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In this issue

Rob Siebers, Editor

Tuberculosis (TB) ranks in the top ten causes of death worldwide. Despite a lot of research, the diagnostic testing and the epidemiological investigation to control the spread of TB still require significant resources of clinical microbiology laboratories. In this issue, Indira Basu from LabPlus, Auckland provides a brief overview of the past, present and future of the different diagnostic technologies used in tuberculosis, covering the time-intensive culture-based technologies, along with the newer molecular technologies that have revolutionised TB diagnosis. Also described are the technologies that rapidly determine the drug susceptibility or resistance of the bacterium, *Mycobacterium tuberculosis*, and the technologies of molecular typing that help in an outbreak or prevent further spread of the disease. The emerging technology of whole genome sequencing of *Mycobacterium tuberculosis* and its possible applications in TB diagnosis are discussed. This review, together with international publications in this area were submitted by Indira for Fellowship of the NZIMLS.

Lynnette Jones and Michael Legge from the University of Otago have previously shown that paralysis following spinal cord injury leads to compromised fatty acid metabolism. In this issue they further report that fasting plasma free fatty acids and glycerol were significantly elevated in spinal cord injured individuals most likely caused by adipose tissue lipolysis and may explain the development of insulin resistance and type 2 diabetes mediated by free fatty acids.

Recurrent spontaneous miscarriage is one of the most common complications during pregnancy. Currently, karyotyping is considered the gold standard to detect underlying chromosomal abnormalities. Recently more rapid techniques not requiring cell culture, such as QF-PCR, have been used to derive a faster diagnosis. In this issue, researchers from Iran used this technique to determine the frequency of 13, 18, 21 X and Y aneuploidy of spontaneous miscarriage in the Mazardaran Province in Iran. Twenty-two cases of aneuploidy were detected from 92 samples with trisomy as the most common finding. QF-PCR might play a major role in aneuploidy screening and be considered as a valid alternative to the full karyotype.

Ibrutinib is an effective novel drug that is very effective against chronic lymphocytic leukaemia even in the relapsed setting. Despite this, a rare proportion of patients treated with ibrutinib will still undergo Richter Transformation (RT) to high-grade disease. In this issue, Annabella Yee, a 4th year BMLSc student from the University of Otago, and colleagues present a case where a patient underwent RT after having stable disease for 4.5 years on ibrutinib. Ibrutinib-resistance in this case was likely driven by an acquired *TP53* and other genetic aberrations.

The University of Otago offers summer studentships to health and biomedical science undergraduate students, including BMLSc students, in order to undertake a short-term research project during the academic summer break (10 weeks) in the Dunedin, Christchurch and Wellington campuses. In this issue, Charlotte Wu, a 4th year BMLSc student from the University of Otago, and colleagues determined whether these studentships resulted in peer-reviewed publications. They found that over a seven-year period about a third of students achieved at least one peer-reviewed publication and 40.4% of these were named first author.

The Qualified Medical Laboratory Technician (QMLT) qualification of the NZIMLS has been in existence for over 40 years with varying changes in structure and routes of qualifying. Progressively the QMLT has evolved into a multidisciplinary qualification with currently 12 disciplines that may be examined. In this issue, Michael Legge considers the future of the QMLT qualification and alternatives to the current QMLT are discussed.

Of interest presently within the medical laboratory science field is the burgeoning role of the medical laboratory science practitioner, or clinical laboratory scientist. In this issue, Lauren Eddington from Dunedin discusses a new qualification now available at select American universities called the Doctorate of Clinical Laboratory Science (DCLS). The main distinguishing feature is the inclusion of clinical expertise as a component of the qualification. In an accompanying commentary, Michael Legge gives a perspective of the Clinical Scientist training programmes in the UK, Australia and New Zealand. The UK and Australian requirements for Clinical Scientists are much more rigorous than the DCLS. The DCLS provides a qualification, which falls into the realm of education on the quality of patient care and is intended to be a “generalist” qualification. The “Fellowships” have extensive supervised specialist training with the expectation of creating experts and consultants within a given field of pathology, thereby creating “specialists”.

In the April 2019 issue, the NZIMLS President, Terry Taylor was featured in an occasional article to highlight his role in the organisation. In this issue, Jillian Broadbent, the CPD Co-Ordinator is featured showing the diverse nature of this position. Future issues of the Journal will feature other prominent persons and positions within the NZIMLS.

Recently a prominent member of our profession passed away and his obituary, written by Brian Millar, is in this issue. Desmond James Philip joined the staff of Auckland Hospital laboratory in 1946 and moved to Middlemore Hospital laboratory in 1952, retiring in 1988. Des served the Institute for 16 years as Council Member, Treasurer, Vice President and President. He was a Fellow of the NZIMLS and was made a Life Member for his contributions to the profession.

Two more highly experienced New Zealand medical laboratory scientists have been appointed to the Journal’s Editorial Board. They are Jillian Broadbent from Canterbury Health Laboratories (and CPD Co-Ordinator) and Sujata Hemmady from LabPlus, Auckland (and Region 1 Representative on the NZIMLS Council). We look forward to their contributions and experience for the future of the Journal.

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REVIEW ARTICLE

The past, present and future of tuberculosis diagnostics techniques

Indira Basu

ABSTRACT

According to a recent World Health Organization report, tuberculosis (TB), an ancient disease with an enormous global impact, still ranks in the top ten causes of death worldwide. Pulmonary TB, where patients show symptoms like cough, fever, night sweat and weight-loss is highly contagious and poses serious public health concerns. If diagnosed in a timely manner nearly all patients infected with TB can be cured. However, TB remains one of the world's big healthcare-related challenges since treatment usually requires daily intake of multiple antibiotics for a prolonged period of six months. As such patient compliance, as well as tolerance to this multi-drug therapy, poses a big issue in many cases, resulting in relapse or development of drug-resistance. The increase in resistance to the different front-line TB drugs has led to the spread of multi-drug resistant tuberculosis (MDR-TB) and further prevents TB control. MDR-TB creates a higher burden on clinical management requiring prolonged treatment, and monitoring of adverse side-effects from alternate therapy. In addition, presence of co-morbidities (HIV, immunocompromised status, diabetes) can further complicate cure. Despite a lot of research, the diagnostic testing and the epidemiological investigation to control the spread of TB still require significant resources of clinical microbiology laboratories as well as public health services.

This review article provides a brief overview of the past, present and future of the different diagnostic technologies used in tuberculosis. It covers the time-intensive culture-based technologies, along with the newer molecular technologies that have revolutionised TB diagnosis with faster turn-around-time. This review article also describes the technologies that rapidly determine the drug susceptibility or resistance of the bacterium, *Mycobacterium tuberculosis,* the causative agent of TB; and the technologies of molecular typing that help in an outbreak or prevent further spread of the disease. It discusses research that has contributed to further understanding of the biology of various strains in the *Mycobacterium tuberculosis* complex, and their prevalence in humans globally and in New Zealand. Finally, the emerging technology of whole genome sequencing of *Mycobacterium tuberculosis* and its possible applications in TB diagnosis is discussed.

Keywords: Tuberculosis, epidemiology, New Zealand, TB drugs, drug-resistance, diagnostic tools, TB typing, application of WGS.

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Epidemiology of tuberculosis

Highly infectious tuberculosis (TB) is primarily a pulmonary disease that has been a human scourge for many centuries. TB was known in the 18th and 19th centuries as consumption, since the disease "consumes" or causes drastic weight loss of the afflicted individual over the course of the disease progression. The onset of symptoms in pulmonary cases is usually weight loss, lethargy, night fevers and a cough – which is usually productive and may include visible blood. Coughing expels droplets of respiratory secretions containing the bacteria into the surrounding air, which can be inhaled to infect others making TB highly contagious. While primary infection is generally confined to the lower respiratory tract, in some patients the bacteria can spread to other sites via the blood stream, leading to disseminated or miliary tuberculosis, which if undetected and untreated, can lead to death.

Tuberculosis can also manifest clinically by affecting various other extra-pulmonary sites. Tuberculosis has claimed many lives over the centuries including Jane Austen in the 18th century, Fredric Chopin in the 19th century, and New Zealand's very own Katherine Mansfield in the 20th century. TB continues to remain a major challenge for definitive global prevention and cure and continues to take the lives of millions in the developing world, even in the 21st century. TB is one of the top 10 causes of death worldwide (1). It ranks above HIV/AIDS in the top 10 causes of death globally due to a single infectious agent (1). In 2017, TB was responsible for an estimated 1.3 million deaths among HIV-negative people (1). The global emergence of antimicrobial resistance as well as co-morbidity with HIV/AIDS poses additional challenges for tuberculosis control. On a positive note, the World Health Organization (WHO) set a target to "End TB" by 2035 considering the development of better therapeutic interventions like new antimicrobials, better diagnostic methods through more advanced technologies and better preventatives, for example, the improvement of vaccines through better understanding of disease pathogenesis (1).

According to the latest ESR report, the 2015 notification rate of tuberculosis in New Zealand was 6.2 per 100,000 (2). Hence, New Zealand has a low burden of tuberculosis compared to the nations in the Indian subcontinent, South East Asia, and Africa which share 84% of the global burden of TB according to the WHO report for 2018 with >10,000 estimated TB cases per year (1).

Mycobacterium tuberculosis, the causative agent of tuberculosis

In 1882 Dr Robert Koch shared his discovery of *Mycobacterium tuberculosis* or tubercle bacillus, the causative agent of tuberculosis with the scientific community (3). Thus he presented his ground breaking postulate in microbiology, famously known after him as the Koch's postulate, where he showed a causal relationship between a microorganism and

a disease. He won the Nobel Prize in Physiology or Medicine in 1905 for this discovery.

M. tuberculosis is quite different from other bacteria because of its slow growth rate when cultured. Specimens from patients suspected of infection with *M. tuberculosis* are cultured for a maximum of 6 weeks, if the bacterium does not grow. Another special characteristic of *M. tuberculosis* is its thick and waxy outer layer that makes it impervious to usual staining techniques used for other bacterial detection and groups it under "acid-fast bacillus" (AFB) as a result (4).

Currently, the genus *Mycobacterium* consists of more than 200 species (5, 6). *Mycobacteria* can be divided into the following groups based on the clinical manifestation of the disease in humans and growth characteristics *in vitro* - *Mycobacterium tuberculosis* complex (MTBC) causing tuberculosis in humans, *Mycobacterium leprae* causing leprosy; *Mycobacterium ulcerans* causing Buruli ulcer; and those referred to as nontuberculous mycobacteria (NTM) (6) that can be sub grouped as rapid growers (grows in <7 days) and slow growers (grows in >7days). MTBC causing tuberculosis is comprised of human adapted pathogens and animal adapted pathogens (7). The human adapted ones are *Mycobacterium tuberculosis sensu stricto*, *M. africanum* isolated mostly from West Africa (8) and *M. canettii* isolated only from the Horn of Africa (9). Among the various animal adapted pathogens, *M. bovis* cause tuberculosis in cattle. In the late 19th and early 20th centuries *M. bovis* caused around a quarter of all human cases of tuberculosis when humans ingested contaminated dairy products (10). In spite of pasteurization reducing the risk of transmission of *Mycobacterium bovis* from infected cattle to humans from the 1940s, *M. bovis* transmission from animals to humans has been reported in New Zealand in recent times (11). Other animal adapted pathogens include *M. caprae* causing tuberculosis in goats, *M. microti* in rodents like voles, *M. orygis* in antelopes, *M. pinnipedii* in seal and sea lions, *M. mungi* in banded mongooses, *M. suricattae* in meerkats, the dassie bacillus in hyrax and the chimpanzee bacillus (7).

M. tuberculosis has a long history of co-evolution with its human host (12, 13). Phylogenetic analysis of MTBC culture isolates based on phylogenetic markers, large sequence polymorphisms (LSP) or regions of difference (RD) showed that *Mycobacterium tuberculosis* comprises several phylogeographical lineages (Figure 1a) (14). Among *Mycobacterium tuberculosis* clinical isolates, the main human-adapted strain lineages are classified into Lineages 1–7 according to their geographic distribution. Indo-Oceanic or Lineage 1 is known as the "ancestral" or "ancient" strain and the rest of the "modern" strains are East Asian or Lineage 2, East African-Indian or Lineage 3, Euro-American or Lineage 4 and Ethiopian or Lineage 7 (14). Lineages 1–4 comprise the majority of human-adapted strains which are responsible globally for human TB cases, while West African 1 or Lineage 5 and West African 2 or Lineage 6 are restricted to West Africa and are traditionally known as *M. africanum* West Africa 1 and 2, respectively. These seven phylogeographic lineages are associated with particular geographic regions and differ among others, in virulence, biological fitness and propensity to acquire drug resistance (7).

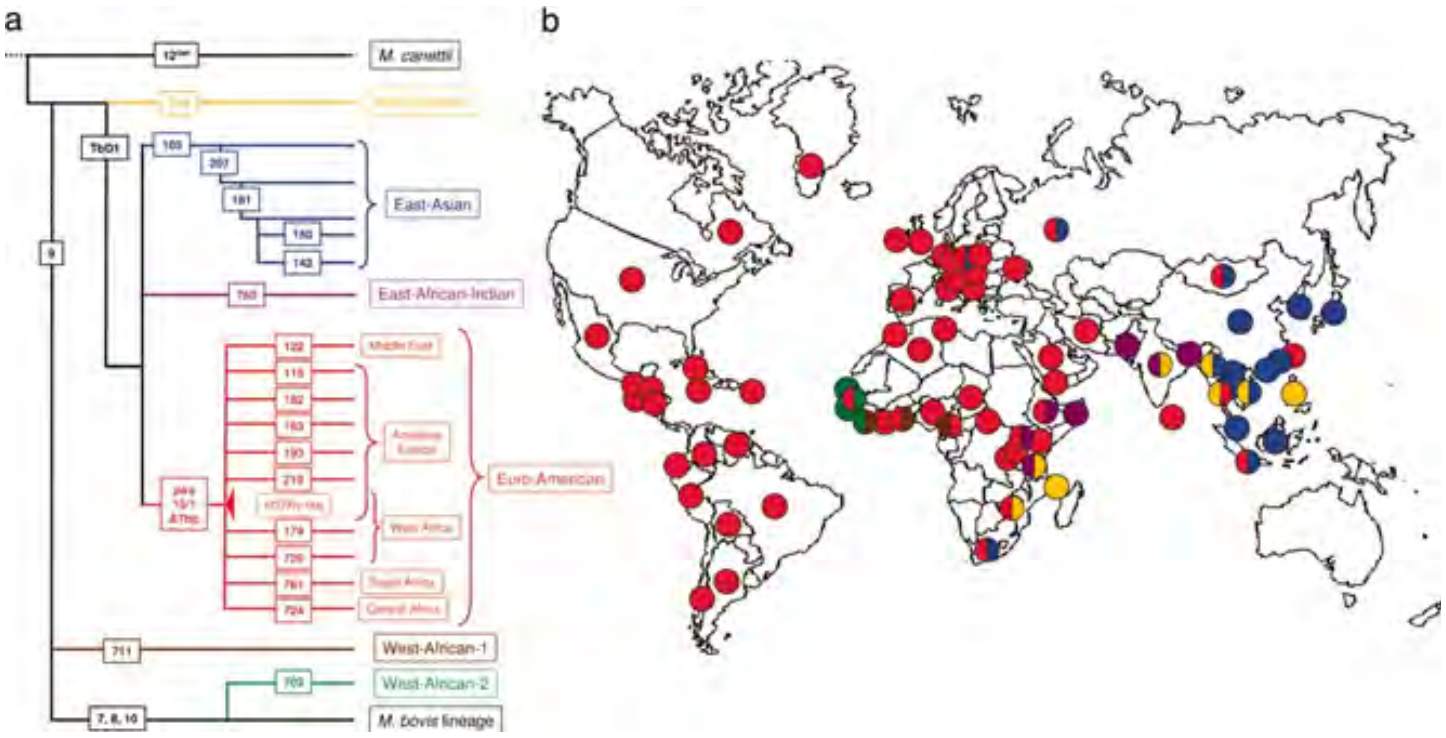

Figure 1. The global population structure and geographical distribution of *M. tuberculosis*.
(a) Large scale polymorphism (LSPs) define a global phylogeny for *M. tuberculosis*. The names of the lineage-defining LSPs or regions of difference are shown in rectangles. The geographic regions associated with specific lineages are indicated.
(b) The six main lineages of *M. tuberculosis* are geographically structured. Each dot corresponds to 1 of 80 countries represented in the global strain collection. The colours of the dots relate to the six main lineages defined in Fig. 1a and indicate the dominant lineage(s) in the respective countries.
Reference: S Gagneux *et al.* Variable host–pathogen compatibility in Mycobacterium tuberculosis. *Proc Natl Acad Sci USA* 2006; 103(8): 2869-2873. Copyright: (2006) National Academy of Sciences.

Lineage 4 is the most widespread lineage of *M. tuberculosis* causing human tuberculosis globally (Figure 1b). A more recent study by Gagneux and his collaborators further investigated the global distribution of *M. tuberculosis* lineage 4 (15) using a wider collection of clinical isolates. In this study, employing molecular whole genome sequencing based phylogenetic techniques on the global collection of lineage 4, the authors inferred that the global success and spread of lineage 4 is a consequence of both biological and social phenomena. Furthermore, they also show that the lineage 4 *of M. tuberculosis* is genetically diverse. Based on the phylogeography of lineage 4, the authors deduced a distinction between globally represented "generalist" sublineages and geographically restricted "specialist" sublineages within lineage 4 (15).

A collaborative study was undertaken in 2012 to ascertain the distribution of MTBC lineages in New Zealand and also to examine whether particular lineages correlate with patient ethnicity, country of origin and multi drug resistant tuberculosis (MDR-TB) (16). In this study, techniques similar to that used in the paper by Gagneux and co-authors in 2006 (14), i.e. large sequence polymorphism (LSP) and single nucleotide polymorphism (SNP) analyses, were applied on all representative laboratory confirmed *M. tuberculosis* stains isolated in New Zealand in 2010 and 2011. This study showed that New Zealand *M. tuberculosis* isolates are dominated by lineage 4 (Figure 2) (16).

In this study, it was found that among patients who were immigrants to New Zealand, the predominant lineages corresponded to highly prevalence lineages in the country of origin while lineage 4 was predominantly isolated in New Zealand-born individuals (Figure 3).

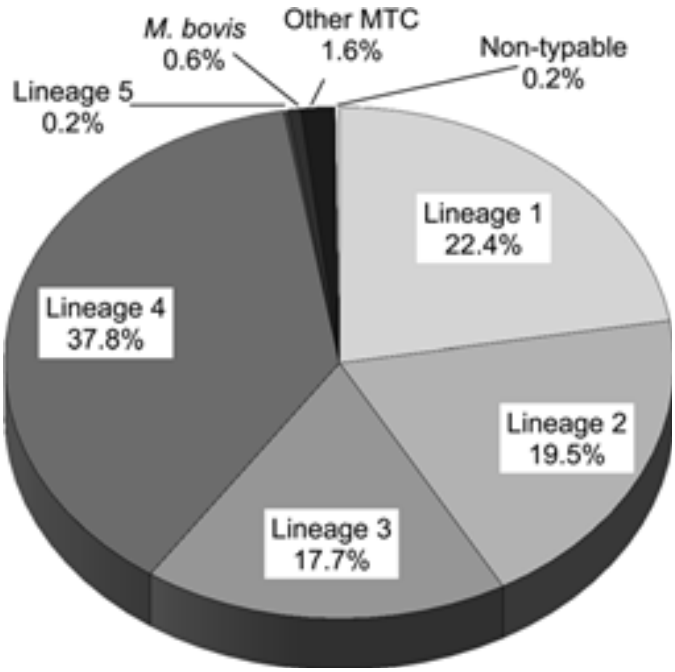

Figure 2. Relative abundance of MTB phylogenetic lineages in New Zealand.
MTC = *M. tuberculosis* complex. Reference: Bower JE, Freeman JT, Basu I, O'Toole RF. Phylogenetic lineages of uberculosis isolates in New Zealand and their association with patient demographics. *Int J Tuberc Lung Dis* 2013; 17(7): 892–897. Reprinted with permission of the International Union against Tuberculosis and Lung Disease. Copyright © The Union.

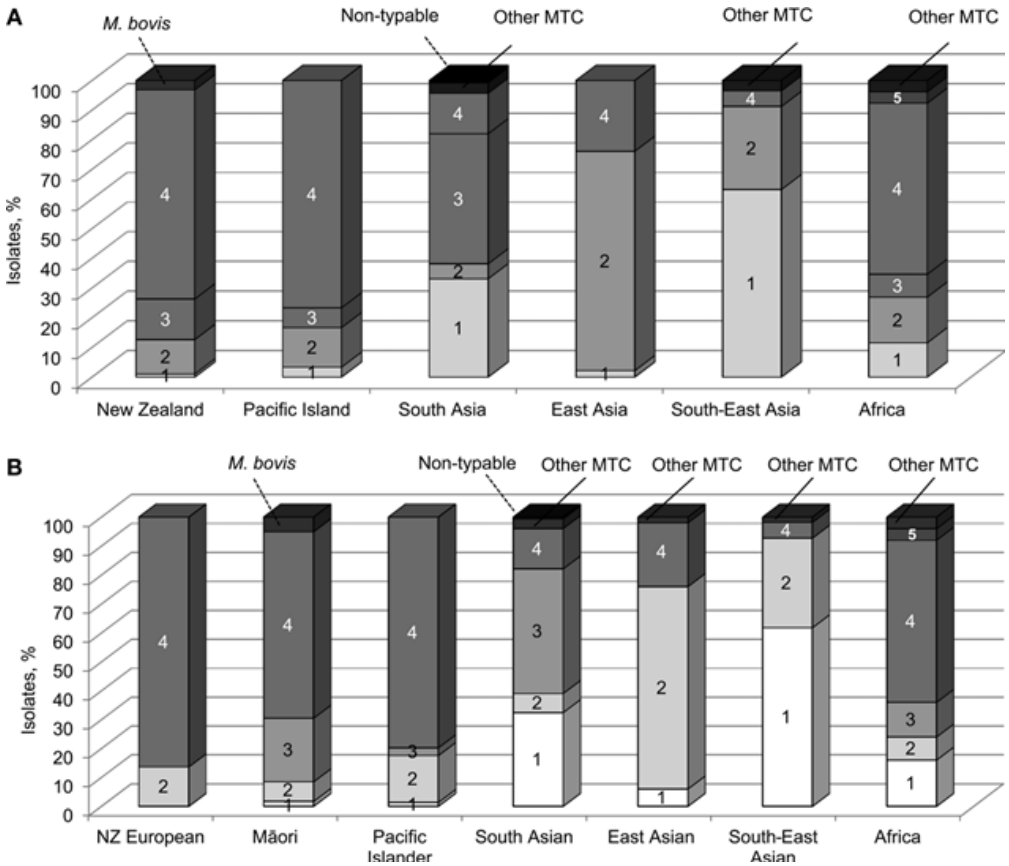

Figure 3. Relative abundance of phylogenetic lineages according to (A) country of origin and (B) ethnicity.
(MTC = *M. tuberculosis* complex). The numbers inside the bars represent the lineages. Reference: Yen S, Bower JE, Freeman JT, Basu I, O'Toole RF. Phylogenetic lineages of tuberculosis isolates in New Zealand and their association with patient demographics. *Int J Tuberc Lung Dis* 2013; 17(7): 892–897. Reprinted with permission of the International Union Against Tuberculosis and Lung Disease. Copyright © The Union.

