recommends that before treatment with anti-EGFR antibody therapy, patients with mCRC should have their tumour tested for mutations in KRAS exon 2 (codons 12 and 13) AND: • NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146) This is an interesting extension to testing as mutations in NRAS comprise a small proportion of patients with mCRC (approx. 2%). While RAS gene mutations predict that patients may have worse outcomes from the addition of either cetuximab or panitumumab to chemotherapy, the failure to detect RAS mutations does not guarantee that a patient will benefit. Therefore other biomarkers are needed to determine the best treatment for patients with mCRC because the efficacy of anti-EGFR antibody therapy, even in the RAS wild-type population, is modest.


Abstract

DNA yield from tissue samples in surgical pathology and minimum tissue requirements for molecular testing

Authors: Austin MC, et al.

Summary: This study sought to assess tissue adequacy to yield enough DNA for complex molecular assays. Slide-based measurements were used to investigate the relationship between processed tissue volume and DNA yield and DNA yield by A260 from 366 formalin-fixed, paraffin-embedded tissue samples submitted for EGFR, KRAS, and BRAF testing. All samples with a volume >8 mm³ yielded at least 1 μg of DNA; over 80% of samples providing less than 1 μg of DNA were less than 4 mm³ in volume. Tissue samples of 9 mm³ are expected to yield >1 μg of DNA 99% of the time.

Comment: This very useful paper offers practical advice on how low you can go with the volume of formalin-fixed, paraffin-embedded tissue before you don’t have enough DNA for common adjunct molecular testing for KRAS, EGFR and BRAF. A DNA yield of 1.0 μg or greater was obtained from 90% of the tissue samples with a volume of 4 mm³ or greater and 100% of tissue samples with volume 8 mm³ or greater. The authors suggest that this translates to a minimum of two 1 cm cores from an 18-gauge needle.


Abstract

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Research Review publications are intended for New Zealand health professionals.
Abstract


77.0) and 83.1% specificity (95% CI 79.4-86.3).

CI 28.4-45.7), with 66.2% sensitivity (95% CI 54.0-

of preeclampsia within 4 weeks of 36.7% (95%

subsequent week of 99.3% (95% CI 97.9-99.9) with

cohort, an sFlt-1:PlGF ratio

was identified as having important predictive value

assessed to predict the presence of preeclampsia

the absence of preeclampsia within 1 week after

36 weeks 6 days of gestation). Low sFlt-1:PlGF ratios

preeclampsia was suspected (24 weeks 0 days to

absence or presence of preeclampsia in the short

growth factor (PlGF) ratio that would predict the

aim of markers associated with antimicrobial resistance directly

from urine samples using next generation sequencing

Authors: Graham RM, et al.

Summary: This study assessed next generation sequencing of Neisseria gonorrhoeae from clinical specimens for generating information on epidemiological genotyping and antimicrobial resistance (AMR) markers. DNA was extracted, enriched for microbial DNA and sequenced from 13 urine specimens testing positive for N. gonorrhoeae by nucleic acid amplification testing (NAAT). After filtering out sequences aligned to the human genome, the remaining sequences were de novo assembled and the resulting contigs searched for regions of interest. MLST and NG-MAST alleles were assigned according to the schemes at PubMLST.org and NG-MAST.net, respectively. A sufficient number of N. gonorrhoeae sequence reads were obtained from 11 of the 13 samples in order to provide full coverage of the genome at a depth of 6-130x. Each of these samples yielded complete MLST and NG-MAST sequence types. In searching for 10 different AMR markers in genes associated with reduced susceptibility to several antimicrobials, both previously reported and novel mutations were identified.

Comment: Whilst the wily gonococcus continues to cause alarm with its ever-increasing levels of AMR, the shift to diagnosis of gonorrhoea by NAAT has unfortunately limited the availability of isolates for epidemiological typing and phenotypic antimicrobial susceptibility testing. This small study offers the exciting alternative of sequencing the entire genome of N. gonorrhoeae directly from clinical samples using next generation sequencing. Using total DNA isolated from 13 urine-Cobas PCR media (Roche) specimens (positive for N. gonorrhoeae using the Roche Cobas 4800 CT/NG test), this study showed that 11 of the 13 samples generated a sufficient number of sequence reads to provide complete MLST and NG-MAST sequence types as well as 10 different AMR markers. Enrichment of the DNA samples for microbial sequences was essential. Whilst not yet cost-effective, this approach does demonstrate a means to produce valuable epidemiological typing and antimicrobial sensitivity testing directly on clinical NAAT samples.


Epidemiological typing of Neisseria gonorrhoeae and detection

Abstract

Comment: This prospective study sought to develop a 2-h algorithm for use of hs-cTnI in the early triage of patients with possible AMI. Testing was conducted on the Architect STAT high-sensitivity troponin I (Abbott Laboratories) on serum collected at presentation to Accident & Emergency and again after 2 h. Rule-out criteria were defined as a maximal hs-cTnI concentration within the first 2 h of <6 ng/L and an absolute change within the first 2 h of <2 ng/L. For rule-in of AMI the optimal thresholds were either a maximal hs-cTnI value within the first 2 h of >64 ng/L or an absolute change in hs-cTnI within the first 2 h of >15 ng/L. One limitation is that the patients in this study were in the Accident & Emergency and further studies are required to quantify the performance of this algorithm in patients with lower (Primary Care) or higher (e.g. Coronary Care Unit setting) pre-test probability.


Epidemiological typing of Neisseria gonorrhoeae and detection

of markers associated with antimicrobial resistance directly

from urine samples using next generation sequencing

Authors: Graham RM, et al.

Summary: This study assessed next generation sequencing of Neisseria gonorrhoeae from clinical specimens for generating information on epidemiological genotyping and antimicrobial resistance (AMR) markers. DNA was extracted, enriched for microbial DNA and sequenced from 13 urine specimens testing positive for N. gonorrhoeae by nucleic acid amplification testing (NAAT). After filtering out sequences aligned to the human genome, the remaining sequences were de novo assembled and the resulting contigs searched for regions of interest. MLST and NG-MAST alleles were assigned according to the schemes at PubMLST.org and NG-MAST.net, respectively. A sufficient number of N. gonorrhoeae sequence reads were obtained from 11 of the 13 samples in order to provide full coverage of the genome at a depth of 6-130x. Each of these samples yielded complete MLST and NG-MAST sequence types. In searching for 10 different AMR markers in genes associated with reduced susceptibility to several antimicrobials, both previously reported and novel mutations were identified.

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