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Making Education Easy

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Abbreviations used in this issue

CEA = carcinoembryonic antigen
HPV = human papilloma virus
MALDI-TOF = matrix-assisted laser desorption ionisation time of flight
NAAT = nucleic acid amplification test
TB = tuberculosis
THC = Δ^9-tetrahydrocannabinol

Welcome to the 18th edition of New Zealand Laboratory Medicine Research Review.

In this review, highlights include cost-effective molecular testing for Trichomonas vaginalis infection, the use of iron studies to predict *HFE* mutations, a comparison of multiplex polymerase chain reaction assays for respiratory virus detection, a rapid molecular test for *Mycobacterium tuberculosis* Rangipo strain in New Zealand, a new high-throughput mass spectrometry-based test for HPV, the utility of urinalysis tests to rule out the need for urine culture, THC exposure among police officers during raids on cannabis growing operations, a non-invasive test for recurrent urothelial carcinoma surveillance and the prognostic significance of CEA elevations during adjuvant chemotherapy for colon cancer.

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

Kind regards,

Dr Collette Bromhead
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Audit of Trichomonas vaginalis test requesting by community referrers after a change from culture to molecular testing, including a cost analysis

Authors: Bissessor L, et al.

Summary: Robust molecular platforms have improved the detection of Trichomonas vaginalis infection over culture methods but are more costly. Recently, LabTests in Auckland replaced culture methods with the Aptima nucleic acid amplification test (NAAT) for Trichomonas vaginalis detection. In order to contain costs, rather than testing all vaginal swabs for Trichomonas vaginalis as previously, the Aptima NAAT was employed on specific request and as reflex testing in the presence of risk factors (age and gender, other sexually transmitted infections). An audit was performed to evaluate outcomes following the change using data collected from August 2015 (microbroth culture and microscopy) and August 2016 (NAAT). In August 2015, 10,299 vaginal swabs were tested with a positivity rate of 0.9%. In August 2016, 2189 urogenital swabs and urines were tested with a positivity rate of 5.3%, leading to a higher number of Trichomonas vaginalis infections detected in the latter period. The number needed to test for one positive result was 111 in 2015 and 19 in 2016. Associated costs per positive result were $902.55 in 2015 and $368.92 in 2016.

Comment: Another insightful cost-benefit analysis on the move from culture to molecular techniques from Dr Arlo Upton's team at LabTests Auckland. They first demonstrated higher sensitivity of the Aptima NAAT for Trichomonas vaginalis detection, which is generally known for all NAATs over culture for this organism. However, it is only cost effective to move to "by request only" testing instead of screening all vaginal swabs, and it is predictably taking clinicians time to adjust to this new regime.

Reference: N Z Med J. 2017 Jun 16;130(1457):34-37.
Abstract

Using iron studies to predict *HFE* mutations in New Zealand

Authors: O'Toole R, et al.

Summary: The diagnosis of hereditary haemochromatosis is complicated by the frequent absence or non-specific nature of symptoms and other conditions that may affect biochemical markers of iron overload. In order to inform evidence-based recommendations for hereditary haemochromatosis laboratory testing in New Zealand, the authors assessed the correlation between iron studies (serum ferritin, transferrin saturation, serum iron and serum transferrin) and *HFE* genotype. Of 2388 patients genotyped for common HFE amino acid substitutions (C282Y, H63D and S65C) in Wellington from 2007 to 2013, 62% of HFE genotyping tests were ordered solely on the basis of elevated serum ferritin and only 11% of cases had had a C-reactive protein test performed to rule out an acute-phase reaction. On receiver operator characteristic curve analysis, serum ferritin performed poorly as a predictor of *HFE* genotype. Transferrin saturation showed the strongest association, with values ≥45% predicting *HFE* mutations with the highest sensitivity and specificity. Serum ferritin >1000 µg/L was present in one at-risk patient (C282Y homozygote) whose transferrin saturation was <45%. The authors developed an evidence-based laboratory testing algorithm based on a transferrin saturation ≥45%, a serum ferritin ≥1000 µg/L and/or a family history of hereditary haemochromatosis, which identified all C282Y homozygotes in this study.