It was also noted in the same study that lineage 2 predominantly accounts for MDR-TB cases in New Zealand from 2002 to 2011 (Figure 4) (16). This is consistent with reports showing the Beijing sublineage belonging to "ancient" lineage 2 are more virulent, have better transmission capability and are MDR-TB isolates (17).

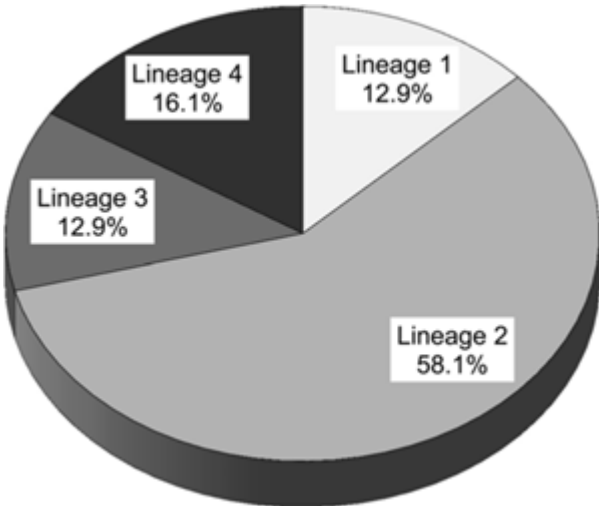

Figure 4. Relative distribution of multidrug-resistant isolates of the *Mycobacterium tuberculosis* complex, 2002-2011 with respect to genetic lineage.
Reference: Yen S, Bower JE, Freeman JT, Basu I, O'Toole RF. Phylogenetic lineages of tuberculosis isolates in New Zealand and their association with patient demographics. *Int J Tuberc Lung Dis.* 2013; 17(7): 892-897. Reprinted with permission of the International Union Against Tuberculosis and Lung Disease. Copyright © The Union.

Anti-tuberculous therapeutics and emergence of resistance to anti-tuberculous drugs

Most bacterial infections are cured by a short course (a week or two) of antibiotic monotherapy. In contrast, an abundance of scientific evidence shows better outcomes for tuberculosis with a long-term and multidrug therapeutic approach, due to the slow growth and dormancy of a sub-population of MTBC (18). It takes at least 6 months to successfully treat uncomplicated drug-sensitive pulmonary TB. For a *M. tuberculosis* isolate which is fully susceptible to first line drugs, the regimen prescribed during the first two months consists of a daily dose of two bactericidal drugs, isoniazid and rifampicin, along with ethambutol which inhibits actively multiplying bacilli and pyrazinamide, a bacteriostatic agent that inhibits semi-dormant bacilli located in acidic environments such as TB lesions. After the intensive phase of the first 2 months with a daily dose of these 4 drugs, the sterilising phase in the following four more months comprises a daily dose of isoniazid and rifampicin. This long-term therapy with the combination of "first line drugs" has been shown to achieve bacteriological eradication (Table 1) (19).

Drug resistant TB results from inadequate or incomplete treatment that selects for drug-resistant bacilli (acquired resistance), and those resistant organisms can be transmitted to other individuals (primary resistance). Second-line drugs are used to replace the ineffective first-line drugs in complicated cases of drug resistant TB. The second line drugs are more costly and can lead to more adverse side effects. In case of multi-drug resistant tuberculosis [MDR-TB, defined as being resistant to both isoniazid (INH) and rifampicin (RIF)] the first two treatment options undertaken are with the higher generation fluoroquinolones (FQ), such as moxifloxacin or levofloxacin and one of the second line injectable drugs (SLIDs) which includes aminoglycosides and the cyclic peptides (20). Other drugs can be used according to the WHO guidelines (20). Details about drugs commonly used in treatment for drug-sensitive and drug-resistant tuberculosis discussed above, their mode of action and the mutations commonly implicating resistance are compiled in Table 1.

Drug resistance is concerning, as it requires more complexity with regards to the length of treatment, monitoring of adverse side-effect of the toxic second-line drugs and the cost of the treatment. An increase in multi-drug resistant TB (MDR-TB) has added to the global concern of tuberculosis. New Zealand has a low burden of MDR-TB at an average annual rate of 1.2% among culture-positive TB cases (2). WHO define extensively drug-resistant (XDR) TB as a multi-drug resistant tuberculosis that is also resistant to any one of the FQs and one of the SLIDs. New Zealand has only one recorded laboratory-confirmed case of an XDR-TB in 2010 (21).

The primary diagnostic tool used in a basic microbiology laboratory is smear microscopy of respiratory specimens predominantly induced sputum, with staining for acid-fast bacilli (AFB). This is simple, rapid and inexpensive but lacks sensitivity and varies greatly based on the AFB burden in sputum, with 1000–10,000 colony-forming units (CFU)/ml required for reliable detection (22). Culture of respiratory specimens is considered the gold standard for detection of pulmonary tuberculosis and requires only 10 CFU/mL to be positive in liquid culture (23). However, culture of *M. tuberculosis* must be performed in a high containment laboratory and requires expensive equipment. Solid medium-based cultures like the egg-based Löwenstein–Jensen (LJ) medium have a good specificity but require 3–6 weeks to grow (24).

In the recent years, the capacity for culture-based TB diagnosis has improved due, in part, to the new policy recommendations of the WHO (25) on the use of liquid-based culture medium, for example, the modified Middlebrook 7H9 broth base Mycobacteria Growth Indicator Tube (MGIT) from Becton Dickinson. Irrespective of smear results, which give an indication of the bacterial burden, the mean time to detection in the MGIT broth is 15.3 days for all mycobacteria (26). According to the WHO policy guidelines for any positive liquid cultures, all reference mycobacterial laboratories need to use a rapid and affordable method for the identification of the mycobacterial species in order to differentiate *M. tuberculosis* complex from non-tuberculous mycobacteria (NTM) (25). This differentiation is predominantly based on a secreted protein called the MPT64 that is released during the growth of *M. tuberculosis* complex in culture medium.

Several simple and easy to use immunochromatographic tests (ICT, strip test in cassette) have been developed based on detection of this MPT64 antigen in the culture medium (27). In the reference mycobacterial laboratory in LabPLUS the Becton Dickinson (BD) MGIT TBc (MTBC) Identification Test Rapid test that detects the protein MPT64 in liquid or solid cultures is used for the rapid identification of *M. tuberculosis* complex. However, in the period following the adoption of BD MGIT TBc Identification Test, six MGIT culture isolates gave a negative result when this ICT was used for detection of MTBC. Since there was a high suspicion of TB in these 6 cases, the isolates were tested further with Xpert MTB/RIF PCR targeting the rpoB gene and were found positive for MTBC. These 6 isolates were further analysed to address the discrepancy in result obtained from the two tests. On sequencing the mpt64 gene, which codes for the MPT64 antigen, mutations were detected in the mpt64 gene in these false-negative cases due to deletion, insertion or substitution of bases. These mutations prevent the secretion of the MPT64 antigen in the culture medium and consequently prevent its detection by the MGIT TBc Identification rapid test (28). In spite of this constraint, this ICT is easy-to-use, rapid and affordable. Hence an algorithm has been implemented subsequently at the mycobacteriology lab in LabPLUS. If there is a high clinical suspicion of TB, a MGIT culture with a negative ICT result is further run through Xpert MTB/RIF PCR (with a different target) to rule out the presence of MTBC in the liquid MGIT culture (28). This prevents the misidentification of tuberculosis from a MGIT culture.

Table 1. Anti-tuberculous drugs.

Drug name (abbreviation)	Class of drugs	Mode of action	Mutation in gene(s) conferring resistance
Rifampicin (RIF)	Rifamycin derivative	Interferes with RNA synthesis by binding to the ß subunit of the RNA polymerase	*rpoB*
Isoniazid (INH)	Pyridine [a pro-drug that requires activation by the catalase/peroxidase enzyme KatG, encoded by the *katG* gene, to exert its effect]	Inhibits synthesis of mycolic acids through the NADH-dependent enoyl-acyl carrier protein (ACP)-reductase, encoded by *inhA*	*katG, inhA*
Ethambutol (EMB)	Ethylenediamine	Inhibition of arabinogalactan biosynthesis in cell wall	*embB*
Pyrazinamide (PZA)	Pyrazine; an analogue of nicotinamide	Converts pyrazinamide to pyrazinoic acid, which disrupts the bacterial membrane energetics inhibiting membrane transport	*pncA*
Moxifloxacin/ Levofloxacin	Fluoroquinolone (FLQ)	Inhibition of topoisomerase II or DNA gyrase involved in DNA synthesis	*gyrA* *gyrB*
Kanamycin/ Amikacin	Aminoglycoside (second line injectable drugs; SLIDs)	Inhibition of protein synthesis	*rrs, eis*
Capreomycin/ Viomycin	Cyclic peptide (second line injectable drugs; SLIDs)	Inhibition of protein synthesis	*rrs*

Several simple and easy to use immunochromatographic tests (ICT, strip test in cassette) have been developed based on detection of this MPT64 antigen in the culture medium (27). In the reference mycobacterial laboratory in LabPLUS the Becton Dickinson (BD) MGIT TBc (MTBC) Identification Test Rapid test that detects the protein MPT64 in liquid or solid cultures is used for the rapid identification of *M. tuberculosis* complex. However, in the period following the adoption of BD MGIT TBc Identification Test, six MGIT culture isolates gave a negative result when this ICT was used for detection of MTBC. Since there was a high suspicion of TB in these 6 cases, the isolates were tested further with Xpert MTB/RIF PCR targeting the rpoB gene and were found positive for MTBC. These 6 isolates were further analysed to address the discrepancy in result obtained from the two tests. On sequencing the mpt64 gene, which codes for the MPT64 antigen, mutations were detected in the mpt64 gene in these false-negative cases due to deletion, insertion or substitution of bases. These mutations prevent the secretion of the MPT64 antigen in the culture medium and consequently prevent its detection by the MGIT TBc Identification rapid test (28). In spite of this constraint, this ICT is easy-to-use, rapid and affordable. Hence an algorithm has been implemented subsequently at the mycobacteriology lab in LabPLUS. If there is a high clinical suspicion of TB, a MGIT culture with a negative ICT result is further run through Xpert MTB/RIF PCR (with a different target) to rule out the presence of MTBC in the liquid MGIT culture (28). This prevents the misidentification of tuberculosis from a MGIT culture.

The phenotypic drug susceptibility testing (pDST) of MTBC can take 2 to 4 weeks, since it is constrained by the slow growth characteristics of *M. tuberculosis*. Therefore, it is time-consuming, requires advanced and sophisticated laboratory infrastructure, qualified staff and strict quality assurance mechanisms. Moreover pDST can be challenging and in resource-limited areas it can be cost-prohibitive. Yet, it is still considered the gold standard for drug resistance detection. Phenotypic DST uses critical concentrations which is defined as the lowest concentration of an anti-TB drugs in vitro that will inhibit the growth of 99% of phenotypically wild type strains of MTBC. Critical concentrations of anti-TB agents is used to determine the susceptibility or resistance of a culture of MTBC (29).

To address the growing global need for prompt and accurate detection of tuberculosis, the use of genotypic drug susceptibility testing (gDST) took off with the introduction of the Cepheid Xpert MTB/RIF test. It is a commercial, automated, real-time PCR based-technology which detects the presence of MTBC as well as RIF resistance (30). The introduction of Xpert MTB/RIF test was a “game- changer” in TB diagnosis since the result is available with minimal hands-on technical time and less than 2 hours of PCR from direct respiratory specimens like induced sputum. Cepheid Xpert MTB/RIF assay, endorsed by WHO, has become a valuable first-line test for the detection of *M. tuberculosis* and rifampicin resistance, especially in resource constraint countries. More than 95% of RIF resistant mutations are associated with mutations in the *rpoB* gene and the majority of the mutations in *rpoB* gene are found within an 81 base pair (bp) RIF-resistance determining region (RRDR). Hence Xpert MTB/RIF PCR technology integrates this 81bp mutational hot-spot in the *rpoB* gene, and thus not only detects the presence of MTBC DNA in a clinical specimen but also whether there is a RIF resistance -associated mutation in the RRDR hot-spot resulting in presumptive MDR-TB. Mycobacterial resistance to RIF mostly occurs together with resistance to INH and hence resistance to RIF is considered as a surrogate marker for MDR-TB (31).

However, during routine diagnostic testing at LabPLUS, the Xpert MTB/RIF showed false-positive rifampicin resistance in one-third of cases tested between December 2009 and November 2011 (32). Sequencing of the rpoB gene around the mutational hot-spot revealed that the test incorrectly assigned rifampicin resistance to silent mutations in the rpoB gene that do not cause any phenotypic change. It was a cautionary finding that underscores the importance of confirmatory testing by sequencing of the rpoB gene in rifampicin resistance positive tests before reporting the rifampicin result. This confirmation was required to rule out instances where silent mutations occur. In response to multiple similar reports in the literature about false-positive RIF results, Cepheid has further modified the Xpert MTB/RIF system to overcome this problem and recently introduced the Xpert MTB/RIF Ultra. The newer version uses two multicopy targets, IS6110 and IS1081 to detect the presence of *M. tuberculosis* which has enabled a lower limit of detection (33). Furthermore the Xpert MTB/RIF Ultra uses the melting of the rpoB gene RRDR for detection of RIF resistance, making it more reliable for predicting specific mutations (34).

Another common molecular technology used in gDST for detection of resistance to RIF and other first and second line drugs are the Hain Line probe assays (LPA) - Genotype MTBDRplus and Genotype MTBDRsl. These tests involve a multiplex PCR amplification followed by reverse hybridisation where single-stranded amplicons bind to specific probes attached to the LPA strips. The visualised band patterns on the strips are then interpreted by a manual comparison with a printed template for the absence/presence of wild-type and mutant bands. Genotype MTBDRplus (both versions 1.0 and 2.0) includes the 81bp mutation hotspot of rpoB for RIF resistance as well as the mutational hotspots in katG and inhA genes for INH resistance. Hain Genotype MTBDRsl version 1.0 detects mutational hotspots in rrs, gyrA and embB genes implicated in the resistance to SLIDs, FQ and ethambutol respectively. The new version 2.0 of MTBDRsl does not carry the embB gene. In its place, the MTBDRsl assay incorporates probes to detect mutations in gyrB and the promoter region of the eis gene, which are associated with resistance to FQs and SLIDs, respectively (Table 2) (35). Genotypic DST technology is successfully used on AFB smear-positive respiratory specimens as well as MTBC culture isolates (36). The commercial diagnostic tests for pDST and their targets and specific application are tabulated below (Table 2).

Table 2. Commercial molecular diagnostic platforms for gDST and their targets.

Type of Therapy	Drug name	Mutation in gene(s) conferring resistance	Commercially available molecular diagnostic platforms for mutation detection
First line	Rifampicin	*rpoB*	Xpert MTB/RIF (Ultra), Hain Genotype MTBDR*plus* (V 1.0 and 2.0)
	Isoniazid	*katG, inhA*	Hain Genotype MTBDR*plus* (V 1.0 and 2.0)
	Ethambutol	*embB*	Hain Genotype MTBDR*sl* (V 1.0 only)
	Pyrazinamide	*pncA*	No
Second line	Moxifloxacin/ Levofloxacin	*gyrA, gyrB*	Hain Genotype MTBDR*sl* *gyrA* (V1.0 and V2.0); *gyrB* in V2.0 only
	Kanamycin/ Amikacin	*rrs, eis*	Hain Genotype MTBDR*sl* *rrs* (V1.0 and V2.0); *eis* in V2.0 only
	Capreomycin/ Viomycin	*rrs*	MTBDR*sl* (V1. 0 and V2.0)

TB typing

M. tuberculosis has a homogenous genome that harbours little genetic diversity and as such is referred to as monomorphic bacterial pathogen. Most current genotyping techniques used to study the epidemiology of *M. tuberculosis* are based on regions of the genome that have variation (polymorphism) like mobile or repetitive genetic elements (37). TB genotyping, when combined with epidemiological data, has been used as a proxy for TB transmission. The proportion of cases that belong to a genotype cluster are influenced by the discriminatory power of the genotyping approach used. The three most commonly used genotyping methods include (1) insertion sequence 6110 (IS6110) based restriction fragment length polymorphism (RFLP), (2) spacer oligonucleotide typing or spoligotyping and (3) mycobacterial interspersed repetitive units - variable tandem repeat loci (MIRU-VNTR).

In IS6110-RFLP, the mycobacterial genomic DNA is digested with a restriction enzyme (PvuII), that cleaves the IS6110 sequence into fragments, which are, in turn, then separated by gel electrophoresis. The bands are then transferred to a membrane, and Southern blot hybridization is carried out with a labelled probe complementary to part of the 3´end of the IS6110 sequence. As a result, every visualized fragment represents a single copy of IS6110 surrounded by different lengths of flanking DNA sequences (38). The IS6110-RFLP method is highly discriminatory and reproducible, and its profiles are stable over time. However, strains with fewer than six IS6110 insertion sites have a limited degree of polymorphism.

Direct repeat (DR) locus is a chromosomal region that contains between 10 to 50 copies of a 36bp direct repeat separated by spacer DNA with various sequences each of which is 37 to 41bp. Spoligotyping detects spacer sequences interspersed with direct repeats (DRs) in the genomic region uniquely present in members of *M. tuberculosis* complex. Strains differ in terms of the presence or absence of specific spacers, the pattern of spacers in a strains can be used for genotyping. Spoligotyping requires a lot less DNA than IS6110-RFLP and can be expressed in a digital format (39).

Variable number of tandem repeats (VNTR) are novel minisatellite-like structure in the genome composed of 40bp to 100bp repetitive sequences which in *M. tuberculosis* is named "mycobacterial interspersed repetitive units" (MIRU). These are scattered in 41 locations throughout the genome of *M. tuberculosis*. MIRU-VNTR based genotyping interrogates multiple genomic loci, mostly in intergenic regions, which contain variable numbers of DNA tandem repeats. Twelve of these 41 locations show polymorphisms in copy number of non-related *M. tuberculosis* isolates. The principle of this typing system is PCR analysis of 12 variable tandem repeat loci with specific primers complementary to the flanking regions followed by gel electrophoresis. The size (in base pair, bp) of the amplicon reflects the tandem repeat unit and is converted into numerical code to get digital format results in which each digit represents the number of copies at a particular locus. Typing, using these numeric codes, makes the method easier to handle with a large number of strains for comparison. The MIRU-VNTR technique is 100% reproducible, sensitive, and specific for *M. tuberculosis* complex isolates making it a reliable genotyping method. To facilitate its uniform use for global molecular surveillance, a 24-locus format including a discriminatory subset of 15 loci with the highest evolutionary rates, has been internationally standardised in 2006 (40).

TB genotyping helps identify TB patients involved in recent transmissions in combination with epidemiological data. It is a method for monitoring epidemiological trends, and has significant impacts on the control of TB. Genotyping could enable the earlier detection of outbreaks, resulting in more rapid responses and fewer transmissions. For the laboratory, genotyping identifies false-positive cultures that may be the result of mislabeling of specimens, or cross-contamination of cultures, and sometimes can address clinical issues where it could differentiate between treatment failure and re-infection in the same patient. Moreover, in addition to routine molecular epidemiological applications, TB genotyping methods are increasingly also being applied to study evolutionary questions.

Whole genome sequencing and its application in tuberculosis

Advantage is being taken of the new molecular technology of massively parallel sequencing (MPS) or next generation sequencing (NGS). This technology supersedes the "first generation" Sanger sequencing which targets limited number of DNA sequences at a time. The MPS/NGS technology has been hailed as a highly advanced molecular technology capable of sequencing the entire genome of microbial pathogens including Mycobacterium tuberculosis. It provides a lot of information about the genome of the MTBC than is available from the current commercial diagnostic molecular technologies. Whole genome sequencing is now being undertaken on every laboratory confirmed MTBC isolates in the Mycobacterial Reference Laboratories like the Public Health England in order to gather more information from the whole genome of Mycobacterium tuberculosis and not just the mutation hotspots involved in resistance to anti-tuberculous drugs. Recently this tool has been used for genotypic prediction of *M. tuberculosis* susceptibility to first line drugs and correlated with phenotypic susceptibility to those drugs (41). Thus it is becoming an invaluable technology for gathering information about resistance to multiple drugs used to treat tuberculosis (42). WGS technology is also being used for molecular typing of the *M. tuberculosis* strains to help Public Health in contact tracing (43).

Locally, WGS was used to retrospectively study the first and only XDR *M. tuberculosis* strain in New Zealand. Genomic DST determination based on WGS data was compared to the results obtained from pDST and other commercial gDST technologies previously used to characterise this isolate. WGS provides a higher resolution in gDST of the XDR MTBC isolate (44). In another instance, WGS of a New Zealand multidrug-resistant *M. tuberculosis* belonging to lineage 3 was compared to that of a drug-susceptible New Zealand MTBC isolate also belonging to lineage 3. Both isolates have identical genotypic profile using the 24-locus MIRU-VNTR. MIRU-VNTR data suggested that these cases were linked epidemiologically. But WGS data analysis showed these two isolates were not genotypically identical and hence not epidemiologically linked. This showed the limitation of MIRU-VNTR typing for *M. tuberculosis* in some cases of infection with specific lineage (like lineage 3) of *M. tuberculosis*. (45).

In spite of the low burden of tuberculosis in New Zealand, there is still significant active transmission of tuberculosis. This is especially caused by one specific MIRU-VNTR based genotypic cluster of *M. tuberculosis* complex, informally called the Rangipo (based on the region from which the first laboratory confirmed case was isolated) strain belonging to this cluster. Whole genome sequencing (WGS) was used to investigate the acquired functional advantages of the Rangipo strain over others in their ability to transmit and cause tuberculosis in New Zealand. The data obtained from WGS suggest that strain specific virulence factor variations are important for the successful spreading of the Rangipo strain in New Zealand (46).

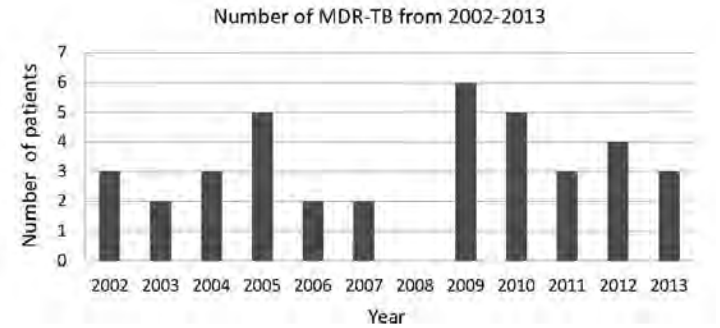

Figure 5. Number of MDR-TB patients in New Zealand confirmed by laboratory from 2002 to 2013. Reference: Basu I, Bower JE, Roberts SA, Henderson G, Aung HL, Cook G, *et al*. Utility of whole genome sequencing for multidrug resistant *Mycobacterium tuberculosis* isolates in a reference TB laboratory in New Zealand. *N Z Med J* 2018; 131 (1487): 15-22. Reprinted with permission of the New Zealand Medical Journal. Copyright © New Zealand Medical Association.

New Zealand has a low-burden of MDR-TB (Figure 5). From 2002 till 2013, there were 38 laboratory confirmed cases of MDR-TB. A retrospective study was undertaken to compare the pDST results of the MDR isolates from 2002 till 2013 with the gDST results obtained from the Cepheid Xpert MTB/RIF and Genotype MTBDR*plus*. The comparison highlighted discrepancies in the results for resistance to RIF and INH between pDST and gDST in 12/38 (31.5%) MDR-TB isolates (47).

Using WGS technology, additional information was obtained on the drug resistant markers, which addressed the discordance between results from the pDST and the gDST results. In 3/12 MDRs, with discordant result, WGS result showed mutations outside the target *rpoB* gene hotspot for RIF resistance, RRDR which went undetected in the Cepheid Xpert MTB/RIF and Genotype MTBDR*plus* tests since these technologies look at RRDR regions only.

In the remaining 9/12 MDRs, mutations were either detected only in the *inhA* gene implicated in low level INH resistance though all 9 MDRs showed high INH resistance by pDST (48) or no mutation was detected in *katG* or *inhA* genes using these commercial platforms. Using WGS, additional mutations were

detected in *katG*, and/or *inhA* genes outside the mutation hotspot or altogether in other putative genes implicated in INH resistance.

These results emphasized the utility of WGS in a reference mycobacterium laboratory in a country like New Zealand with a low burden of MDR-TB to supplement the diagnostic molecular tests and to assist in a rapid but accurate diagnosis and appropriate management of MDR-TB and to maintain a low burden (47).

The current bottleneck in application of this WGS technology in routine diagnostic work is analysing the data. WGS data analysis benefits from bioinformatics skills acquired with specialised training. Lack of such training among clinical scientists and microbiologists pose an impediment for its clinical adoption as the introduction of WGS will be hampered by the complexity of data and its analysis. Most clinical laboratories handling such data have developed in-house pipelines for analysis with in-house bioinformatic support. This, in turn, can hinder standardization among laboratories embracing this technology and accreditation like ISO 15189 essential for clinical diagnostic laboratories performing and interpreting WGS (49). Moreover the huge amount of data generated requires robust and secure information technology (IT) infrastructure for transfer and storage of data. Hopefully, with time and with the development of easy-to-use automated analysis pipelines and databases, scientists in clinical laboratories without bioinformatic training will be able to analyse whole genome sequence data in the not-so-distant future.

AUTHOR INFORMATION

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ORIGINAL ARTICLE

Fasting plasma fatty acids and glycerol in spinal cord injured males

Lynnette M Jones and Michael Legge

ABSTRACT

Fasting plasma free fatty acids and glycerol were analysed from spinal cord injured individuals and able-bodied controls. Both analytes were significantly elevated ($p<0.0005$) when compared with the able-bodied controls. This significant elevation is most likely caused by adipose tissue lipolysis and provides a direct *in-vivo* explanation for the development of insulin resistance and type 2 diabetes-mediated by free fatty acids.

Keywords: fatty acid,glycerol,spinal cord injured,metabolic syndrome.

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INTRODUCTION

Associated with the trauma of a spinal cord injury and subsequent paralysis are the physical changes to the body below the lesion. The loss of motor function inevitably leads to significant muscle wasting and an increase of fat mass above and below the lesion (1,2). This combined loss of muscle mass and an increase in adiposity has been identified as a leading cause for the increased development for cardiovascular disease (3). In addition, the metabolic changes brought about in spinal cord injured (SCI) individuals and their inter-relationship with the development of metabolic disease has become well established, frequently leading to the development of diabetes and metabolic syndrome (4). While there is a strong metabolic evidence for risk factors consistent with metabolic syndrome such as glucose intolerance, hyperinsulinaemia, insulin resistance, dyslipidaemia and onset of obesity (5), the fundamental mechanism causing the metabolic dysfunction remains elusive.

Previously we have reported that by using factor analysis there was a strong association between body fat, glucose tolerance, dyslipidaemia, insulin resistance and fasting post-load carbohydrate variables (4). Recently, we identified that there were significant differences between fasting serum fatty acid profiles for non-injured controls and SCI individuals (6). From these data, we were able to calculate that there were significant changes in the desaturases and elongase activities in the SCI population when compared with the able-bodied controls leading to the conclusion that fatty acid metabolism is compromised in the SCI population.

The aim of the present investigation was to further understand the metabolic mechanisms that lead to poor health outcomes for SCI individuals. To undertake this, we have investigated the relationship of plasma free fatty acids and glycerol in the same groups that we had previously identified the changes in the desaturases and elongases (6).

METHODS

Participants and outcome measures

The present study was completed using plasma from control and SCI individuals from our previous study (6). Data on biophysical information, spinal injury classification, DEXA results and physical activity has been previously published (6,7). Briefly, all participants (n=20 in each group) were males and were matched for age, height, weight, BMI and physical activity, where $p>0.05$ for each parameter. Ethical approval was obtained from the Regional Health Funding authorities, Otago and Canterbury Ethics Committees.

Fatty acid and glycerol analysis

Following an overnight fast, blood samples were collected between 8am and 10am from all participants. A total of 10ml of blood was collected in to an EDTA tube from each participant. Following centrifugation, plasma was pipetted in to Eppendorf tubes and stored at -80^{o}C until analysis. Plasma samples were analysed for total free fatty acids (ROCHE Diagnostics GmbN, Nonnenwald 2, Germany) and glycerol (RANDOX Laboratories Ltd, Ardmore, Ireland) according to the manufacturer's instructions.

Statistical analysis

Means and standard error of the mean were calculated for all measured variables. Independent Student t-tests were conducted to identify difference between groups. To determine any association between free fatty acids (FFA) and glycerol, Pearson's product moment correlations were run for each group and also for combined data. Data were analysed using the Statistical Package for the Social Sciences (SPSS) v22.0 (IBM, Version 22.0, Armonk, NY, USA). Statistical significance was accepted at $p<0.05$.

RESULTS

A significant Shapiro-Wilk test of normality of distributed mean free fatty acids and glycerol data were log-transformed for analysis. We have reported the mean and standard error of the mean for ease of understanding and the confidence intervals for the log-transformed independent t-test. The log-transformed 95% confidence intervals were free fatty acids = 0.75 and 1.03 and for glycerol 0.36 and 0.54 respectively, for SCI and controls.

Both the free fatty acids and the glycerol results were significantly higher in the SCI group (free fatty acids 0.26 ± 0.03mmol.L^{-1} and glycerol 0.30 ± 0.01m.L^{-1} when compared with the controls (free fatty acids 0.03±0.001mmol.L^{-1} and glycerol 0.11± 0.01±0.01mmol.L^{-1} [$p<0.0005$ for both analytes]).

DISCUSSION

Skeletal muscle atrophy and increased intramuscular fat (IMF) below the lesion is a consistent finding following a spinal cord injury. This major metabolic change is believed to be a causative agent in the development of insulin resistance and the genesis of the metabolic syndrome often associated with SCI (1), and is considered to be a significant factor in the failure of skeletal muscle to utilize glucose, thereby initiating significant changes in fat metabolism, including increasing IMF (1,2,8). Previous investigations relating to lipid changes associated with

SCI has linked cholesterol, lipoproteins and triglycerides with the associated SCI pathology (9-11). However, these are not direct metabolic analytes that would be associated with IMF and the underlying basis for the development of metabolic disease in spinal cord injured individuals.

From our previous work we identified a clear dysregulation of fatty acid metabolism in the spinal cord population investigated. In brief, fatty acid profile analysis revealed several significant differences in the composition of individual plasma fatty acids consistent with the predisposition to Type 2 diabetes and metabolic syndrome (6). Using these data, we were able to calculate that the enzymes responsible for fatty acid metabolism, the desaturases and elongase, had significantly higher activity in the SCI individuals compared to the controls. These findings are consistent with the involvement of this group of enzymes in Type 2 diabetes and metabolic syndrome (6,12) leading to us to consider whether plasma free fatty acids and glycerol would be similarly modified in the SCI group, which would be consistent with the development of insulin resistance (13,14).

Although it is well established that there is a transition following injury from lean tissue mass to fat mass, little is known relating to this transition or the biochemical sequalae following these major metabolic changes. Whilst it is established that in the non-spinal cord injured and obese there are regional differences in the major fat deposits (15,16) it would seem that there may well be differing criteria relating to metabolic changes in fat deposits in the SCI. These changes are reflected in the current work. Both plasma free fatty acids and glycerol were significantly elevated in the SCI compared to the able-bodied controls despite being equally matched for BMI and weight (6) providing evidence for a mechanism for the development of free fatty acid mediated insulin resistance (17). The significant elevation of both plasma free fatty acids and glycerol in the present work is consistent with the report by Mahendran (18) whereby both elevated analytes were implicated in the development of insulin resistance resulting from the lipolysis of adipose tissue and the net consequence of insulin inhibition of the anti-lipolytic effect previously described by Jensen *et al* (19). The net result would be an increase in hepatic glucose production and a diminished inhibition of glucose production by insulin with the simultaneous increase in skeletal muscle insulin resistance by the elevated free fatty acids (13,20).

In summary, there is an increase in fat mass and a decrease in lean tissue mass in the SCI (6). Associated with this transition is progressive denervation leading to reduction of capillaries and a progressive loss of mitochondria (21). The consequential loss of the microvasculature and the possible development of dysfunctional mitochondria (22) will have a significant impact on the remaining muscle tissue for the metabolism fatty acids and glycerol. At present, we can only speculate that the significant elevation of both analytes is the lipolysis of the adipose tissue as indicated by the elevation of plasma glycerol (23). However, Romijn and Fliers (24) have proposed that shifting the balance of the autonomic nervous system modulating both the sympathetic and para-sympathetic nervous system, will alter fat and glucose metabolism in adipose tissue. Shifting the balance of this control (such as in SCI) disrupts the metabolic and endocrine responses of adipose tissue will initiate insulin-resistance states, type-2 diabetes and dyslipidaemia.

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ORIGINAL ARTICLE

Determination of the prevalence of chromosomes 13, 18, 21, X and Y aneuploidy in spontaneous miscarriage, in Mazandaran Province, Iran

Kosar Babaee, Abolhassan Rezaee, Seyedeh Elham Norollahi, Faeze Gharibpoor, Ali Akbar Samadani

ABSTRACT

Background: Chromosomal abnormalities are recognized as a major factor contributing to pregnancy loss and accounts for about 50% of all spontaneous miscarriages. Karyotyping of miscarriage samples is the gold standard for aneuploidy screening, however, it is often unsuccessful due to the high tissue culture failure rate. In contrast, quantitative fluorescent PCR (QF-PCR) is an inexpensive and reliable method for aneuploidy screening, however, it is not routine for spontaneous miscarriage aneuploidy screening. The objective of this study was to determine the frequency of 13, 18, 21 X and Y aneuploidy of spontaneous miscarriage in Mazandaran Province, Iran.

Methods: This study was carried on 92 samples taken from patients with one or more spontaneous miscarriages and each sample was tested by QF-PCR. Aneuploidy screenings were performed amplifying four short tandem repeats (STRs) on chromosomes 21, 18, 13, two pseudo-autosomal, one X linked, as well as the AMXY and SRY.

Results: Aneuploidy was detected in 22 samples with trisomy as the most common finding. Trisomy 13 was the most common aneuploidy (5 cases), followed by trisomy 21 (4 cases), 18 and monosomy 18 (4 cases), trisomy X (3 cases) and monosomy 13 (2 cases).

Conclusion: Identifying the genetic etiology of pregnancy loss can provide important information for couples future reproductive plan. In this way, QF-PCR might play a major role in aneuploidy screening and be considered as a valid alternative to the full karyotype.

Key words: quantitative fluorescent PCR, aneuploidy, STR, spontaneous miscarriage, trisomy.

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INTRODUCTION

The incidence of spontaneous miscarriage in confirmed pregnancies is 10-20% (1,2). Recurrent spontaneous miscarriage is one of the most common complications during pregnancy with a prevalence of 0.3% and is defined as the occurrence of two or more continuous fetal losses prior to 20 weeks of gestation (3). Despite the emotional distress and burden imposed by spontaneous miscarriage on couples and health systems, the causal agents of about 50% of recurrent spontaneous miscarriage is still unclear. Some well-known factors include infection, chromosomal abnormalities, and endocrine, immunological and anatomic factors (4-6). The roles of chromosomal abnormalities, including numerical and structural, are well documented in early pregnancy loss. It is estimated that abnormalities in the number of chromosomes is responsible for more than 96% of spontaneous miscarriages related to chromosomal abnormalities. Trisomies, because of nondisjunction meiosis and maternal age, are considered as one of the most frequent chromosomal etiologies related to spontaneous miscarriage. The most common autosomal trisomies are in chromosomes 18, 21 and 13, X and Y (7,8).

Generally, cytogenetic analysis of amniotic fluid, chorionic villus sampling and occasionally fetal blood sampling are used to detect underlying chromosomal abnormalities. Hence various diagnostic techniques such as karyotype analysis, fluorescence in situ hybridization (FISH), quantitative fluorescent PCR (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA) have been developed. Currently, karyotyping, as a highly accurate test, is considered as the gold standard method. However, its applicability in daily practice is limited as this method is cell culture based resulting in a prolonged reporting time of 10-14 days. Considering that faster diagnosis can relieve anxiety in parents, more rapid methods not requiring cell culture have received great attention.(9,10).

QF-PCR is a relatively new, cell culture free and less expensive method. Its principles are based on amplification of short tandem repeat (STR) DNA markers in certain chromosomes and results can be obtained within 48 hours with high diagnostic accuracy (11,12). Considering the rapidity and accuracy given by QF-PCR, we aimed to use this technique to determine the prevalence of common aneuploidy of chromosome 13, 18, 21, X and Y in samples from spontaneous miscarriages in the North of Iran. These results could provide important information for future pregnancy consultations and rule out other spontaneous miscarriage related factors.

METHODS AND MATERIALS

Samples

In this cross-sectional study, 92 female subjects with a history of at least one miscarriage were retrospectively enrolled after informed consent. Maternal data, including maternal and gestational age at the time of the spontaneous miscarriage, number of living children parity, spontaneous miscarriages and related marriages, were collected and confirmed by a gynecologist and genetic specialist. This project was approved by the Ethics Committee, Vice Chancellor Deputy of Azad University of Tonekabon Branch (Code: 930437114).

DNA extraction

Samples were derived from amniotic fluid, chorionic villus sampling and fetal curettage. Placental and fetal tissues were manually dissected free from any remaining maternal tissue and preserved at -4ºC for a maximum of 24 hours before DNA extraction. DNA was extracted from amniotic fluid, chorionic villus samples or fetal curettage samples using a salting out method. Briefly, 500µl lysis buffer was added to samples followed by addition of sodium dodecyl sulfate and proteinase K and incubated at 56ºC for 3 hours. Afterwards, centrifugation at 13,000 RPM/2min was done. Sodium acetate was added to the supernatant (1:1 ratio) and a second centrifugation at 8,000RPM/20min was performed. DNA was extracted by adding 96% ethanol to the supernatant followed by 70% ethanol washing. Concentration and quality of DNA was evaluated by 1% agarose gel electrophoresis and 260/280 nm absorbance ratio on a Pico drop spectrophotometer (UK).

QF-PCR

QF-PCR was performed using the KBC-AneuQuick Kit (KBC, Iran). With this kit 25 STR markers, segmental duplications (SDS) and SRY gene, Chromosomes 13, 18, 21, X and Y can be determined simultaneously in a single reaction. The composition of each PCR tube was as follows: master mix 5µl, primer mix 0.5-1µl, Taq 1 µl, patient DNA 100-200ng and up to 20µl distilled H_2O. Amplification products were analyzed with an ABI 3130XL 5 color genetic analyser. Results were interpreted by comparison with the normal graph.

RESULTS

Characteristic of participants

Overall, we assessed 92 amniotic fluids and fetal tissues for chromosomal anomalies by QF-PCR. The mean age of patients at time of spontaneous miscarriage was 29.6 years (±5.3) and most cases (41.3%) were in the age range of 25-29 years. The mean gestational age at the time of the spontaneous miscarriage was 14.5 weeks (±7.7). Most spontaneous miscarriages (50%) occurred in the second trimester (13-28 weeks; Figure 1). 26% of cases were related couples and the remainder were non-related. Most cases (63%) had no living children at time of spontaneous miscarriage, 28% and 9% had one and two children, respectively. Characteristics of participants and laboratory findings are listed in Table 1.

Table 1. Characteristic of participants. Data are presented as mean (±sd) or number (%).

Variable	
Age (years)	29.6±5.3
Gestational age at spontaneous miscarriage	14.5±7.7 weeks
Relative marriage	Related-couples: 24 (26%) Non-related couples: 68 (74%)
Number of spontaneous miscarriages	At least one miscarriage: 71 (76%) Two or more miscarriages: 21(24%)
Number of living children parity	No children: 58 (63%) One or more children 34 (37%)

QF-PCR analysis

Using QF-PCR, we observed aneuploidy in 22 samples as follows: trisomy 13 (n=5), trisomy 18 (n=4), trisomy 21 (n=4), trisomy X (n=3), monosomy 18 (n=4) and monosomy 13 (n=2) (Figure 2). Analysis of extracted DNA showed satisfactory concentrations and OD260/OD280 ratio values. Results of six random samples is shown in Table 2. Results are interpretable only when at least two markers of each chromosome show two peaks (1:1 ratio) and the rest show a single band. Specific markers were used in the case of trisomy status (3 band 1:1 ratio or 2 bands 2:1 ration) or monosomy (single band). The result of a female X-trisomy case is shown in Figure 3. AMXY region is composed of only one peak and the lack of Y chromosome markers in DYS391 and SRY region, suggesting a female fetus. DXS7132, HPRT, DXS6801 and DXYS267 regions showed a 2:1 ratio, which represents a biallelic trisomy (Figure 4).

Table 2. DNA concentrations and OD ratios of extracted DNA in six random samples.

Sample	DNA concentration (ng/µl)	OD260/OD280
01	340.21	1.9
02	230.2	1.8
03	830	2.0
04	290	2.0
05	410	2.1
06	150	1.9

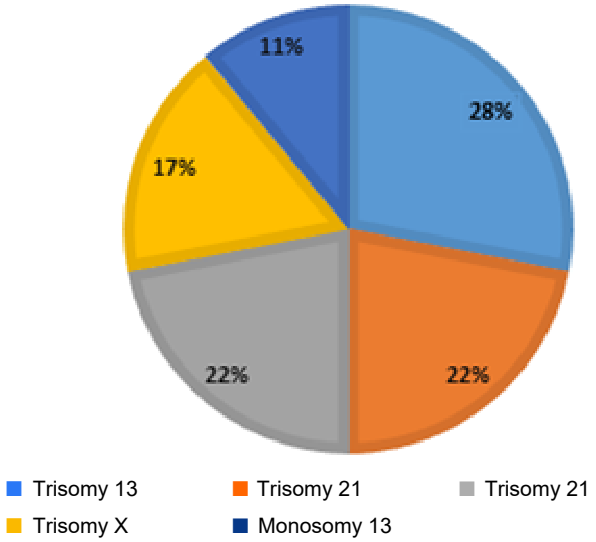

Figure 1. Incidence and types of aneuploidies samples using QF-PCR.