Comment: Very proud to bring you this paper which is the result of the hard work and elegant analyses performed by Rebecca O'Toole (now of Wellington SCL) during her master's study with me. It shows the immense value that exists within laboratory databases if only research questions could be asked of it! Essentially, we showed that transferrin saturation ≥45% should be the mainstay indication for *HFE* genotyping in the absence of family history or significant symptoms. Ferritin is not a reliable marker as it is also elevated in inflammation and infection, but a lot of GPs are still ordering expensive *HFE* genotyping on the basis of mild elevations.

Reference: Intern Med J. 2017 Apr;47(4):447-454.

Abstract

Comparison of the fast track diagnostics respiratory 21 and Seegene Allplex multiplex polymerase chain reaction assays for the detection of respiratory viruses

Authors: Barratt K, et al.

Summary: Respiratory virus testing in increasingly performed using real-time multiplex polymerase chain reaction assays. While they offer automated analysis in a closed tube system, they are limited by low throughput resulting from reduced multiplexing ability. This study compared the performance of the established fast-track respiratory 21 assay (Fast-track diagnostics, Junglinster, Luxembourg) with the new Seegene Allplex assay (Seegene) (Seegene Inc., Seoul, Korea). The latter offers greater multiplexing since multiple targets can be detected in each fluorescence channel and is quicker to perform than previous Seegene respiratory multiplex assays. Of 199 mostly upper respiratory tract samples tested, a respiratory pathogen was detected in 63.8% with the fast-track respiratory 21 assay and in 61.8% with the Seegene Allplex assay. Kappa agreement was in the range 0.87–1 for all targets except human bocavirus and adenovirus. Advantages of the Seegene assay were the simultaneous detection of two gene targets for each of the common Influenza A subtypes, higher throughput (30 samples per run) and automated result calling. Although the fast-track respiratory 21 assay could only test 17 samples per run, validation for use on several different real-time thermal cyclers made it easier to integrate into an existing laboratory system. Overall, the performance of the two assays was similar and both were cost effective compared with in-house multiplex polymerase chain react screening for respiratory viruses.

Comment: Congratulations to Kevin Barratt and his team from CHL for this quality paper that I hope serves to stimulate other laboratory staff around the country into publishing their assay appraisal data. This comprehensive comparison and validation showed pluses and minuses with both platforms, vexing bocavirus results and broke down test sensitivity and specificity for each virus. There is a steady evolution in respiratory virus testing from in-house panels to commercial copies and onto increasingly multiplexed tests. The importance of these tests is in ruling out patients with viral infections and saving precious antibiotics for those who have bacterial infections.

Reference: Br J Biomed Sci. 2017 Apr;74(2):85-89.

Abstract

Rapid molecular diagnosis of the *Mycobacterium tuberculosis* Rangipo strain responsible for the largest recurring TB cluster in New Zealand

Authors: Mulholland CV, et al.

Summary: New Zealand is considered a low-burden tuberculosis (TB) country. However, it has disproportionately high rates of TB in socioeconomically disadvantaged populations such as Māori, in whom the rate of TB is about nine-fold higher than in New Zealanders of European descent. Through molecular typing, around one-third of TB notifications in New Zealand can be assigned to clusters of infection, of which the largest is due to the *Mycobacterium tuberculosis* Rangipo strain. This strain has been responsible for ongoing outbreaks for at least 25 years and is strongly associated with Māori. It appears to be highly transmissible and pathogenic; therefore, a fast and reliable diagnostic test is required for this strain in order to control its transmission. In this paper, the authors reported on their development of a polymerase chain reaction-restriction fragment length polymorphism diagnostic for the rapid molecular diagnosis of the Rangipo strain.

Comment: Small abstract belies big complicated paper! A very comprehensive study from ADHB on their development of a specific typing test for an *Mycobacterium tuberculosis* strain (Rangipo) that is most often associated with Māori. They used whole genome sequencing to find unique single-nucleotide polymorphisms in the Rangipo strain genome that could be exploited in a polymerase chain reaction-restriction fragment length polymorphism assay. This is turn was validated on clinical samples. The sequences discovered could be translated to rapid sputum-based polymerase chain reaction platforms such as GeneXpert.