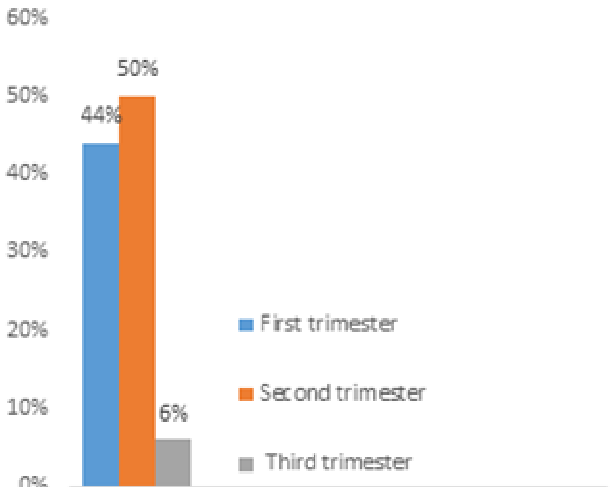

Figure 2. Incidence of spontaneous miscarriage in each trimester

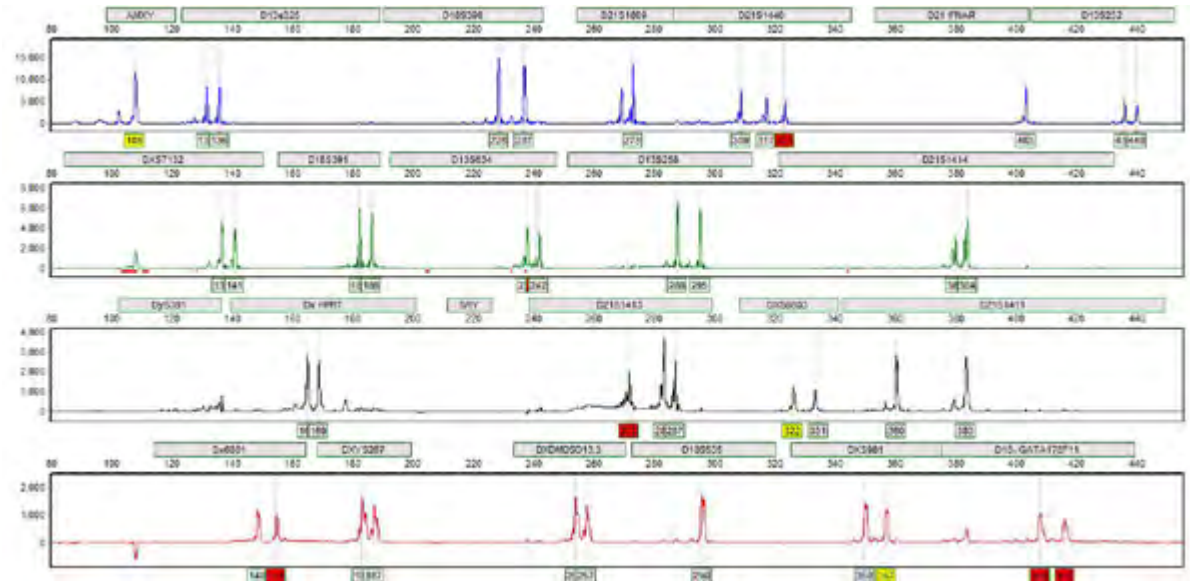

Figure. 3. Electrophoretogram of a female trisomy case, from this graph it can be determined that, D21S809 and D21S1414 sites there are 1: 2 heterozygous (bi-allelic trisomy), D21S1446 and D21S1413 have three peaks with 1: 1: 1 ratio (triallic trisomy), IFNAR region is homozygous. Considering all markers associated to chromosome 21, existence of three copies of it is confirmed.

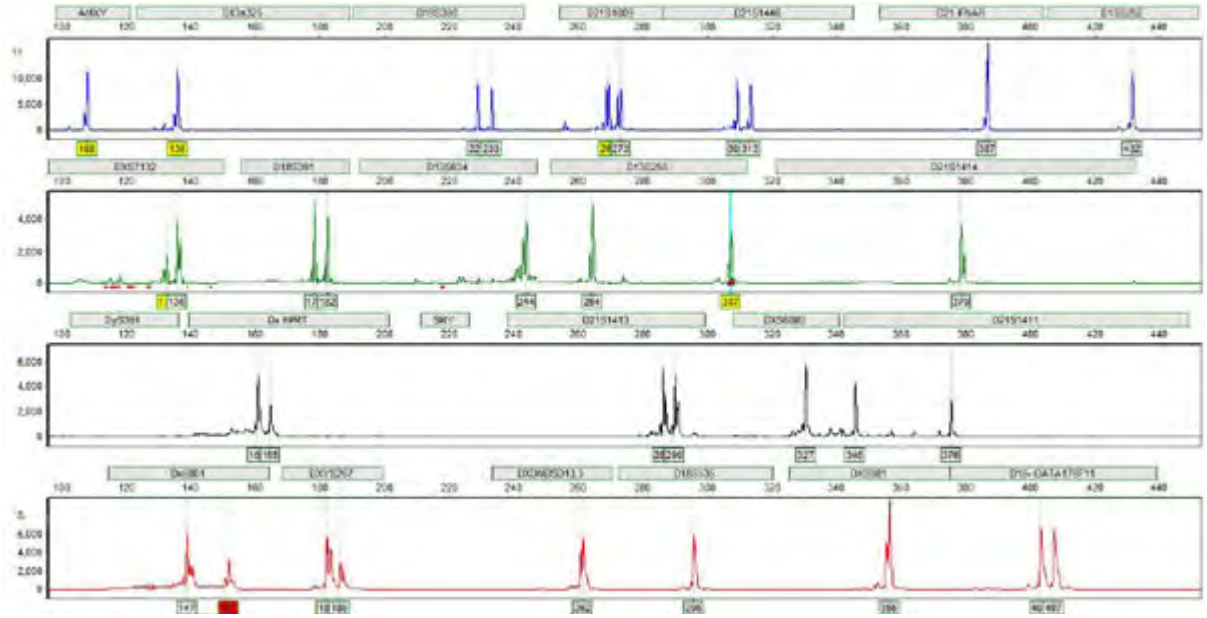

Figure 4. A triple X case, the absence of peaks in the SRY and DYS391 regions and a single peak at AMXY indicates lack of Y chromosome. DXS7132, HPRT, DXS6801 and DXYS267 regions show a ratio of 2: 1, which is indicative of biallelic trisomy.

DISCUSSION

Chromosomal aneuploidy is accounted for in 23 to 61% of spontaneous miscarriages (9). In this study, using QF-PCR, 92 samples were investigated for aneuploidy of chromosomes 13, 18, 21, X and Y. 24% of samples had aneuploidy. Among them trisomy was the most common aneuploidy. This finding is consistent with other studies, introducing trisomy as a major etiology of spontaneous miscarriages caused by chromosomal abnormality (9,13). Accordingly, studies have shown that trisomy of 14,15,16 and 22 are responsible for earlier miscarriages compared to more common aneuploidies, including 13,18,21 and sex chromosomes (9). However, in our study only the latter group of chromosomes were investigated and it was revealed that the majority of cases (50%) had miscarried in the second trimester.

Furthermore, our study identified trisomy 13 as the most frequent aneuploidy. However, in other studies conducted in Iranian population trisomy 21 was more prevalent. Which is not surprising as trisomy 21 is the most frequent chromosomal abnormality in humans (11,14-16). Although QF-PCR is the preferred test for detection trisomy 21, the common invasive sampling methods such as chorionic villus sampling and amniocentesis are associated with a 1% risk of fetal loss (17). Optimising sampling methods and developing less invasive techniques are more attractive choices. Interestingly, Cioni *et al* demonstrated that QF-PCR can identify chromosomal numerical abnormalities while using intrauterine lavage (a less invasive sampling method obtaining trophoblast cells through intrauterine lavage) (18).

Following the introduction of QF-PCR, numerous studies have been conducted to validate this method in identifying chromosomal abnormalities. It's been shown that QF-PCR can detect trisomies, triploidies and maternal cell contamination as accurately as karyotyping (19). The two latter abnormalities cannot be assessed by other rapid methods, including MLPA, BACs-on-beads and array comparative genomic hybridization (aCGH). However, QF-PCR lacks the ability to identify other chromosomal abnormalities (12). Consequently, combined

models of QF-PCR and other rapid tests, including aCGH/QF-PCR and MLPA/QF-PCR have been developed and are shown to have a higher diagnostic ability compared to each test alone and other tests, including karyotyping (12,13).

Although this is the first investigation of aneuploidy in spontaneous miscarriage samples by QF-PCR in Northern, Iran, it has some limitations that should be addressed, the relative low sample size and lack of results confirmation by standard tests are among them. Finally, we suggest the QF-PCR method for determination of aneuploidies in a larger sample size and in other provinces of Iran. Results can be useful for evaluating genetic factors accounting for spontaneous miscarriages.

In conclusion, QF-PCR might play a major role in aneuploidy screening and be considered as a valid alternative to karyotyping. A genetic diagnosis for pregnancy loss can provide important information for future reproductive advice.

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CASE STUDY

Richter Transformation post-ibrutinib treatment of relapsed small lymphocytic lymphoma—a case study

Annabella C Yee, Kern Y Chai and Shingi Chiruka

ABSTRACT

Chronic lymphocytic leukaemia or small lymphocytic lymphoma (CLL/SLL) is a disease of the elderly, characterised by proliferation of monoclonal mature B-cells, predominantly in peripheral blood (CLL) and/or lymph nodes (SLL). It is a heterogeneous disease with a variable clinical course and a proportion of patients eventually develop relapsed or refractory disease after first-line treatment. Ibrutinib is a novel drug that is very effective against CLL compared to conventional chemoimmunotherapy, even in the relapsed setting. Despite this, a rare proportion of patients treated with ibrutinib will still undergo Richter Transformation (RT) to high-grade disease. RT is characterised by transformation of the CLL/SLL cells to large lymphoma cells, most commonly diffuse large B cell lymphoma, usually accompanied by a rapid increase in constitutional symptoms, nodal volume and cytopenias.

We present a case where a patient underwent RT after having stable disease for 4.5 years on ibrutinib, and currently remained stable on salvage rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) therapy. Ibrutinib-resistance in this case is likely driven by an acquired *TP53* and other genetic aberrations and less likely by ibrutinib *per se*.

Keywords: relapsed chronic lymphocytic leukaemia; small lymphocytic lymphoma; diffuse large B-cell lymphoma, Richter Transformation, ibrutinib.

N Z J Med Lab Sci 2019; 73: 54-58

INTRODUCTION

Chronic lymphocytic leukaemia or small lymphocytic lymphoma (CLL/SLL) is the most common leukaemia in the western world and predominantly a disease of older individuals with a median age of 72 years. While CLL/SLL is well-characterised by monoclonal mature B cells proliferation of certain immunophenotypes (1), the prediction of its clinical course can be a challenge because approximately 7% eventually relapse to refractory or relapsed (R/R) CLL/SLL (2). More recently, there is an increase use of monotherapy agents such as ibrutinib for these relapsed cases. Although this is a potent treatment option, a high rate of discontinuation due to various adverse events or progressive disease still occurs in up to 36% of the patients (3). Amongst those who developed resistance to ibrutinib, they can progress to the more aggressive large lymphoproliferative cells known as Richter Transformation (RT). This is generally suspected when a patient with CLL/SLL develops constitutional symptoms, rapidly enlarging lymph nodes and/or hepatosplenomegaly (4). The majority of these transformations are to diffuse large B-cell lymphoma (DLBCL) that are clonally related to the underlying CLL/SLL, with outcomes that are usually more dismal than its clonally unrelated counterpart (3,5). RT is rare and only accounts for about 4% of ibrutinib cessation (3,5). Its median overall survival rate is 2.6 to 3.5 months, compared to more than 17.6 months for those without transformation (3,4). Currently, the role of ibrutinib in RT development seemed controversial due to the scarcity of long-term outcome studies post-ibrutinib therapy (5,6). In this case report, we presented a case of an aggressive RT phenotype, likely driven by an acquired *TP53* mutation, which was absent during the CLL/SLL phase. The accumulation of additional genetic aberrations led to transformation to DLBCL and lost sensitivity to ibrutinib.

CASE REPORT

A 60-year-old female, with no medical history apart from colonic diverticulosis, treated hypertension and dyslipidaemia, presented in January 2013 with a short history of a painless swelling in the right side of her neck. At the time, she had no constitutional symptoms and her full blood count (FBC) and lactate dehydrogenase (LDH) was normal. She underwent a fine needle aspirate of the enlarged node which revealed a clonal population of B cells that expressed typical CLL/SLL immunophenotype. A staging CT scan revealed multiple areas of low volume lymphadenopathy in the neck, axilla, upper abdomen and external iliac chain, with the largest nodes measuring approximately 2 cm. An excision biopsy of the right-sided cervical lymph node revealed CLL/SLL with a low proliferation index of < 5%. A bone marrow biopsy confirmed low-level involvement by flow cytometry, consistent with Binet A disease staging (i.e. low risk and 12-year survival) (7). Given she was asymptomatic with no bulky disease, a “watch-and-wait” approach was adopted.

In October 2014 she developed drenching night sweats, hoarse voice and difficulty swallowing. A restaging CT was undertaken showing mild interval enlargement at all nodal station including Weldeyer’s ring (a ringed arrangement of lymphoid tissue surrounding the pharynx), with the largest nodes still measuring < 3cm in the external iliac regions. Her FBC remained normal and was restaged as Binet B disease (three or more areas of lymphoid tissue enlargement) (7). She was treated with fludarabine, cyclophosphamide and rituximab (FCR) chemotherapy which was poorly tolerated due to multiple admissions with diverticulitis and grade 3 nausea and vomiting. Her treatment was stopped after 4 cycles, by which time she had achieved a partial remission on March 2015 as determined on a CT scan.

In September 2015 she developed progressive disease with worsening lymphadenopathy at all nodal areas as seen on CT scan. A left axillary lymph node biopsy revealed large coalescing proliferation centres, with an increased Ki-67 index of 35%, consistent with accelerated phase SLL. The patient was commenced on ibrutinib monotherapy (420mg once daily) with good control of disease for about 4.5 years. During this time, the patient had recurrent upper respiratory tract infections due to hypogammaglobulinaemia which was successfully treated with intravenous immunoglobulin.

In January 2019 her lymphocyte count started rising with the appearance of blastoid cells on peripheral blood film (Figures1 and 2), and the emergence of new small lymphadenopathy. A restaging CT scan showed widespread lymphadenopathy above and below the diaphragm, with the largest nodes localising to the external iliac chain measuring 64 x 37 mm. RT to DLBCL was histologically confirmed via excision biopsy of an axillary node with a Ki-67 index of 80%, p53 positivity by immunohistochemistry but without MYC, BCL-2 or BCL-6 gene rearrangements by fluorescent hybridisation in situ. Congruously, the Ki-67 index of the clonal population (measured by immunostain) was now 80%, which was markedly higher compared to diagnosis (< 5%) or during the accelerated phase (35%). The patient was started on salvage R-CHOP chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone) with the aim of proceeding to an autologous stem cell transplant (Au-HCT). Her mid-treatment CT scan after 4 cycles of R-CHOP chemotherapy showed a modest reduction in nodal volume, consistent with stable disease. The timeline of her diagnosis is summarised in Figure 3, and immunophenotyping and cytogenetic results are shown in Tables 1, 2 and 3.

Figure 1. Lymphocyte counts over time. Dotted lines: Lower and upper reference interval of 1 and 4x10^9/L. Red arrow: Richter Transformation diagnosis on 15 January 2019

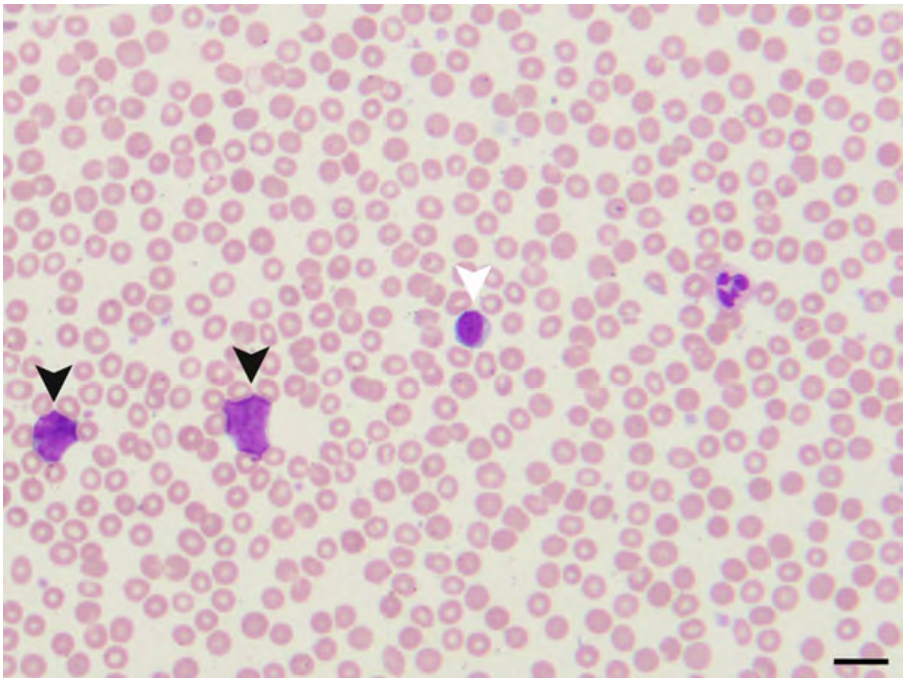

Figure 2. Peripheral blood smear of RT on 7 February 2019. Black arrow: Large pleomorphic cells in RT with prominent nuclei. White arrow: small lymphocytes. A neutrophil is displayed on the right side of the field. Scale bar: 20 µm.

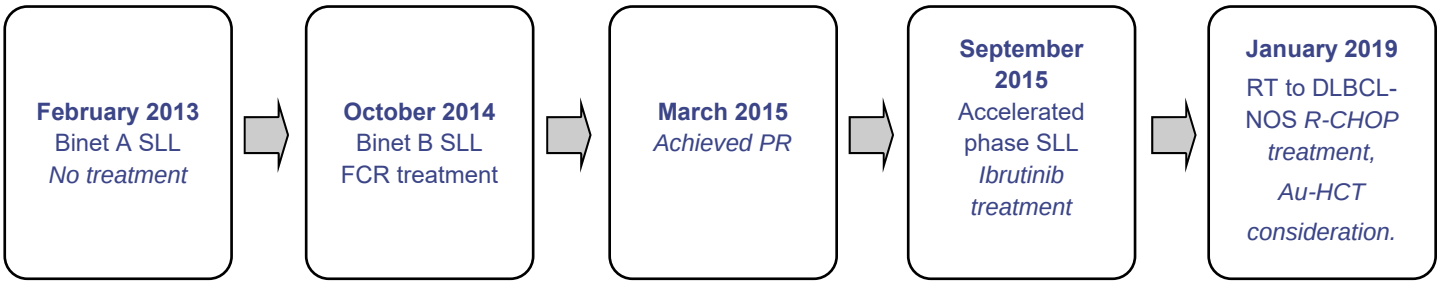

Figure 3. Timeline of diagnosis, progression and treatment. CLL: Chronic lymphocytic leukaemia, SLL: Small lymphocytic lymphoma, PR: Partial remission, RT: Richter transformation, DLBCL-NOS: Diffuse large B-cell lymphoma not otherwise specified, Au-HCT: Autologous haematopoietic stem cell transplant.

Table 1. Flow cytometry results.

Markers	Found in	January 2013* (Initial SLL)	September 2015* (Accelerated phase SLL)	January 2019† (RT)
CD5	B cells	Positive	Positive	Positive
CD23	B cells	Positive	Positive	Positive
CD20	B cells	Positive	Weak positive	Weak positive
CD38	Atypical B cells	Positive	Partial, 64%	Negative
FMC7	Mature B cells	Positive	Negative	Negative
CD10	CALLA, follicular	Negative	Negative	Negative
CD200	Early B cells, CLL antigen	ND	ND	Positive
Kappa	B cells	0%	0%	Negative
Lambda	B cells	90%	88%	Weak positive

*LDH levels were within the normal reference interval. †LDH levels were elevated >1.5x upper limit of normal. CALLA: Common acute lymphoblastic leukaemia antigen. ND: not done.

Table 2. Cell surface markers.

	Cell markers	January 2013 (%)	September 2015 (%)	January 2019 (%)
T cells	CD3	9	11	24
	CD4 (CD3+)	6	8	17
	CD8 (CD3+)	2	2	6
LGL/NK cells	CD56 (CD3-)	ND	ND	5
B cells	CD19	90	88	63

Table 3. Immunohistochemistry and FISH results for prognostic markers.

Markers	January 2013 (Initial SLL)	September 2015 (Accelerated phase SLL)	January 2019 (RT)
Ki67	10-15%	35%	80%
FISH	No 11q (ATM) deletion, no trisomy 12, no 13q deletion, no 17p (p53) deletion	ND	No MYC, BCL-2 or BCL-6 gene arrangements

DISCUSSION

The conventional treatment of R/R CLL rarely leads to durable remissions, so the discovery of targeted therapy, such as ibrutinib, becomes a more common treatment option for those affected. Ibrutinib is generally well tolerated with fewer toxicities compared to conventional retreatment with fludarabine-based chemotherapy for relapsed cases. This is particularly advantageous as CLL/SLL tends to affect an older population, usually with limited medical fitness. This treatment targets Bruton's tyrosine kinase (Tyrosine-protein kinase BTK involved in B-cell development), by inhibiting this enzyme that plays a role in signalling pathways of B-cell receptor. Currently, it is a convenient, first-in-class, once-a-day oral therapy that is indicated in treatment naïve (TN) CLL patients with high-risk del17p and/or *TP53* mutations and R/R CLL patients. Two recent phase 3 multi-centre clinical trials: RESONATE (PCYC-1112) and RESONATE-2 (PCYC-1115), compared the outcomes of TN and R/R CLL treated with ibrutinib and an anti-CD20 agent, ofatumumab. It was discovered that progressive-free survival (PFS) and overall survival (OS) are significantly prolonged when treated with ibrutinib irrespective of baseline cytogenetic risks (3). Similar findings were demonstrated when compared to another anti-CD20 agent, rituximab (8). In the cross-trial study, RT seemed to be a frequent occurrence amongst R/R CLL patients, when compared to a TN setting. This observation suggests that prior lines of therapy are a risk factor for developing RT (3). Hypothetically, this phenomenon may represent clonal evolution of CLL cells under the selective pressure of treatment, and/or the acquisition of genetic mutations due to cytotoxic chemotherapy.

The overall incidence of RT is 1 to 9% in patients with established CLL/SLL and the median time to transformation is 1.9 years after CLL/SLL diagnosis, with a wide range of 0 to 6.8 years (4). Despite the wide range, similar rates of RT were observed amongst R/R CLL treated with novel targeted agents, namely: ibrutinib, idelalisib or venetoclax (9). This finding, combined with the observation that they are typically seen in heavily pre-treated patients, suggests that these agents minimally impacts the rate of transformation, lending further weight to other chemo-immunotherapies being a larger risk factor for RT (6,10).

Conversely, the PFS and OS seemed to be unaffected in patients who received up to two prior lines of therapy. It is noted that there is limited generalisability of this observation as the prior lines of therapy were quite varied. Nevertheless, those who received first- or second-line therapy may use ibrutinib as subsequent therapy relatively safely. For these patients, the median duration of treatment was up to 4.2 years (3). The duration of treatment found is superimposable to this case report, albeit being at the higher end of this range.

Supporting our hypothesis is the study of clonal evolution of FCR-treated CLL/SLL with ibrutinib (11). In this study, the investigators show that ibrutinib favours selection and expansion of ibrutinib-resistant subclones that were already present before treatment initiation. These subclones also present with additional driver mutations such as *TP53* aberrations. The heterogeneity of genetic changes associated with chemotherapy has always been known, owing to this is the presence of low-level persistent disease. The best response achieved by the majority of the patients on ibrutinib seemed to be partial remission (PR) only until discontinuation of treatment (3). This is reflected in the cumulative incidence estimates for RT that increases from 4.5% at 12 months to 6.5% at 18 months of post ibrutinib treatment (12). The increasing incidence seen is in-line with the development of drug resistance with longer-term therapy that is "less aggressive" in nature.

Recent studies have identified acquired BTK^{c481S}, BTK^{T316A} and phospholipase C-γ2 (*PLCG2*) mutations to be molecular drivers of ibrutinib resistance. These mutations affect the binding of ibrutinib to the receptors and subsequently affecting BCR signalling and enabling cellular proliferation. However, much work needs to be done, as it remains unclear whether there is a causal relationship, and how these mutations are related to RT, despite these variants being present in all ibrutinib-resistant cases (13).

Unfortunately, many current studies on ibrutinib have limited follow-up periods and variable stringency of RT diagnosis between centres, hence, no definitive conclusion can be drawn. Furthermore, the risk factors for developing RT remain poorly defined in the literature, although many molecular factors have been described (4). This is largely because transformed cells in RT tend to acquire genetic lesions that are heterogeneous in both number and spectrum (14). The current literature suggests that RT is more likely due to poor risk cytogenetics at CLL/SLL diagnosis, with these patients more likely to receive ibrutinib treatment, rather than attributed to ibrutinib therapy itself (5).

The use of ibrutinib is becoming more prevalent in the treatment of CLL/SLL, therefore, it is expected that there will be more ibrutinib-resistant cases in the future. Thus, more work needs to be done to better characterise the patients who undergo RT post-ibrutinib use. Ultimately, this information could be used to guide salvage strategies for these patients.

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BRIEF COMMUNICATION

Publication outputs from summer student projects at the University of Otago

Charlotte Duen-Yi Wu, William Levack and Rob Siebers

ABSTRACT

Background: The University of Otago provides summer studentships to undergraduate students to undertake a short-term research project in the medical and biomedical sciences.

Objectives: To determine whether these studentships resulted in peer-reviewed publications.

Methods: PubMed, SCOPUS and the University of Otago Publications databases were searched for publications arising from summer studentship projects over a seven year period (2009/2010-2015/2016). Recorded were the publication titles, year of publication and names of journals. Impact factors of journals were sourced from the Web of Science database (2017) and recorded whether the student was the first named author. Results were evaluated by the Mann-Whitney U test with statistical significance set at the p 0.05 level.

Results: Of 1319 students, 465 (35.3%) achieved at least one peer-reviewed publication and 40.4% were listed as 1st author. Publications from Dunedin students were on average in journals with a significantly higher impact factor than students from Christchurch or Wellington ($p<0.0001$).

Conclusions: Summer studentship projects at the University of Otago provides students with research experience and resulted in about one third of them achieving peer reviewed publications.

Keywords: students, research, publication.

N Z J Med Lab Sci 2019; 73: 59-61

INTRODUCTION

The University of Otago offers summer studentships to health and biomedical science undergraduate students, including BMLSc students, in order to undertake a short-term research project during the academic summer break (maximum 10 weeks). These summer student projects are generally complete short scientific research projects in the health and biomedical sciences. Potential benefits arising from these projects are scientific publications, which in addition to contributing to expert knowledge, can benefit the academic supervisors in terms of development of their research portfolio and career development.

To our knowledge, the rate of publications arising out of these summer studentship projects from the University of Otago is unknown. A previous study at the University of Auckland showed that 31.6% of students undertaking a summer studentship in the medical and health sciences achieved at least one publication (2). Previous research has also shown that 40.4% of Bachelor of Medical Sciences with Honours [BMedSc(Hons)] theses at the University of Otago resulted in peer-reviewed publications (3). The aim of our study was to determine the peer-reviewed publication output arising from summer student projects at the University of Otago.

METHODS

Details of summer studentship projects over a seven year period (2009/2010 to 2015/2016) were collected from the three main campuses (Dunedin, Christchurch and Wellington) of the University of Otago's Health Science Division. We recorded the students' names, project titles, date of studentship and supervisors names. We then searched for the students and their supervisors' names on the University of Otago's publication database (https://corpapp.otago.ac.nz/publications/search/input/), and the PubMed and SCOPUS databases for any peer-reviewed publications arising from the project. Publications were verified that they came from the University of Otago.

We recorded the earliest publication related to the summer projects, based on the project title and information in the lay report. We recorded the publication title, year of publication, journal name and determined if the student was the first named author or co-author. The impact factor of journals was sourced from the 2017 Web of Science database (Clarivate Analytics, Philadelphia, United States).

All data were entered into an Excel spreadsheet and the results were presented as proportions. Impact factor data are presented as the median with the interquartile range. Differences between campuses for publication rates and impact factor were evaluated by the Mann-Whitney U test with statistical significance set at the p 0.05 level. Ethical approval was not required as this was a retrospective analysis of data that is publicly accessible.

RESULTS

Across the three campuses, a total of 1319 students undertook summer studentships between 2009/2010 and 2015/2016. Of these 1319 students, 465 students (35.3%) achieved at least one peer-reviewed publication from their summer student project. Overall, 40.0% of the 465 students with peer-reviewed publications were listed as first author. There was no statistically significant difference in publication rates or first authorship rate between the three campuses (Table 1).

Of the 465 publications, 356 were in a journal with an impact factor. Publications from Dunedin students were on average in journals with a significantly higher impact factor than students from Christchurch or Wellington (Table 2).

Table 1. Peer-reviewed publications by summer students.

	All campuses (1319 students)	Dunedin (756 students)	Christchurch (333 students)	Wellington (230 students)
Achieved publication	465 (35.3%)	276 (36.5%)	104 (31.2%)	85 (37.0%)
Publication with impact factor	356	212 (76.8%)	84 (80.8%)	60 (70.6%)
First authorship	186	113 (40.9%)	38 (36.5%)	35 (41.2%)
In New Zealand Medical Journal	37	16 (5.8%)	10 (9.6%)	11 (12.9%)

Table 2. Journal impact factors of summer students publications.

	n	Median	Interquartile	Range
Dunedin	212	3.761*	2.707-4.979	0.855-15.210
Christchurch	84	2.600**	1.785-4.000	0.752-7.422
Wellington	60	2.212	1.492-3.258	0.748-8.855

*p=<0.00001 compared to Christchurch and Wellington.
**p=0.093 compared to Wellington

DISCUSSION

Our study has shown that about a third of summer studentship projects resulted in at least one peer-reviewed publication. It should be noted that there is no requirement for publication in peer reviewed journals for the successful completion of summer student projects at the University of Otago. Rather, scientific and lay reports are required. Of the students with a publication, 40% were named 1st author. Publications from the Dunedin campus were generally in a higher impact factor journal than from Christchurch and Wellington campuses. Thirty-seven (8.0%) of all publications were in the New Zealand Medical Journal.

The reason for the higher impact factor of publications from Dunedin is not known. It may be that those publications were in more specialised journals, such as molecular pathology, that attract higher impact factors than general medical journals. However, we did not determine whether there was a difference between the three campuses in the type of journals in which the reports were published.

A previous study at the University of Auckland also showed a similar publication rate arising from summer studentship as our study (31.6% and 35.3%, respectively). Another study at the University of Otago showed that 40.4% of BMedSc (Hons) theses resulted in at least one peer reviewed publication (3). This rate is very similar to the rate of publications arising from summer student projects at the Universities of Otago and Auckland. This finding was pleasing as the timeframe for summer studentships project is much shorter than for BMedSc (Hons) theses (10 weeks vs one academic year).

We were unable to determine the reasons for the lack of publications from about two thirds of the summer student research projects. This could be due to the short duration of the projects 10 weeks). We noted that a number of projects were for outside organisations that required a confidential report, while other studies appeared to be pilot or feasibility projects, meaning that data from these projects may have primarily aided in the design of larger future studies rather than producing findings worthy of publication in their own right. Ideally to determine the reasons for non-publications, we would have had to requested information from the supervisors. However, this was outside the scope of our study due to time constraint of our study (summer studentship). A number of students who did not achieve peer reviewed publications did, however, achieve authorship in abstracts of scientific conference proceedings.

Considered that some articles may take a number of years to be published, we collected data only up to the 2015/2016 period. It is possible that some projects from the later time period studied were still in the process of being prepared for publication or under consideration.

A small number of projects were supervised by non-academic supervisors and this may have contributed to the publication rate. However, a previous study found that the supervisor's professional background (clinical versus academic) had no impact on the publication rate arising from BMedSc(Hons) theses (3).

It was pleasing to see that over a third of summer studentship projects resulted in at least one peer-reviewed publication and that some publications, especially those from Dunedin, were published in journals with a high impact factor. Evidence from this study is that the University of Otago's summer studentship programme as a whole makes a substantial contribution to scientific knowledge internationally and is a great opportunity for undergraduates to gain research experience and potentially publication. It may also stimulate their future research involvement. Furthermore, it enhances supervisors' research portfolios and career development.

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DISCLOSURE

Rob Siebers is Editor of the New Zealand Journal of Medical Laboratory Science. He had no input in the editorial process or decision. This was handled by the Deputy-Editor, Michael Legge.

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South Pacific Congress Breakfast Session

POSITION PAPER

The future of QMLT qualifications: a position paper

Michael Legge

ABSTRACT

The Qualified Medical Laboratory Technician (QMLT) qualification has been in existence for over 40 years with varying changes in structure and routes of qualifying. Progressively the QMLT has evolved in to a multidisciplinary qualification with currently 12 disciplines that may be examined. Key to the qualification is the development of a two-stage qualification system; a discipline-based examination and the completion of a supervised Practical Assessment (logbook), which must be completed while training in the designated laboratory before the qualification is awarded. In this 'Position Paper" the future of the QMLT qualification is considered and alternatives to the current QMLT are discussed. As this is a position paper it does not reflect any policy in relation to the QMLT, rather it may open the discussions of how pathology laboratories may be staffed in the future.

Key words: QMLT, examinations, graduates, future workforce.

N Z J Med Lab Sci 2019; 73: 62-64

BACKGROUND

In 1969 the NZIMLT (NZIMLS) assumed responsibility for the Qualified Technical Assistant (QTA) and the Qualified Technical Officer (QTO) examinations that had previously been organised by the Auckland School of Technology. For the first examinations a total of 35 candidates sat the examination, which comprised of two, two hour written papers. By 1973 there were 159 candidates taking 11 different disciplines and four QTO examinations. However, over the following years the number of QTA candidates progressively declined and by 1995 there were 62 candidates. The number of candidates for QTO peaked at eight in 1975 and the Institute ceased to offer the QTO examinations after 1980.

The early structure of the QTA examination differed from that provided today. Subjects included Animal Husbandry and Intravenous Solution Preparation and prospective candidates working in narrow discipline fields could apply to train for a "Special Certificate" if a suitable syllabus was prepared by their Charge Technologist and approved by the Examinations Committee. A General Certificate was also available for small laboratories and was across disciplines. By 1992 the NZIMLS was finding it difficult to find people to both review syllabi and act as examiners. A NZIMLS Workshop in 1993 decided that all disciplines should have a ''component' common to all disciplines plus a discipline specific component and that the examination should be a single three-hour paper. This forms the basis of the current QMLT examination currently offered by the NZIMLS.

Disciplines provided in the current QMLT examination

There are 12 discipline-based examinations for QMLT. These are given below:

Biochemistry
Component Processing (Transfusion Science—Blood Products)
Donor
General
Haematology
Histology
Immunology
Microbiology
Mortuary
Phlebotomy
Specimen Services
Transfusion Science

Overview of the current position

The NZIMLS currently offers all the disciplines potentially each year. Prospective eligible candidates must enroll for the QMLT examination by May in the year they intend to undertake the examination. This provides the discipline specific database for the required number of disciplines to be examined and for the examiners to be contacted and contracted. Irrespective of the number of candidates for any discipline each discipline-based examination is new every year based on the Common Syllabus and the discipline-based syllabus. A discipline specific moderator then moderates the draft examinations, when refinements are made. The Examinations Committee then considers all examination papers and any queries are returned to the examiners. The Examination Committee prior to each examination being accepted subsequently checks any refinements. All discipline-based examinations must be consistent with each discipline-based syllabus.

Notwithstanding the examination process, there is a discipline-based Practical Assessment (logbook), which must be completed over two years, and a requirement for work-based 2000 hours in conjunction with the Practical Assessment (logbook). Although the examination can be sat after six months in the laboratory, overall it will take approximately two years to fulfill the requirements for a qualified QMLT.

The total number of candidates (both pass and fail) to sit the QMLT examinations from 2013 to 2018 and the percentage fail rate is shown in Table 1 below.

Table 1. Total number of candidates and fail rates.

Discipline	Number of candidates	% fail rate
Biochemistry	20	0
Donor	30	6.7
General (2018)	2	0
Haematology	16	18.8
Histology	25	8.0
Immunology	3	0
Microbiology	26	3.9
Mortuary	16	6.3
Phlebotomy	430	26.0
Specimen services	193	19.2
Transfusion science	5	20.0
Transfusion science (blood products)	5	40.0

Considerations with the current QMLT examinations

The current QMLT examination system covers a wide range of disciplines for overall relatively small numbers of candidates in any one year (Phlebotomy and Specimen Services excepted). Frequently in the discipline-based examinations there are only one or two candidates in any one year. A secondary consideration relates to the introduction of new discipline-based papers that have either been by-passed by technology or no uptake. The purpose of this Position Paper is to provide context around future discussions relating to the QMLT.

Options for the QMLT in the future

Listed below are some broad options for the QMLT examination process and considers the relationship to the future workforce. Each option is discussed on its own perceived merit and is not ranked in any priority. Other options may be possible and it is not intended to cover endless options or hybridisation of options.

i. Retain the status quo.
ii. Provide a single General examination for selected disciplines.
iii. Merging of certain specialist papers.
iv. Medical Laboratory Science Technician without the QMLT.
v. Who can provide the QMLT qualification?
vi. Future considerations in the pathology workforce.

i. Retain the *status quo.*

This is clearly the simplest option and has worked well over the years. However, a leading issue is that the examination system does not cover the overall costs for providing the examination. This makes the present qualification system vulnerable to external changes, for example changes that may be required by the Medical Sciences Council (MSC) such as provision of the overall qualification system and potential changes to the CPD model already in place. In addition, from year to year there are relatively small numbers of candidates for most of the disciplines. A further consideration is the variability of the technologies being used in laboratories, which may compromise some candidates especially from smaller laboratories.

ii. Provide a single General examination for selected disciplines

Of the 12 disciplines currently available for examination, six have the potential to merge in to a single multidisciplinary examination: Biochemistry, General, Haematology, Histology, Immunology and Microbiology. This could simply be styled on the merger of the Common Syllabus and the new General Syllabus to provide a single discipline of “General Pathology”. In general, this qualification would require a basic knowledge of discipline-based subjects as outlined in the current General Syllabus. For the larger laboratories rotation through the disciplines may be problematic, however, the level of knowledge required can be largely obtained by private study and contact with laboratory staff. The Practical Assessments (logbooks) would be retained, and two possible options may be considered for the use of the Practical Assessments (logbooks). The first is to provide a Practical Assessment (logbook), which covers the general application of the six disciplines, like the current Practical Assessment (logbook) provisions for the General Syllabus and training. The second option would be to use a discipline specific Practical Assessment (logbook). Leading up to and following completion of the examination candidates would be employed in their discipline specific positions and the successful completion of the Practical Assessment (logbook) and the sign-off for competencies would be completed following the required number of hours.

iii. Merging of certain specialist papers.

There are six disciplines that ‘stand-out’ from the above proposal: Donor Services, Mortuary, Phlebotomy, Specimen Services, Transfusion Science and Transfusion Science (Component Processing). Mortuary may still have to be considered as a single discipline due to legal and more detailed anatomy requirements. Could Phlebotomy and Specimen Services be merged into a “Pre-Analytical” discipline based subject and examination but separated by their respective Practical Assessments (logbooks)? Similarly, could the two Transfusion Science disciplines be merged for a common examination but differentiated by the post-examination process of specific Practical Assessments (logbooks) and competency assessment? Donor Services may have to be a ‘stand-alone’ discipline based subject or alternatively could the examination be merged with Phlebotomy and Specimen Services?

iv. Medical Laboratory Science Technician without QMLT?

The present QMLT qualification is finite. There are no further options for advancement from the qualification including using the qualification for entry in to science-based qualifications in either the Polytechnics or the Universities. Would this lack of potential advancement influence recruitment to the future workforce and what might be needed for such workforce (considered in section vi)? Even with the QTO qualification there are no further career options other than an increase in pay. A second question is whether there is sufficient recruitment to ensure a continuing QMLT workforce?

This raises the issue whether the profession as whole should be a graduate-only profession? Here there are two options based on the assumption that there will be an eventual decline in QMLT recruitment to specific disciplines. First, is that the profession is a BMLSc only profession with recruitment into either Technician or Scientist roles but advancement between the two would be seamless i.e. a BMLSc Technician would be able to apply for any vacant scientist positions. The second option would be a dual graduate profession whereby the BMLSc would qualify as scientists (but would have the option of being employed as technicians), and science graduates (BSc, BBioMedSci, etc) who would be employed as Technicians (without the option of becoming a Scientist unless a suitable conversion course was undertaken). Collectively, over time this combination would replace the QMLT qualifications and positions. In effect both options are already in operation but to a smaller scale.

v. Who can provide the QMLT qualification?

This question has a clear answer in that the NZIMLS is the only professional organization with the knowledge and infrastructure to provide the training and necessary professional rigor for the QMLT training and qualification. However, the financial model is weak and provision of the services is not matched by the income from the examinations etc. Should the current financial position of the NZIMLS change there would be two options to raise the fees relating to the true cost of providing the service or to discontinue the qualification process? The latter would ultimately lead to a potential workforce crisis if an alternative strategy were not considered.

In addition, offering the possible option of 12 examinations each year for small numbers of candidates may compromise academic and technical knowledge standards especially if the discipline-based syllabus becomes limited by new technologies. Although the examiners and moderators are paid for their services the examination system is still based on obtaining sufficient volunteers with suitable knowledge to set and mark the examinations, which is not part of their normal work. Both the numbers of candidates and the nature of the qualification process would not be a qualification that system tertiary institutions would consider.

vi. Future considerations in the Pathology workforce.

There will be continuing changes in the pathology workforce as new equipment and technologies are introduced. The use of large multichannel analysers will continue to develop, which decreases individual specialist discipline testing. The question arises whether the NZIMLS should be looking to the increasingly seamless pathology laboratory testing systems and how to 'adjust' qualifications accordingly. Should there be a single qualification for "Blood Sciences" which would encompass Biochemistry, Haematology and Immunology? Cellular Pathology could include both Histology and Mortuary while Microbiology may remain as a 'stand-alone' discipline but with increasing specialist technology. Within the next five years there will be a significant increase in the request and use of genetic services and a related change to diagnostic pathology. Liquid DNA biopsy use will increase and as next generation sequencing prices tumble, these or related technologies will be used increasingly for patient care. Diagnostic molecular pathology in conjunction with digital anatomical pathology will undoubtedly change the way current anatomical pathology is undertaken and whether a medically trained pathologist would be at the centre of these changes? The use of personalised medicine is likely to increase particularly in relation to the use of pharmacogenomics with increased applications in the treatment of cancer. Pre-disposition testing is likely to increase and the worldwide epidemic of obesity, diabetes and diseases associated with ageing will continue to rise for the foreseeable future. Point of care testing will increase as more sophisticated devices become available, which may also include genetic screening tests. Examples of such changes will require significant improvement and better security for Laboratory Information Management Systems (LIMS) and faster communication channels. Pre-analytical requirements will still be needed but perhaps at a more sophisticated level.