Reference: Diagn Microbiol Infect Dis. 2017 Jun;88(2):138-140.

Abstract

Detection of HPV subtypes by mass spectrometry in FFPE tissue specimens

Authors: Kriegsmann M, et al.

Summary: The need to detect high- and low-risk human papilloma virus (HPV) subtypes in a time- and cost-effective manner led the authors to develop a new mass spectrometry-based test system. A high-throughput matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometer was used to detect and genotype 19 HPV subtypes in 46 formalin-fixed paraffin-embedded tissue samples. Results were compared with those obtained using two hybridisation-based test systems: the HPV 3.5 LCD-array kit and the EuroArrayHPV system. The MALDI-TOF mass spectrometry assay detected an HPV infection in 36 (78%) of the samples. Only one HPV subtype was identified in 16 samples (44%) and two to six subtypes were detected in the remaining 20 samples (56%). The overall agreement of all three assays was almost perfect (Cohen's k value: 0.83).

Comment: Hmm, this initially sounds cool, but it turns out you still have to do a DNA extraction, multiplex polymerase chain reaction, automated spotting and then have a suitable MALDI-TOF handy to read the results. Great if you are tech'd up but not cool if you have to buy everything in from scratch. Stick with Linear Array or similar I say.

Reference: J Clin Pathol. 2017 May;70(5):417-423.
Abstract

Performance of urinalysis tests and their ability in predicting results of urine cultures

Authors: Yusuf E, et al.

Summary: Urinalysis has a quicker turnaround time that urine culture and if it can predict the results of culture, urinalysis could inform on the need to culture and expedite treatment decisions. In this study, the performance of urinalysis tests by automated test strip analyser (nitrite and leucocyte esterase) and flow cytometry (bacteria and white cell count) were evaluated in different subpopulations and types of samples, with a positive urine culture defined as $>10^5$ colony-forming units/mL considered the gold standard. A total of 2351 consecutive urine samples were tested and the sensitivity, specificity, positive predictive value and negative predictive value of the urinalysis tests were calculated using contingency tables. Cultures were positive in 14% of samples (95.6% monomicrobial, 74.7% *Enterobacteriaceae*). The nitrite test was the most specific (98.7%) but the least sensitive (43.2%); however, its specificity was reduced in urine from the in-and-out procedure (81.9%). The bacteria count was the most sensitive (91.7%) and also highly specific (87.5%); sensitivity was reduced in infants <24 months (86.1%) although specificity was high (95.9%). Bacteria count sensitivity was also reduced in bag specimen urine (83.3%) and in urine from indwelling catheters (84.7%). Negative predictive value was high for all tests and shown to be higher for the combined flow cytometry tests versus the automated test strip analyser (99.1% vs 97.4%).

Comment: Ruling out and avoiding cultures of urines that will ultimately be negative, or psychic microbiology if you will, is a focus of laboratories for decreasing costs and turnaround time. The authors found in their comparison that nitrite was a specific and bacteria count was a sensitive test for diagnosing urinary tract infection, and flow cytometry had a better performance than the automated test strip analyser in excluding urine to be cultured. The performance of the tests was in general applicable in all subpopulations and all types of samples.

Reference: J Clin Pathol. 2017 Jul;70(7):631-636.
Abstract

Work place drug testing of police officers after THC exposure during large volume cannabis seizures

Authors: Doran GS, et al.

Summary: This study assessed police office exposure to Δ^9-tetrahydrocannabinol (THC) as a result of involvement in the seizure and removal of illegally grown cannabis plants from indoor and outdoor growing operations. Swabs of gloves/hands, chests, and heads/necks of police officers were collected during raids as well as air samples at the sites. Hand swabs showed up to 20-times greater THC exposure in officers removing plants from forest plantations than from indoor operations, mainly attributable to the greater number and size of plants seized at forest sites. No THC was detectable in air samples from cannabis growing houses. However, THC was detected in air samples from the cargo area of storage trucks used in forest raids. Officers removing cannabis plants often sustained cuts, abrasions and ruptured blisters on exposed skin surfaces, especially at forest sites. To assess potential systemic exposure, over 100 urine samples were also collected from officers before and after raids – all were negative for THC.