How will these changes influence workforce qualifications? For the 'higher level' operations the university-based training system (BMLSc, MSc, PhD) is sufficiently adaptable to provide core training in the necessary knowledge and skills. Advanced qualifications such as the RCPA Faculty of Science Fellowship can also provide for consultant scientists with specialist knowledge and clinical interaction. As diagnostic testing becomes more sophisticated the profession may become a graduate-only profession with the ability to understand the more complex testing systems. There will still be the necessity for core day-to-day working knowledge and whether this is undertaken by the QMLT equivalent or a graduate is an open question. A role for QMLT in the future could be in the supervision of point of care testing and quality control systems, however, the future of blood transfusion may change with development such as 'synthetic blood' and the possibility of growing 'engineered' human blood are on the horizon. It is likely that organ transplantation will still have requirements and bio-banking and stem cell technologies may have high priorities.

CONCLUSION

It is always difficult to both 'see in to the future' and make accurate predictions, however changing trends and rapid growth of technologies are likely to contribute to significant changes as to how diagnostic pathology will function in the not too distant future. The position for the NZIMLS is to make assessments of how best to provide for changing trends especially which qualification would be fit for purpose? The QMLT (in its various forms over the years) has provided a core of laboratory workers capable of undertaking discipline-based laboratory tasks under the supervision of scientists. Is it time however, to review the role of the QMLT and the associated NZIMLS qualifications and start to make 'adjustments' for future pathology services?

Note: the views expressed in this paper are those of the author alone.

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NZIMLS RESEARCH GRANT

Up to $5,000.00 funding available to applicants who:

- Have been a member of the New Zealand Institute of Medical Laboratory Science (Inc) for a minimum period of two years
- Are currently employed in an IANZ accredited Laboratory
- Applicants are encouraged to supply a final written report, to be in the hands of the NZIMLS Journal Editor within 12 months of completion of the project for consideration of publication
- The decision of the NZIMLS Council on the awarding of Grants is final and no correspondence will be entered into
- The Application Form is available from www.nzimls.org.nz and must be fully completed

Doctorate of Clinical Laboratory Science programmes in the United States of America

Lauren N Eddington

Of interest presently within the medical laboratory science field is the burgeoning role of the medical laboratory science practitioner, or clinical laboratory scientist. This is a role that involves more clinical responsibilities for medical laboratory scientists who have further appropriate training. This position is a recognised need, to fill the gap between pathologists, laboratories and clinicians. It has the potential to result in financial savings and improved patient care (1). The exact scope, title, and training required, however, is still being established and differs between countries.

To advance interested scientists towards this position a new qualification is now available at select American universities called the Doctorate of Clinical Laboratory Science (DCLS). This is an advanced practise Doctorate which aims to prepare medical laboratory scientists for a new role as key members of the inter-professional health team (1). Yet there is no board certification exam for graduates of these programmes, and no separate professional certification. It has been stated that graduates of these courses will have the 'unusual position' of proving their worth to an employer on the job (2). Three universities in the United States of America now offer the Doctorate of Clinical Laboratory Science. These courses award an advanced practise Clinical Doctorate and are not classic research based PhDs. As part of this award, less than 30% of the overall credits are related to research. There is no formal dissertation or candidacy examination, however, independent research will be produced throughout which may take the form of a dissertation. The main distinguishing feature is the inclusion of clinical expertise as a component of the qualification (2). Another major point to note is that the DCLS is an advanced generalist course, and graduates will be knowledgeable in a range of laboratory departments as opposed to having one specialty.

Rutgers-School of Health Related Professions in New Jersey was the first university to deliver a Doctor of Clinical Laboratory Science programme. They boast that this new course and new healthcare model brings scientists into a front-line role, with graduates being able to attend patient rounds, intervene in diagnostic management, review laboratory utilisation, and provide community intervention. A one-year, full time, clinical practise/residency is part of the course alongside a research project, however, all pre-residency courses are available online (3). The American Society for Clinical Laboratory Science has suggested a list of appropriate locations for residencies including acute and chronic care facilities, nursing homes, physician offices, health insurance and hospital legal departments (2).

The first DCLS students from the University of Texas Medical Branch (UTMB) will graduate in August 2019. UTMB currently has 35 DCLS students enrolled at their institution. This course should take a minimum of three years to complete and is implemented online, supplemented with mandatory on-campus clinicals. The curriculum includes papers on the laboratory specialties of hematopathology, clinical immunology and transfusion, molecular diagnostics, clinical chemistry/toxicology, microbiology/infectious disease, and also includes papers on laboratory management and biostatistics. This option seems to give graduates a more management focussed training. The four 'clinical' modules that represent the student's clinical experiences, are each four weeks long and held at the UTMB-Galveston campus only currently. Graduates will be awarded a Doctorate of Science in Clinical Laboratory Science degree on completion (4). Both Rutgers and UTMB are still in the process of applying to the National Accrediting Agency for Clinical Laboratory Sciences for accreditation (3,4).

According to an American Society for Clinical Laboratory Science publication in 2016, nine other American universities intended to develop their own DCLS programmes but did not have formal plans in place at that time. Five universities, including Rutgers and Texas Medical. also offer online papers that have been classified as appropriate for students undertaking the DCLS (2).

The University of Kansas Medical Center (KUMC) will begin their first DCLS course in fall 2019 (2). This course is intended to take three years and will cover six laboratory subspecialties in chemistry, immunology, hematology, immunohematology, microbiology and molecular diagnostics. It will also include a research project and a one-year clinical residency (5). As part of the course approval process KUMC assessed the demand of these future course graduates, and many consulted 'agreed that these graduates could not come soon enough' (6). All three universities list the same six programme goals:

- Provide patient-centered, customized consultation services on appropriate test selection and interpretation for the purpose of clinical decision making among the interprofessional health care team and for the patient.
- Monitor laboratory data, test utilization, and diagnostic testing processes in individual patients and populations using informatics and analytics to reduce diagnostic errors, improve efficiency, and reduce costs.
- Conduct research and apply evidence to demonstrate clinical utility of laboratory tests and algorithms and to improve the quality, efficiency, and safety of the overall diagnostic testing process.
- Educate health care providers, patients, their families, and the general public about the indications, best evidence, patient preparation, and interpretation of clinical laboratory testing, including home self-testing.
- Direct laboratory operations to comply with all state and federal laws and regulations, as well as guidelines determined by professional boards of licensure, and certification/accreditation agencies
- Participate in public and private health policy decision making at all organization and government levels using best evidence (3,4,5).

With the graduates of these courses now entering the workforce it will be interesting to see the integration of medical laboratory scientists into the wider health care team. The first nationwide graduate of the DCLS completed the course in May of 2018 from Rutgers University. She currently works using her qualification, rounding with clinical teams and offering consultation on test requests. As the position is so new and does not yet have a specific title, she holds three different job titles including being lab manager for two different specialties.

Regarding this, the programme director of the Rutgers DCLS course, has stated 'As a profession, we need to establish a position title for the DCLS related to their advanced practice responsibilities, one of my thoughts is 'clinical laboratory consultant' (7).

A point of interest in future will be how these graduates and their professional roles compare to those who have undergone alternative further training; specifically comparing these general laboratory specialists, to scientists who are experts in their chosen laboratory department. It remains to be seen whether these graduates necessitate a new job title, what that will be, and if this leads to the development of a new scientist's certification scope in the United States of America.

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Comment on the Doctorate of Clinical Laboratory Science Programme

Michael Legge

The above article correctly captures the intent of the relatively new American qualification. However, from the UK, New Zealand and Australian perspective how does that Doctorate fit within the context of the Clinical Scientist qualifications currently available from the Royal College of Pathologists (RCP) in the UK and the Royal College of Pathologists of Australasia (RCPA)?

In the UK it has been possible for a number of years to apply for training as a Clinical Scientist and gain Fellowship of the RCP. This is a formal training programme and has an extensive specialist training programme. The Clinical Scientist is someone who has undertaken a three-year training programme comprising of a defined discipline-based research project and a structured workplace training programmeme with first year rotating through the disciplines. Successful completion results in a MSc in Clinical Science. The successful graduates are qualified to undertake complex laboratory and clinical roles. They would normally enter the programme with an Honours degree in Biomedical or related sciences and are paid during the training. The next level is the Consultant Clinical Scientist, which is a five-year specialist Doctoral training programmeme in conjunction with RCP, which includes research, leadership and specialist education. Typically, this would be the transition from the Clinical Scientist to an individual fulfilling highly complex roles as clinical science experts, clinical leaders, directors and innovators and would qualify as Fellows of the RCP and usually have completed a PhD during this time. The qualification route is two years to take the Part 1 and an additional three years to take the Part 2 specialist examinations. The specialist examinations are identical to those taken by medically qualified candidates. In the National Health Service, the non-medical FRCPath is on the same salary scale as medical consultants. Recently an alternative route for Clinical Scientists has been introduced via the experiential route, however, it is too early to know how this will develop. All levels of Clinical Scientists are required by law to be registered with the Health and Care Professions Council as Clinical Scientist after first receiving certification from the Association of Clinical Scientists. It is possible to take this qualification from overseas but there is a requirement for some work periods in the UK.

For Australia and New Zealand, about ten years ago the RCPA agreed to create the Faculty of Science whereby non-medical scientists can qualify as Fellows of the Faculty of Science (FFSc). Unlike the UK RCP however, the RCPA has a separation between medical and non-medical so the qualification post-nominal is FFSc(RCPA) unlike the UK which is FRCPath for all graduates. Fundamentally the qualification is similar to the UK qualification. Applicants with an appropriate Bachelor's degree and five year post-graduate workplace training may apply to enter the training discipline based programmeme with the first two years to complete the Part 1 and a further three years to complete the specialist Part 2 examinations. There is a requirement to complete a research dissertation and the expectation that there are publications resulting during the training period.

An Honours BSc would normally be given a one to two years reduction for entry based on relevant experience. A candidate with a PhD in an appropriate discipline and two-years post-PhD experience plus a minimum of six international peer reviewed publications as first author may be given direct entry into Part 1. A research route to Fellowship is available to both non-medical and medical candidates and would require a PhD or MD plus thirty publications in internationally discipline based journals. Reciprocal arrangements for the Fellowship qualification with the UK have not been sought, and the New Zealand Medical Sciences Council has no registration criteria for this occupational group. Australia has no registration requirements for Medical Laboratory or Clinical Scientists. The qualification can be taken within the Pacific-Asia area providing evidence for appropriate training facilities and supervision can be provided.

Taken overall it can be seen that the UK and Australian requirements for Clinical Scientists are much more rigorous than the Doctorate of Clinical Laboratory Science (DCLS).

The DCLS is a three-year degree with direct entry from a Bachelor's degree. It provides a qualification, which falls into the realm of education on the quality of patient care. The DCLS is anticipated to provide an interface between the laboratory and the clinician relating to specimen advice and collection, POCT, introduction of new tests, consultation on test outcomes, laboratory quality control, education and compliance issues, advice on research and is intended to be a "generalist" qualification. The "Fellowships" have extensive supervised specialist training with the expectation of creating experts and consultants within a given field of pathology, thereby creating "specialists".

Michael Legge is a Fellow of the Faculty of Science and a Member of the Faculty of Science Committees and Principle Examiners. He is the Principle Examiner for Basic Pathological Sciences within the Faculty.

South Pacific Congress 2019 - Preliminary Programme

TUESDAY 17 SEPTEMBER					
0730-1800	Registration				
0900-0930	**Opening Ceremony** Presentation of AIMS Fellowship Awards Welcome from AIMS and NZIMLS Presidents				
0930-1030	**Saal-Foley Lecture** **Robyn Wells**				
1030-1100	***Morning Tea & Opening of Industry Exhibition***				
	Concurrent sessions				
	Pre-Analytical	**Microbiology What's New In Multi-Resistance?**	**Transfusion**	**Biochemistry**	**Management**
1100-1130	Pre-Analytical Errors as part of the Medical Laboratory Science teaching program **Dr Ian Cassady**	Gonorrhoea **A/Prof David Whiley**	Five focus points for the ARCBS **Dr James Daly**	Post analytical issues critical values **Robert Flatman**	Measuring competency **Alan Wainwright** *IBMS*
1130-1200	Antigen Vs Antibodies. What Do Doctors Really Want? **Alana Jenkins**	Tuberculosis **Dr Sushil Pandey**	Platelet Reference Laboratory Testing **Gail Pahn**	Informatics EQA **Derek Holzhauser**	Competency Guideline Development In Australia
1200-1230	Problem With Babies: Common Sources Of Pre-Analytical Error In Paediatric Sampling **Dr Donna Rudd**	Enteric Bacteria **Dr Patrick Harris**	Innovations In ADF Transfusion Practice: Frozen Platelets, Freeze-Dried Plasma And Whole Blood. **Prof Michael Reade**	Are There Changing Issues In Post-Analytical Outcomes? **A/Prof Mike Legge**	NZ CPD and The Regulatory System **Jillian Broadbent**

1230-1330	*Lunch, Posters & Industry Exhibition*				
	Concurrent sessions				
1330-1500	**Pre-Analytical**	**Molecular/ Genetics**	**Haematology/ Coagulation**	**Immunology**	**Education/ Training**
1330-1400	Putting Your Best Team Forward. **Angela Coriat**	Molecular Testing In Your Lab **Fleur Francis**	POCT Devices **Andrew Sargeant**	Alarmins And Gut Danger Signals From Diet And Microbiota **Prof Pete Smith**	Simulation Training In Canada **Christine Nielsen**
1400-1430	Electronic Ordering **Ajesh Joseph**	What Is Genomic Testing And What Does It Tell Us? **Ben Lundie**	The Efficacy of Fibrinogen Concentrates **James Winearls**	Immune dysregulation in Inflammatory Bowel Disease **Dr Jakob Begun**	Education Biomedical Scientists For The Future **Marie Culliton**
1430-1500	Case Studies From Specimen Services (CSR) **David Kendall**	Somatic Variantplex Blood Cancer Assay For Screening Of Variants Associated With Myeloid Malignancies **Dr Hnin Aung**	Too Much Of A Good Thing **Dr Matt Harwood**	Coeliac Disease **Dr James Daveson**	Regulation Of Medical Laboratory Science Practitioners In New Zealand, Current Experience And Future Directions **Don Mikkelsen**
1500-1530	**Afternoon Tea & Industry Exhibition**				
1530-1615	New Ovarian Cancer Test **Dr Lucy Shewell**				
1615-1700	The Malaria Vaccine Project **Dr Danielle Stanisic**				
1700-1900	**Industry Gala Function**				

WEDNESDAY 18 SEPTEMBER

0730-1700	Registration
0730-0845	**Meet the Experts Breakfast Sessions** "Certification NZ and Australian style" **Don Mikkelsen** & **Lee Riddout** "Literature Reviews" **Prof Catherine Pickering** "Blood Cell Morphology" **Lyndall Dial**
0900-1000	Why We Need Iron? **Prof Nathan Subramaniam**
1000-1030	**Morning Tea & Industry Exhibition**

	Concurrent sessions				
	Pre-analytical	**Coagulation**	**Transfusion**	**Biochemistry**	**Serology**
1030-1100	Cutting Edge Technology for Phlebotomy **Annette Bissett**	QC **Paul Zerafa**	First trimester screening **Marie Culliton**	Emergency Chemistry Troponin **Dr Gus Koerbin**	Diagnosis of Zika infection **Carmel Taylor**
1100-1130	Flock swabs and the collection of NPA samples **Fleur Francis**	Coagulation troubleshooting **Robert Freeman**	Foetal DNA testing **Helen O'Brien**	Glucose **Greg Ward**	Q Fever: Diagnostic Dilemma **Kristin Muir**
1130-1200	CX Bladder (NZ) PCR for the early detection of bladder Cancer. **Jane Kendall**	Case Studies **Joanne Beggs**	Case studies **Deborah Longmore**	Keeping Safe – Perspectives on laboratory and patient safety over the last half century **Don Mikkelsen**	Q Fever: Case Presentations **Andrew Miles**
1200-1330	**Lunch, Posters & Industry Exhibition**				
1300-1330	**AIMS AGM**				
	Concurrent sessions				
	Pre-analytical	**Education Workshop**	**Molecular**	**Haematology**	**Virology**
1330-1400	Sample collection at an outreach clinic **Katie Edmondson**	AIMS Research engagement scheme **Anne-Marie Christensen & Prof Catherine Pickering**	POC molecular style **Michelle Williamson**	The good, bad and the ugly – lymphoma in the blood **Lyndall Dial**	Respiratory viruses **Prof Kirsten Spann**
1400-1430	Managing the errors and issues of specimens coming from remote areas. **Sam Hornsby**	Literature reviews **Prof Catherine Pickering**	LAMP Testing For Malaria **Craig Williams**	Lymphomas **Dr Kate Hill**	Picornaviruses among us: the new, the old and the threats emerging **A/Prof Ian Mackay**
1430-1500	Testing sexual health samples and case studies **Fleur Francis**			HD immunotherapy and markers **Dr Colm Keane**	Ebola virus
1500-1530	**Afternoon Tea & Industry Exhibition**				
1530-1615	Certification project update **Lee Riddout**				
1615-1700	Talking about my generation **Christine Nielsen**				
1900-2330	**Conference Dinner**				

THURSDAY 19 SEPTEMBER					
0830-1600	Registration				
0930-1030	Melanoma Genetics **Dr Ken Dutton-Register**				
1030-1100	**Morning Tea & Industry Exhibition**				
Concurrent sessions	**Proffered Papers**	**Proffered Papers**	**Proffered Papers**	**Proffered Papers**	**Management**
1100-1130					How To Manage Underperforming and Difficult Staff **Wendy Branthwaite**
1130-1200					
1200-1230					
1230-1315	**Lunch, Posters & Industry Exhibition**				
1315-1400	**Bloodisloe Cup** Is Wine Good Or Bad For You?				
Concurrent Sessions	**Imaging/ Microscopy**	**Biochemistry Data**	**Interesting Microbiology**	**Haematology/ Coagulation**	**Transfusion**
1400-1430	The IMB Microscopy Core Facility **Nicholas Condon**	Cybersecurity **Derek Holzhauser**	Interesting Outbreaks From Non-Sterile Stuff. Is There Something In The Water At Gold Coast **Dr Petra Derrington**	Update on TTP	Transfusion Challenges For Regional Labs **Deborah Longmore**
1430-1500	Understanding Cell Migration And Cancer Invasion: The Microscopy Of Movement. **Samantha Stehbens**	Data Mining Principles **Dr Tony Badrick**	The Gut Microbiome **Dr Donna Rudd**	ADAMTS13 Testing **Joanne Beggs**	Case Studies **Tim Stanton**
1500-1530	TBA **David Sester**	Examples Of Data Mining Applied To Questions That Improved Predictions On Pathology And Health **Brett Lidbury**	Infectious Diseases Following The Floods In Townsville **A/Prof Jeff Warner**	ECMO	Case Studies **Kylie Wylesmith**
1530-1600	**Afternoon Tea & Closing of Industry Exhibition**				
1600-1630	Forensic Cases **A/Inspector Ewen Taylor**, *Senior Sergeant, Forensic Services Group, Qld Police Service*				
1630-1700	Forensic Anthropology **Prof Donna MacGregor**				
1700-1715	**Conference Close**				

The Program is subject to change, at the discretion of the 2019 SPC Organising Committee

AIMS / NZIMLS Member Registration

Early Bird Registration *Closes 31 July 2019*	Standard Registration
$900	$1070
• Attendance to all Congress sessions • Access to exhibition • Catering (lunch, morning and afternoon tea) • Industry Welcome Gala Function Ticket • South Pacific Congress Dinner Ticket • Congress satchel, name badge and lanyard	• Attendance to all Congress sessions • Access to exhibition • Catering (lunch, morning and afternoon tea) • Industry Welcome Gala Function Ticket • South Pacific Congress Dinner Ticket • Congress satchel, name badge and lanyard

Non Member/Corporate Registration

Early Bird Registration *Closes 31 July 2019*	Standard Registration
$1080	$1284
• Attendance to all Congress sessions • Access to exhibition • Catering (lunch, morning and afternoon tea) • Industry Welcome Gala Function Ticket • South Pacific Congress Dinner Ticket • Congress satchel, name badge and lanyard	• Attendance to all Congress sessions and exhibition • Access to exhibition • Catering (lunch, morning and afternoon tea) • Industry Welcome Gala Function Ticket • South Pacific Congress Dinner Ticket • Congress satchel, name badge and lanyard

Retained/Retired/Student Member Registration

Early Bird Registration *Closes 31 July 2019*	Standard Registration
$600	$710
• Attendance to all Congress sessions • Access to exhibition • Catering (lunch, morning and afternoon tea) • Industry Welcome Gala Function Ticket • South Pacific Congress Dinner Ticket • Congress satchel, name badge and lanyard	• Attendance to all Congress sessions and exhibition • Access to exhibition • Catering (lunch, morning and afternoon tea) • Industry Welcome Gala Function Ticket • South Pacific Congress Dinner Ticket • Congress satchel, name badge and lanyard

Pre-Analytical Registration

Early Bird Registration *Closes 31 July 2019*	Standard Registration
$650	$750
• 2 day attendance to Congress sessions on Tuesday and Wednesday only • Access to exhibition • Catering (lunch, morning and afternoon tea) • Congress satchel, name badge and lanyard	• 2 day attendance to Congress sessions on Tuesday and Wednesday only • Access to exhibition • Catering (lunch, morning and afternoon tea) • Congress satchel, name badge and lanyard
Social functions are an additional cost	Social functions are an additional cost

Single Day Registration

Early Bird Registration *Closes 31 July 2019*	Standard Registration
$300	$350
• Attendance to all conference sessions on the nominated day of attendance	• Attendance to all conference sessions on the nominated day of attendance
• Access to exhibition	• Access to exhibition
• Catering for the nominated day of attendance	• Catering for the nominated day of attendance
• Congress satchel, name badge and lanyard	• Congress satchel, name badge and lanyard
Social functions are an additional cost	Social functions are an additional cost

Additional Cost Functions

Industry Welcome Gala Function	South Pacific Congress Dinner	Meet the Expert Breakfast Session
$75	$150	$50

You and Me and CPD

Jillian Broadbent

In the April issue of the NZIMLS Journal we read Terry Taylor's tale on being the President of the NZIMLS. Now it's my turn – what it is really like to be the Co-Ordinator for the NZIMLS's CPD programmes. So, where do I fit into the 'lab' world, and what do I know about professional development for Medical Laboratory Science personnel?