Comment: Well, here's a workplace hazard that may evaporate if cannabis is decriminalised! This very comprehensive toxicology study examined the airborne and skin contact risk of THC exposure in New South Wales police who were involved in raids on cannabis plantations, i.e. actually pulling plants out. There are fascinating pictures of raids on houses and forests. Despite very high THC exposure via cuts, blisters and abrasions on their upper bodies, ZERO police urine samples were positive for THC. Which could be reassuring or disappointing depending on your point of view…

Reference: Forensic Sci Int. 2017 Jun;275:224-233.
Abstract

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Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma

Authors: Kavalieris L, et al.

Summary: Due to a high rate of recurrence with urothelial carcinoma of the bladder, current guidelines recommend rigorous, invasive surveillance by cystoscopy for disease recurrence. This study sought to develop and validate an effective non-invasive test with sufficient sensitivity to rule out recurrent urothelial carcinoma and reduce the need for invasive investigations without compromising patient care. A total of 1036 urine samples were prospectively collected from 763 patients undergoing routine surveillance for recurrent urothelial carcinoma of the bladder and randomly stratified to independent development and validation sets. A test combining gene expression, clinical and patient data (designated Cxbladder Monitor) was developed. The internally validated sensitivity of Cxbladder Monitor was 0.93, the negative predictive value was 0.97 and the test negative rate was 0.34. Sensitivity was 0.95 for recurrent disease with high risk of progression (all high-grade disease and low grade, stage T1 or greater disease) and 0.86 for low grade Ta disease. Diagnostic performance was not significantly impacted by patient age, gender, tumour stage or adjuvant bacillus Calmette-Guérin treatment within the last 6 months. False-negative findings were reported in less than 1.5% of samples tested.

Comment: This is the third in a stable of genomic tests created by Pacific Edge and designed to identify or rule out urothelial cancer in patients at various stages of the clinical pathway. This paper examined their test to rule-out recurrence during surveillance. The beauty of their tests is that they combine gene expression with all-important clinical and patient data, genetic and phenotypic essentially, to provide test interpretation. Pacific Edge is the product of research conducted at the University of Otago and they have a unique business model: patient information, interpretation algorithm and genetic testing is all kept in-house. So you can't buy this kit for your lab, but you can refer samples to their fully accredited and state-of-the-art laboratories.

Reference: J Urol. 2017 Jun;197(6):1419-1426.
Abstract

Transient elevation in serum carcinoembryonic antigen while on adjuvant chemotherapy for colon cancer: Is this of prognostic importance?

Authors: Lawrence N, et al.

Summary: Although serum carcinoembryonic antigen (CEA) is used as a biomarker to detect colon cancer relapse following initial surgical or adjuvant treatment, the prognostic significance of transient increases in CEA during adjuvant chemotherapy is unknown. The authors performed a retrospective review of 61 Auckland patients with stage II or III disease who had received adjuvant chemotherapy and for whom sufficient CEA data were available. Patients were followed up for a minimum of 7.4 years or until death. Kaplan-Meier estimates for 5-year survival were higher in the groups with transient CEA elevation and no CEA elevation (95.0% and 85.2%, respectively) than in the persistent CEA elevation group (42.9%). The difference in overall 5-year survival between the transient elevation and no elevation groups was not statistically significant.

Comment: This is a very helpful piece of research in the ongoing battle to find significance in a sea of non-specific laboratory markers. In the setting of possible metastatic disease, this is high stakes for patients and these researchers have thoroughly reassured the field of the non-significance of transient increases in CEA during adjuvant chemotherapy. A great relief for affected patients.

Reference: Asia Pac J Clin Oncol. 2017 Apr;13(2):e124-e131.
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.