I started training as a scientist in 1972 at Auckland Hospital and have been working in the profession ever since. In those days we spent time working full time in biochemistry, haematology, microbiology, histology, blood bank and blood transfusion centre. This was spread over a three-year period whilst also attending Polytech for lectures and labs both as day release and at night school, we were very much part of the working staff force during that time. I then spent two further years specialising in clinical biochemistry qualifying as a Medical Scientist (Technologist) in 1976. After that I worked at Auckland Hospital and Greenlane Hospital Labs and also lectured on specialist biochemistry topics to students at the Auckland School of Medical Laboratory Technology.

In 1986 I moved into the commercial world and worked for Boehringer Mannheim (now Roche) as their South Island representative. This included training staff on Hitachi analysers, being the application specialist for the region and also promoting gene sequencing and PCR products (after undergoing a workshop/training course at Massey University).

Since 1994 I have been working (part time) in the Steroid and Immunobiochemistry Laboratory at Canterbury Health Laboratories and in 2003 I completed my Fellowship (by treatise) of the NZIMLS. I now sit on the Fellowship committee of the NZIMLS with two other Fellows.

All of this has given me a good basis to recognise what is 'professional development' and what is 'part of your role as a Medical Science Practitioner'.

I guess most of you know me as the 'person' who marks the NZIMLS Journal questionnaire so you can gain 5 valuable CPD points. This takes a fair amount of my time as each issue of the journal has an average of about one thousand submissions. It still frustrates me how many of you do not get 10/10 for these questionnaires – it's usually only around 30 - 40 % of people who get 10/10! Please check the following issue of the Journal to see if you are one of those. Remember the questions are designed to prove you have read the ENTIRE article. I don't set the questions for the questionnaire, that is the role of the Journal Editor, but I do get given the questions and the articles they come from before the Journal goes to print. It is my job to find the answers, and submit those to the Editor to see if we agree, and to pass on any comments regarding the questions and their model answers.

If any of you write questions for the NZIMLS Classroom sessions, it is my job to have these questions moderated and the answers checked, before the questions can go 'live' on the website. Any education sessions, meetings and workshops needing CPD approval come to me, and I assess these for relevant professional development content (often with consultation with the CPD subcommittee or the education provider) and assign an approval code and a points value. I answer all the emails you send me regarding your CPD questions and problems, and forward some of these to personnel within the NZIMLS who may be better able to assist you.

Co-ordination of the annual CPD audit is also part of my role. As well as visiting the external auditor and briefing him on changes and updates needed to perform the audit, I also liaise with him during the audit process. The audit report is then tabulated and sent on to the Medical Sciences Council after ratification at the first available NZIMLS Council meeting. It is also uploaded onto the NZIMLS website.

And, have you ever forgotten or missed adding all your CPD points for the previous year before the cut-off date? If you have, you will know that it is my job to sort those points for you …. and yes, you need to pay for this! Any documentation required for those points needs to be scrutinised and approved by me (as if you were being audited) before I can add those points for you. This ensures that the points I am adding are all genuine claims.

I attend the NZIMLS Council meetings (usually four per year), present them with a CPD report and table any queries or suggestions that have come from you, the members. I am your voice for CPD at Council level. The CPD programme is approved by the Medical Sciences Council (MSC) and needs to be reviewed and updated regularly. Meetings with the MSC are sometimes part of this process.

I attend, chair and often present at various NZIMLS educational meetings held during the year, be it the North Island Seminar, South Island Seminar, Special Interest Group Meetings or the Annual Scientific Meeting.

And on top of all that, I also travel and endeavour to visit as many labs throughout New Zealand as time and purpose allows. If you see me in your lab, come along to one of my question/answer/suggestion sessions there, or ask me anything CPD you like when I'm wandering around your lab.

My hours are not regular, I have my phone and laptop with me most of the time, even when I'm travelling around New Zealand or on holiday. I don't have them with me when I'm on the water paddling in a dragon boat or a waka, but they're not far away. The decisions/answers I give you are not my own, they are part of an approved process and I often need to consult with our CPD sub-committee in order to provide a fair and consistent judgement for you that will fit the legislation.

But always remember that I am here for you and to help you fulfil your CPD quota to meet the requirements for your Annual Practicing Certificate (APC). The degree of commitment to this from all of you is incredible and makes me very proud of the determination of all New Zealand lab staff to be continually learning and improving their knowledge in this chosen profession.

AUTHOR INFORMATION

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OBITUARY

Desmond James Philip
27/11/28—23/2/19

Des Philip was one of those almost literally larger-than-life characters, a man of warmth, wit, and wisdom. The presence of around 200 people at Des' funeral service at St Mark's Anglican Church, Remuera, Auckland - including a significant number of current and ex-laboratory staff (and at least one pathologist) from Auckland and also from further afield - showed the respect and love felt for this giant of our profession.

Des was born and raised in Auckland, and attended Mount Albert Grammar. In his youth and early twenties he played club rugby and tennis, and in later life was no mean opponent at table tennis. In 1952 Des married his "princess" – Phyllis Kelly – and enjoyed 54 years of marriage until Phyllis died in 2006. Between them, they raised Dave, Bruce, Lyn, Judi, and Sue; and then extended the family with the additions of Sally and Aggie.

Des joined the staff of Auckland Hospital laboratory in 1946 (where the technician in charge was Doug Whillans). He moved to Middlemore Hospital laboratory in 1952, where there was a staff of about 4-5; when Des retired in 1988 the staff numbered around 150.

When Des started his career in the laboratory it was glass test tubes and mouth pipetting, monocular microscopes, reusable glass syringes with needles that needed re-sharpening, Sahli haemoglobin measurements, and red cell counts in Neubauer counting chambers. Prothrombin clotting times (now INR) were done using thromboplastin produced by the lab staff from brain tissue from the mortuary. Pregnancy testing was done using live rabbits and later frogs. Staff were rotated around the hospital labs at Auckland, Cornwall Park, National Women's, Green Lane, and Middlemore, usually on a three-monthly basis.

One of Des' characteristics was his sense of humour. He was known to introduce new lab trainees into a urine test – putting his finger over the top of a test tube of urine, inverting it, then licking his finger. Of course, he used his index finger to invert it, and then licked his middle finger.

I also recall Des speaking at a lab conference one year, about the proliferation of laboratory automation. "In fact," said Des, "automation is even getting out into the community. My wife and son and I were in a shopping mall, and outside a chemist's shop was a set of scales, with a sign saying that it spoke your weight. My wife got on; it said '54 kilos – next please.' My son got on; it said '84 kilos – next please.' I got on; it said 'One at a time please.' "

Des always wore a bow tie in the laboratory. There were two possible reasons for this. Prior to taking up wearing bow ties, he wore a conventional tie (as we all did back then!); but Des' ties were highly colourful, prompting remarks about the 'pot at the end of the rainbow.' The other (more likely!) reason – while staining a batch of blood films over the sink, where Leishman stain and buffer were mixed together by gentle blowing, one of Des' conventional ties came loose and was dragged through the batch of staining slides, causing irreparable damage to said tie.

Des also took on the role of Santa at Middlemore at Christmas and went round the children's wards with sweets and his inimitable jollity. And with Des' wisdom there was no need in those early days for an H.R. department. He became laboratory manager at Middlemore around 1970, a position he held for the next 18 years until his retirement.

As well as his lab duties, Des also served the wider profession in a variety of ways. He was first elected onto the Council of the New Zealand Association of Bacteriologists in 1960, the year in which the name changed to the New Zealand Institute of Medical Laboratory Technicians (NZIMLT). Later the word Technician was replaced by Technologist and some years later the name changed again to the New Zealand Institute of Medical Laboratory Science (NZIMLS) to more accurately reflect the wider range of disciplines it represented. Des served 16 years on the NZIMLS Council, seven as Treasurer, six as Vice president and three as President.

In 1975 the NZIMLT Travel Award was instigated to enable an official representative of the NZIMLT to attend international laboratory conferences. Des was the first recipient of this award in 1978, and travelled to the 12th International Congress in Edinburgh, Scotland. Two years later at the 14Th IAMLT Congress in Durban, South Africa he was elected to the International Council. He was elected President in 1988 and served three years in this capacity. During this time he also took on the onerous task of Editor of the IAMLT Journal "Med Tec International".

In addition to his activities with both the NZIMLS and IAMLS Des was also president of the Medical Laboratory Technologists Registration Board in NZ for 13 years (1976—1988) and was a key person in the introduction of TELARC'S accreditation programme for medical testing laboratories. He is the only person to have been invited twice to deliver the prestigious Thomas Pullar Memorial Address at the NZIMLS Annual Conference. He was both a Life member and a Fellow of the NZIMLS. Perhaps the high point of Des' career can be summed up in his own words - "I got the greatest kick after I retired out of seeing medical technology become a university degree course – how many years and hours and meetings did it take to get there?"

As well as his professional life, Des was heavily involved in church activities – his Christian faith was very real, and outgoing. He was involved with several churches over the years, including Church of Christ, Baptist, and finishing his life as an Anglican – as Des and I agreed once when discussing this: 'Different label, same Boss.' At Otahuhu Baptist for example Des was a parishioner, deacon, and organist – Des loved his hymns and other church music, his bass voice could be heard in the congregation at St Mark's, and he had both piano and organ in his home.

After his retirement Des and Phyl bought a section and built a house at Clark's Beach, getting involved of course with church life there too. While there Des modified a Nissan van into the camper van variety so he and Phyl could travel round NZ in it. In the late 1990s family circumstances meant another move, back to Auckland – where Des and Phyl bought another empty section in Blockhouse Bay and built their third (and last!) house on it, where Des lived until his death.

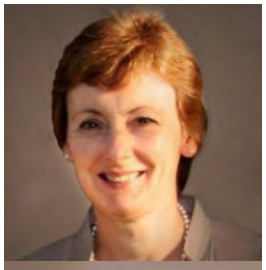
Des didn't fear death. Beyond the peace given to him by his Christian faith, he didn't want to live to an age where he became completely dependent on others for his welfare. Despite his ongoing issues with cellulitis, arthritis and just plain old age, he remained active, still driving himself around. And his mental faculties remained as good as they ever were. Des suffered a cardiac arrest on February 22nd; he died in the early hours of February 23rd with his family around him.

Kua hinga he totara i te wao nui a Tane.
A mighty totara has fallen in the forest of Tane.

I acknowledge with thanks the contributions of David Philip, Judi Sporle (nee Philip), Lynette Anderson (nee Philip), Nancy Thompson (nee Clarke), Marilyn Eales, and John Buchanan, in supplying material for this tribute.

Brian Millar (ex NZIMLS, now Director of Music at St. Mark's Anglican Church, Remuera)

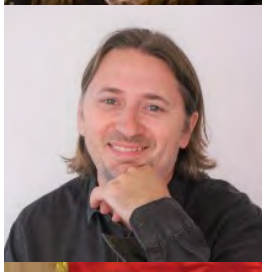
Snapshot of SPC Speakers



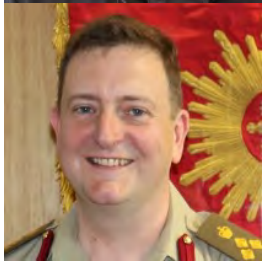
RobynWells
President of the Australian
Institute of Medical Scientists

Don Mikkelsen
Counties Manukau Laboratories

Christine Nielsen
The Canadian Society
for Medical Laboratory Science



David Whiley
University of Queensland
and Pathology Queensland

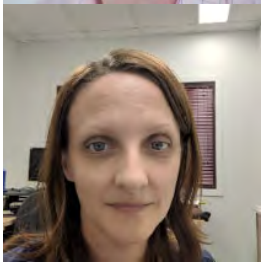


Michael Reade
Australian Army

Alana Jenkins
Queensland Health

Danielle Stanisic
Griffith University

Tony Badrick
The Royal College of
Pathologists of Australasia



Katie Edmondson
Contact Tracing Support

Science Digest

Contributed by Michael Legge

Does the gut microbiome affect mood?

Progressively more is being discovered about the gut microbiome and human health. Historically the beneficial interaction of gut bacteria for human health has been known in relation to the synthesis of vitamin B12 plus biotin, folate and short chain fatty acids. In addition, gut bacteria are important for the extrahepatic circulation, for creating a wide range of metabolites and mediating the metabolism of drugs. In addition, the gut microflora form part of the immune system discouraging infection by exogenous bacteria. Disruption of the gut microflora has implication for both nutrition and the body's defense mechanisms. In addition to these functions there is increasing evidence for other interactions between the gut microflora and regulatory systems in the body. In a recent publication from collaboration between Belgian and Dutch researchers the significance of the gut microflora was identified as linking to mental health (1). Analysis of the fecal microbiome from patients known to have clinical depression (n=1054) were correlated with Quality of Life (QoL) outcomes and mood scores. Analysis of the microbiome identified that *Faecalibacterium* and *Coprococcus* correlated with higher QoL outcomes and *Dialister* and *Coprococcus spp* were depleted in depression. Additionally, the presence of *Butyricoccus* was linked to antidepressant treatment. The researchers used a module-based analytical framework which assembled a range of neuro-active products based on microbial metabolism. From this they identified that there was potential to synthesize 3,4-dihydroxyphenlacetic acid, a precursor of dopamine, which also positively correlated with mental health QoL. In addition, they also identified microbial metabolism capable of producing gamma-aminobutyric acid which correlated with depression. The authors concluded that a symbiotic relationship exists between the gut-brain axis.

Who was "Jack the Ripper"?

Over the space of three months in 1888, five women were brutally murdered in London in what has become known as the case of "Jack the Ripper". Although there were many suspects at the time all five murders remained unresolved despite the police considering five prime suspects. In 1888 DNA had not been discovered and forensic analysis such as finger printing and scene photography were in their infancy. In a recently published paper from the UK two researchers have investigated the use of minimal destructive technologies for sample recovery in conjunction with mitochondrial (mt) and genomic DNA analysis on a shawl, which is the only remaining evidence from a woman murdered by "Jack the Ripper" (2). A police sergeant kept the shawl in 1888 following the murder and it remained in a box and was stored in the Metropolitan Police Crime Museum, London. The two current investigators treated the shawl as a crime scene investigation. The silk shawl was initially imaged under different light sources and various stains were located. The stains were identified as blood, possible imprints from body organs and semen. mtDNA was amplified from the blood and organ stains and greater than 70% of the two sets indicated they were from the same person. Animal bloodstains were excluded from all the bloodstains. The stains identified as presumptive semen were confirmed as male and using SNPs, known to be linked to human skin, hair and eye colour, identifying that the semen came from a male with brown hair and brown eyes. The phenotypic data derived from the semen stain analysis matches the only known eye witness account of the killer and fitted one of the police main suspects at the time, Aaron Kominski, a Polish barber who was known to have serious mental health problems and was frequently placed in institutions by other family members. Although not in the current paper the authors have indicated that there was a genomic DNA match with semen and current living relatives of Aaron Kominski. The authors of the research strongly emphasize that it was never the intention to solve the "Jack the Ripper" murders, rather to demonstrate that a series of molecular technologies could be used on very small sample sizes for both historic and modern murder cases.

A 102 year old *Vibrio cholera* culture provides historical information on disease patterns.

From 1899 to 1923 the sixth global cholera pandemic created significant fear of the disease that may have affected British troops in Egypt (British Expeditionary Force, BEF) during the 1914-1918 war. The causative agent for the pandemic was *Vibrio cholarae* serogroup O, which differs from the causative organism of the seventh pandemic that started in 1961 which was *Vibrio cholerae* serogroup 01 and 0139. In 1931 a comprehensive analysis of medical statistics was undertaken for the British Army relating to World War 1 and despite the high casualty rates the BEF was largely free of cholera. In 1916 a British soldier was convalescing from diarrheal disease and *Vibrio cholerae* strain "Martin 1" (NCTC30) was isolated from him. Despite the infection the soldier did not demonstrate the deadly effects of the disease. Recent research from the UK has re-investigated NCTC30 to determine the relationship of the infection to the lack of disease severity (3). Using freeze-dried samples of the original culture stock genomic and phenotypic analysis was undertaken and the researchers demonstrated that the NCTC30 organism was not related to the pandemic strain and likely to be serogroup 02. They constructed a pangenome using 197 publically available *Vibrio cholerae spp* and three related *Vibrio spp* confirming the NCTC 30 was a *Vibrio cholerae* isolate. The isolate proved to be difficult to culture and both electron microscopy and molecular techniques failed to demonstrate flagella. The NCTC also lacked the CYXf lysogenic bacteriophage that encodes for the cholera toxin and had reduced susceptibility to ampicillin despite pre-dating antibiotics (penicillin was first reported in 1929 by Flemming). The authors conclude that analysis of historic *Vibrio cholerae* increases the understanding of cholera pandemics and demonstrated the presence of antimicrobial resistance genes prior to antibiotic use.

T cell stemness and dysfunction share a common pathway.

When naive stem cells progress to T cells the extracellular environment is critical to supply nutrients by creating the correct microenvironment and initiate metabolic pathways. The preservation of equilibrium in the microenvironment is essential for normal production of T cells. However, despite the immune response to cancers, tumours can persist despite the presence of tumour infiltrating lymphocytes (TIL), which appear to be dysfunctional but express a continuum of transcriptional, epigenetic and metabolic states. However, cellular necrosis is a common feature of many solid tumours and as the necrosis progresses the cellular contents are released in to the extracellular spaces. In particular cellular potassium release may increase extracellular potassium in excess of 40mM.

Recent research has demonstrated that the elevation of extracellular potassium in the T cell microenvironment disrupts the electrochemical gradient environment, which in turn disrupts the ability of stem cells to create T cells (4). The elevated potassium in the microenvironment directly reduces the capacity of T cell metabolism, resulting in a starvation response limiting histone acetylation of genes and aspects of amino acid metabolism subsequently influencing DNA metabolism and dysfunction of CD8+. However, the authors have identified that after immunotherapy TILs with stem-like behavior can moderate tumour function, and that after immunotherapy TILs with stem cell like behavior can mediate tumour destruction. The authors conclude that their work may explain why certain tumours progress and postulate that this type of research could provide new way to boost T cell stemness in cancer immunotherapy.

An unusual case of neonatal hyperbilirubinaemia.

Amongst the many causes of neonatal jaundice, haemolytic disease of the newborn (HDN) can cause significant issues in neonatal care, caused as a consequence of feto-maternal transfusions resulting in maternal antibodies being created against fetal red cell antigens that have entered the maternal circulation. The maternal antibodies can cross the placenta and destroy fetal red cells leading to anaemia and jaundice. The most common cause of HDN is Rhesus D antibodies being created in a Rhesus D negative mother by a Rhesus D positive fetus, and is largely preventable by administering Rhesus D immunoglobulin to the mother. However, other blood group systems including other components of the Rhesus system are capable of causing HDN. In a joint recent case report from India and Iran the authors identified that an infant presented with anaemia, pallor, splenomegaly and a positive direct Coombs test (5). No ABO or Rhesus D incompatibility was identified and the authors investigated the possibility of a minor blood group incompatibility. The mother's blood group was A RhD positive and e and C negative, the father was B RhD positive and e and C positive, and the infant was B RhD positive and e and C positive. The infant was shown to have anti-e and anti-C antibodies resulting in anti-e and anti-C isoimmunisation, and was successfully treated with phototherapy with no further complications on follow up. The authors conclude that minor blood group incompatibility should always be considered in unexplained early neonatal jaundice and may require exchange transfusions as well as phototherapy.

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THE Pacific WAY

Centre based training courses

Training courses are held at the Wellington Centre throughout the academic year. New Zealand based courses are restricted to eight trainees, with both theoretical and practical training covered within the duration of the course. Trainees wishing to be accepted must be currently employed in a medical laboratory and have had at least two years' experience before attending. Courses cover all aspects of medical laboratory science. Funding is provided by NZAID, New Zealand Red Cross, Norman Kirk Trust, WHO, PPTC or through the country's Ministry of Health.

Biochemistry 20th May – 14th June

A biochemistry course was provided by the PPTC in May / June 2019 at its centre in Wellington, and the following seven students attended:

Name	Country	Laboratory
Atevalu Paea Lino	Tonga	Vaiola Hospital
Silvia Da Cruz Babo	Timor-Leste	Stamford Medical, Dili
Eurosia Olije Cham	Timor-Leste	National Health Laboratory
Dalila Dias Da Conceicao	Timor-Leste	National Health Laboratory
Victor Mapesone	Samoa	TTM Hospital
Patricia Bala	PNG	Port Moresby General Hospital
Richard Tolinao	Solomon's	NRH Hospital

Biochemistry course 2019, staff and students

Remaining for 2019

- Laboratory health & safety; and quality management systems
  1 July – 26 July 2019 (4 weeks)
- Haematology and blood cell morphology
  5 August – 13 September 2019 (6 weeks)
  Microbiology
  23 September – 18 October 2019 (4 weeks)
- Blood transfusion science
  4 – 29 November 2019 (4 weeks)

Teaching and training

To maximise the progress made by laboratories participating in the PPTC's Regional External Quality Assessment Programme, the Centre has expanded its commitment to quality by assisting laboratories to establish and progress the implementation of Laboratory Quality Management Systems into their services. The PPTC is committed to working alongside our neighbouring Pacific Island Ministries of Health and laboratories to enable and strengthen their quest in achieving the quality objectives and goals outlined by the World Health Organization.

Implementing quality standards is a major emphasis for PPTC staff when visiting the many under-resourced laboratories of Pacific Island countries. They play a very pro-active role which often involves assistance in the development of documentation, management audit processes and staff competency records. The standard can be used as a baseline against which progress in improving medical laboratory practices and health status can be measured. The PPTC has extensive experience working in laboratory strengthening throughout the Pacific and it is well respected by Pacific Governments for its ability to understand and work within Pacific cultures.

Through the continued support of the New Zealand Overseas Development Programme, the PPTC has been granted a five-year contract (2016 – 2020) in order to deliver its Pacific Laboratory Quality Accreditation Programme. The activity design, on which the five year contract is based, was formulated through a comprehensive analysis of the issues and state of laboratory services in the Pacific. As a result, decisions were made to target investment to four specific countries as being more likely to achieve sustainable transformational development, than spreading services too thinly across the region as was the case in previous years. Four Pacific countries selected for specific investment include Samoa, Solomon Islands, Tonga and Vanuatu. This activity aligns itself with the New Zealand Aid strategic goals to improve the health of the people in these Pacific countries as a key achievement focus area and investment priority.

The following visits towards accreditation and service development to date involving the selected countries supported by the PPTC's 5-year grant funding arrangement with the NZ Ministry of Foreign Affairs and Trade can be listed as follows:

Tonga

- **Filipo Faiga**
  4th – 8th March: Filipo continued accreditation development towards the International Standard ISO15189 with laboratory staff.
- **Navin Karan**
  8th – 12th April: PPTC phlebotomy training in association with BD NZ. Navin was assisted by Suzanne Montocchio, BD's Core Product Specialist, to deliver a phlebotomy workshop to the Vaiola Hospital laboratory for which the PPTC was extremely grateful.
- **Navin Karan**
  13th – 17th May: Navin provided microbiology training in both Nuku'alofa and Vava'u to strengthen the diagnostic processes within each laboratory.

Solomons

- **Russell Cole**
  5th – 14th March: Russell continued accreditation development towards the International Standard ISO15189 with laboratory staff.
- **Filipo Faiga**
  25th – 29th March: Filipo provided biochemistry training to strengthen the diagnostic processes within the Honiara laboratory.

Samoa

- **Filipo Faiga**
  8th – 12th April: Filipo provided biochemistry training to strengthen the diagnostic processes in the laboratory at Apia.
- **Russell Cole**
  13th – 17th May: Russell visited the laboratory in both Apia and Savaii to provide training in microbiology.

Vanuatu

- **Vichet Khieng and Russell Cole**
  8th – 12th April: Vichet and Russell, visited Vanuatu to carry out an audit of the Santo laboratory as well as the installation and operation of the new Haematology staining machine provided by the PPTC to the Port Vila laboratory.

Papua New Guinea

- **Navin Karan**
  17th – 20th June: The NZIMLS generously contributed once again to the PPTC's Regional External Quality Assurance programme. As the principal teaching and training institution in the medical laboratory sciences for the Pacific region, it is extremely important that the PPTC maintains and supports Pacific Island laboratories through its continued physical presence. This grant assisted the PPTC in the continued monitoring and evaluation of the Port Moresby laboratory that is currently a registered participant in our REQA programme. Navin made a first visit to Port Moresby in November 2017 sponsored by the NZIMLS and returned in June 2019 to reassess the laboratory's EQA Programme and diagnostic processes. Navin also carried out an audit covering the quality management essentials the foundational platform of the International Standard ISO15189. PNG is continuing to improve in REQA with the PPTC's on-site support and so a special thank you goes to the NZIMLS for creating this opportunity.

Laboratory managerial staff and pathologists, Port Moresby Hospital

Guam

- **Phil Wakem and Navin Karan**
  20 - 24th May**:** Both Phil and Navin attended the 6th Association of USAPI Laboratories (AUL) and PIHOA (Pacific Island Health Officers Association) LabNet Meeting in Guam, the theme ***"In pursuit of excellence in quality USAPI laboratory services"*** which provided the basis for both Navin and Phil to present ***"Updates on medical lab workforce development, EQA and LQMS in the Pacific".***

Fiji

- **Russell Cole and Navin Karan**
  3rd – 7th June: Russell and Navin were both invited to the PPHSN's (Pacific Public Health Surveillance Network) regional meeting held in Nadi, Fiji, from 3rd to 7th June the theme of which was "***Linking up the initiatives and scaling up the actions***". The conference gave the delegates present the chance to share views, successes and challenges on laboratory matters likely to hinder disease surveillance. During the conference, Russell as chairperson of the LabNet Technical Working Body, was given the opportunity to update on progress made through LabNet as well as present on the PPTC's laboratory service/accreditation development programme currently under implementation.

Can you help?

If any New Zealand medical laboratories have items of diagnostic instrumentation that have been recently upgraded or continue to be stored in the laboratory but are actually surplus to requirements, the PPTC would be most grateful if such items could be donated through its Centre to Pacific Island laboratories where there is an exceptional need. Pacific laboratories have very restricted budgets and often cannot afford to replace troublesome instrumentation that continues to breakdown and which is often discontinued because it is so outdated.

The PPTC would also welcome teaching resources in terms of wall charts, haematology case studies (stained blood films), projector slides, textbooks and journals (within 10 years of publication) etc , for teaching purposes in the Pacific, if you no longer have a use for them. Any contribution is so valuable to us.

Please contact:
Phil Wakem
Chief Executive Officer
Pacific Pathology Training Centre
P.O.Box 7013, Wellington, New Zealand
E-mail: pptc @ pptc.org.nz or phil @ pptc.org.nz
Tel: 64-4-389 6294 or 027 2305483

Notice of Annual General Meeting 2019

Notice is hereby given of the 75th Annual General Meeting
of the
New Zealand Institute of Medical Laboratory Science (Inc)

to be held at the

Waipuna Hotel and Conference Centre
Auckland
on 17th August 2019

This is a breakfast meeting with breakfast commencing at
7:00am sharp, for a 7:30am meeting start

For catering purposes, you must register for this meeting
(via the North Island Seminar Registration page) on
www.nzimls.org.nz

The AGM registration is located under “Functions”.
(You do not need to register for the North Island Seminar
if you are only attending the AGM)

NZIMLS The New Zealand Institute of Medical Laboratory Science (Inc)

NZIMLS
THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE (INC)

NZIMLS NORTH ISLAND SEMINAR 2019

Saturday 17th August 2019

Waipuna Hotel and Conference Centre

Auckland

Presentation offers:
by June 21, 2019

Contact:
Linda Keat
linda.keat@middlemore.co.nz
Phone: 09-276-0153
Mobile: 0274579918

Registration available at:
www.nzimls.org.nz

Registration:
8:15am to 9:00am
(Tea and coffee available)

Seminar:
9:00am to 5:00pm

Followed by drinks and nibbles until **6pm**

Biochemistry SIG 2019

Saturday 5th October 9am – 5pm

Waipuna Conference Centre, Auckland

Register online at www.nzimls.org.nz

Contact: Lisa Aspin lisa.aspin@labtests.co.nz

NZIMLS
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NZIMLS
Presents the

Anatomical Pathology Special Interest Group Seminar

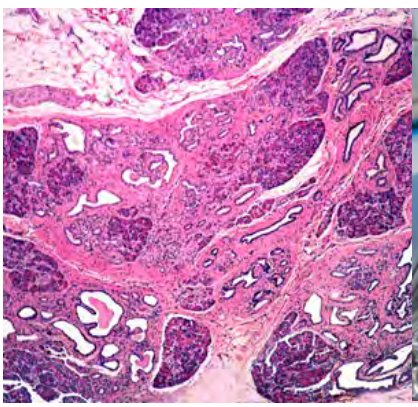
Novotel Auckland Airport Hotel

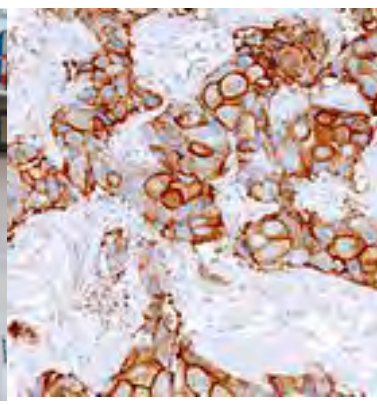
Saturday 5 October 2019

Keen to present a paper? Contact:

Natasha Caldwell
natasha.caldwell@middlemore.co.nz

Registration available at www.nzimls.org.nz





NZIMLS

Presents the

Microbiology

Special Interest Group Seminar 2019

Clinical Education Centre
LabPLUS, Auckland City Hospital
Auckland

Friday 18th October 1-5pm
EUCAST Workshop
ATU, the new "I", difficult bugs, CPE detection

Saturday 19th October 9am-5pm
Special Interest Group Seminar

"You do not know what you will find, you may set out to find one thing and end up by discovering something entirely different."
— Sir Alexander Fleming

On track for Microbiology
Join us in the City of Sails
Presentations welcome
Contact: Tracy Camp
Tcamp@adhb.govt.nz

Registrations open now at: www.nzimls.org.nz

EUCAST WORKSHOP

To be held in conjunction with the
Microbiology Special Interest Group

Friday 18 October 1:00—5:00 pm

Clinical Education Centre, Level 5, Main Building
Auckland City Hospital

Registration available at www.nzimls.org.nz
(via MicroSIG Seminar)

Convenor: Tracy Camp
tcamp@adhb.govt.nz

Antibiograms

Tricky drugs including
fosfomycin, colistin, nitrofurantoin

Dosing

The new "Y"

Distinguishing CPE from CRE, including Pseudomonas and Acinetobacter

ATU and the tricky Haemophilus

Plus more!!

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THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE (INC)

Haematology SIG Seminar 2019

Napier conference centre

Saturday 19th October 2019

Come a get a drop of Red

O+, AB-, cabernet sauvignon, merlot or syrah. The choice is yours.

Who could imagine a better weekend? Come and expand your knowledge and your palate at the same time in the warm sunny surroundings of Hawkes Bay.

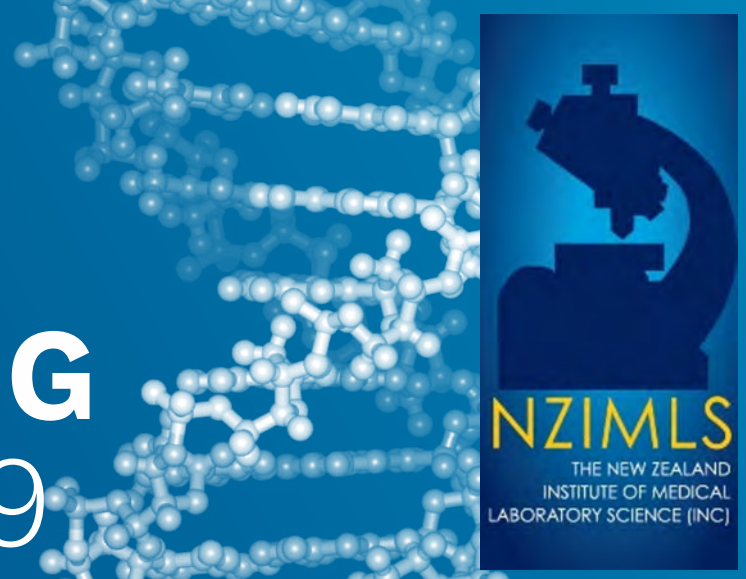
Presenters welcome.

Register online at www.nzimls.org.nz

Contact: Chris Greenwood

chris.greenwood@hawkesbaydhb.govt.nz

Molecular Diagnosis SIG Meeting 2019



Friday 25 October, 2019

The NZIMLS Special Interest Group in Molecular Diagnostics

The University of Auckland
Faculty of Medical & Health Sciences
85 Park Road, Grafton, Auckland

This year's SIG will have a focus on research and the future of molecular diagnostics.

We will also provide an opportunity to visit the LabPLUS & Grafton Clinical Genomic Molecular Laboratories, and see Molecular Diagnostics in action!

Presentations (oral and poster) are invited for the following disciplines:

- Translational Diagnostic Research
- Molecular Genetics
- Biochemical Genetics
- Molecular Virology
- Molecular Microbiology
- Molecular Haematology Cytogenetics

Closing Date for Abstracts:

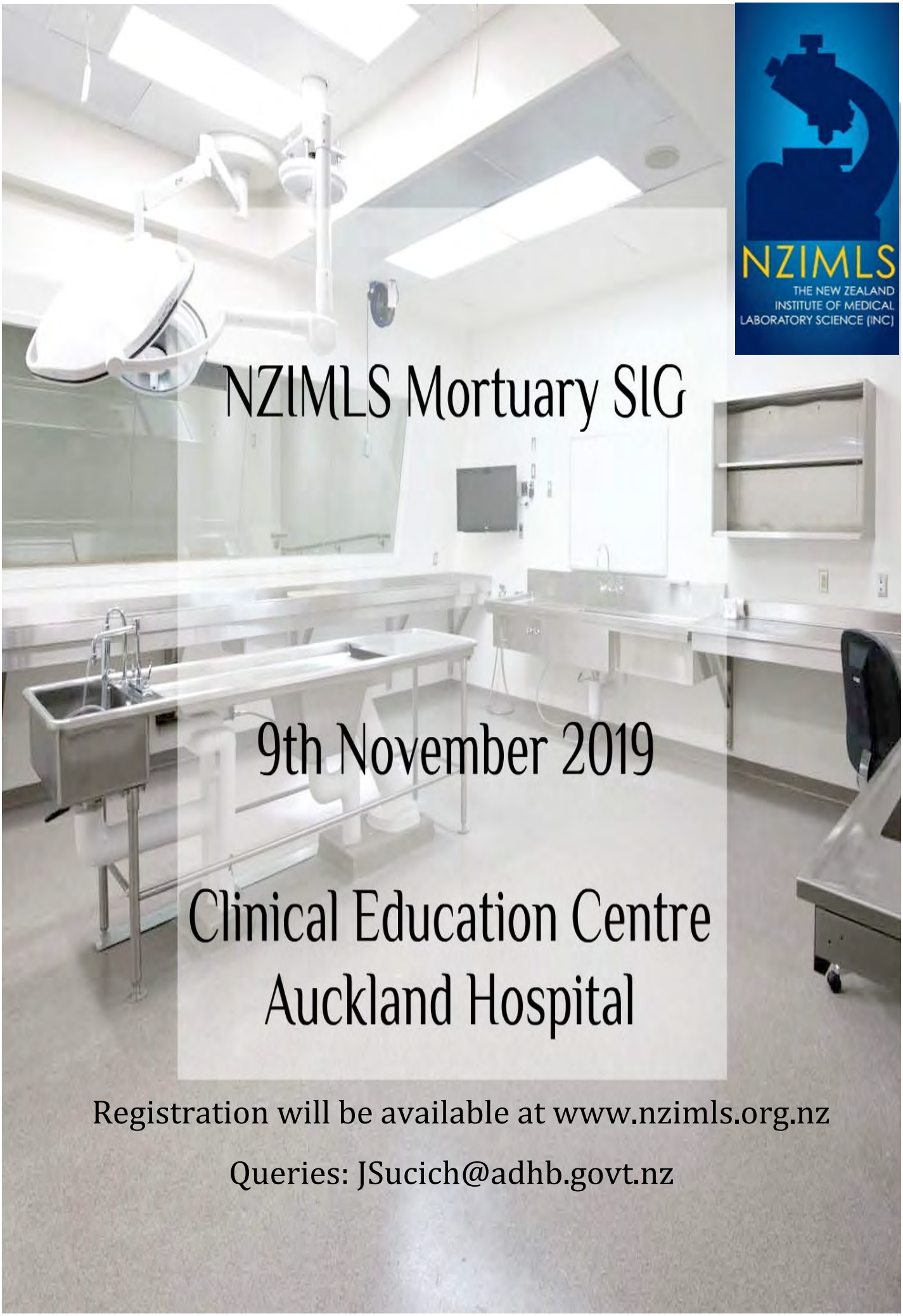
Friday 30th August 2019

More information:

Phillip Shepherd University of Auckland
p.shepherd@auckland.ac.nz

Or; **Roberto Mazzaschi** LabPlus Mol. Genetics
RobertoM@adhb.govt.nz

Register online
www.nzimls.org.nz



NZIMLS
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NZIMLS Mortuary SIG

9th November 2019

Clinical Education Centre
Auckland Hospital

Registration will be available at www.nzimls.org.nz

Queries: JSucich@adhb.govt.nz

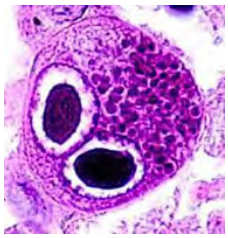
"The Weird & Wonderful"

2019 NZIMLS IMMUNOLOGY SIG SEMINAR

November 9th Commodore Hotel Christchurch

Believe it or Not!

Showcasing the weird and wonderful worlds of Immunology, Serology, Virology & Molecular



Strange but true … Book your adventure …

Calling for unusual case studies & presentations of the extreme, NOW…

Contact: Donna.Mitchell@cdhb.health.nz 03 3640355

Contact: Rodger.Linton@cdhb.health.nz 03 3641229

Registration will be available at www.nzimls.org.nz

Journal Questionnaire

Below are ten questions based on articles and the Science Digest column from the August 2019 issue. Read the articles carefully as most questions require more than one answer. Answers are to be submitted through the NZIMLS website. Make sure you supply your correct email address and membership number. It is recommended that you write your answers in a word document and then cut and paste your answers on the web site.

You are reminded that to claim valid CPD points for successfully completing the journal questionnaire you must submit an individual entry. It must not be part of a consultative or group process. In addition, members who have successfully completed the journal questionnaire cannot then claim additional CPD points for reading the articles from which the questions were derived.

The site will remain open until Friday 18th October 2019. You must get a minimum of eight questions right to obtain five CPD points. The Editor sets the questions but the CPD Co-ordinator, Jillian Broadbent, marks the answers. Direct any queries to her at cpd@nzimls.org.nz.

AUGUST 2019 JOURNAL QUESTIONNAIRE

1. What is the prescribed drug regime during the first two months for a *M. tuberculosis* isolate fully susceptible to first line drugs?
2. What does the WHO define as extensively drug-resistant tuberculosis?
3. What is the algorithm implemented at LabPlus if there is a high clinical suspicion of tuberculosis and why?
4. What are the three most commonly used tuberculosis genotypic methods?
5. What strong metabolic indicators are risk factors consistent with metabolic syndrome?
6. What could be the net result of increased plasma free fatty acids and glycerol in spinal cord injured males?
7. What are some of the well-known factors for recurrent spontaneous miscarriage?
8. The most common autosomal trisomies are in which chromosomes?
9. Which techniques are generally used to detect underlying chromosomal abnormalities in spontaneous miscarriage?
10. Ibrutinib treatment of relapsed cases of chronic lymphatic leukaemia or small lymphatic lymphoma targets which enzyme, by which mechanism, and what role does this enzyme have?

APRIL 2019 JOURNAL QUESTIONS AND ANSWERS

Questions

1. Acute promyelocytic leukemia presentation is characterised by which features?
2. How is the differential diagnosis of acute promyelocytic leukemia made?
3. Which are the most common aberrant antigens expressed in acute promyelocytic leukemia?
4. What is the expected pattern in acute promyelocytic leukemia for the selected 2nd line monoclonal antibodies?
5. What are the typical clinical symptoms of diabetes mellitus?
6. What is the World Health Organisation's for diagnosing pre-diabetes and diabetes mellitus?
7. The Alvarado score for the diagnostic criteria for acute appendicitis is based on what?
8. Patients with the severe infantile onset phenotype of very long chain acyl-coenzyme A dehydrogenase deficiency often develop which conditions?
9. What is the gold standard for diagnosing very long chain acyl-coenzyme A dehydrogenase deficiency?
10. What has become the most accurate maternal serum marken for the three most common trisomies? Name these three trisomies.

Answers

1. Morphological, immunophenotypic, molecular and coagulation.
2. Demonstration of the presence of the PML-RARA fusion gene and the classic t(15:17) (q24:q21) translocation.
3. CD2 and CD56.
4. CD9 positive and CD18 and CD11c negative.
5. Hyperglycemia, polydipsia, polyphagia, polyuria, blurred vision, weight loss, generalised pruritis.
6. Fasting plasma glucose 6.1 – 6.9 mmol/L and ≥ 7.0 mmol/L respectively'
7. Clinical manifestations, C-reactive protein, white blood cell count and leucocytosis.
8. Cardiomyopathy, arrythmia, hypoglycaemia, pericardial effusion, hepatomegaly, and hypotonia.
9. Acylcartinine profiling via LCMSMS with mutation analysis of the ACADVL gene.
10. Free fetal DNA. Trisomy 21, trisomy 18 and trisomy 13.

2019 NZIMLS CALENDAR

Dates may be subject to change

DATE	*COUNCIL*	*CONTACT*
15-16 August	Council Meeting, Auckland	fran@nzimls.org.nz
November/December	Council Meeting, Rangiora	fran@nzimls.org.nz
DATE	*SEMINARS*	*CONTACT*
17 August	North Island Seminar, Waipuna Hotel & Conference Centre	lkeat@middlemore.co.nz
05 October	Anatomical Pathology SIG, Novotel Airport Hotel, Auckland	tanyaf@adhb.govt.nz
18-19 October	Microbiology SIG Seminar, CEC, LabPlus, Auckland City Hospital	tbathgate@adhb.govt.nz
19 October	Haematology SIG Seminar, Napier Conference Centre	Chris.greenwood@hawkesbaydhb.govt.nz
09 November	Mortuary SIG, Henley Room, LabPlus, Auckland City Hospital	jsucich@adhb.govt.nz
09 November	Immunology SIG, Commodore Hotel, Christchurch	rodger.linton@gmail.com

NZIMLS ANNUAL GENERAL MEETING

The Annual General Meeting of the NZIMLS for 2019 will be held in conjunction with the North Island Seminar, Saturday 17 August, Waipuna Hotel & Conference Centre. Commencing 7:00am with breakfast for a 7:30am meeting start.

DATE	*CONFERENCE*	*CONTACT*
17-19 September	South Pacific Congress, Gold Coast Convention Centre, Gold Coast, Australia	fran@nzimls.org.nz
DATE	*MEMBERSHIP INFORMATION*	*CONTACT*
January	Membership and CPD enrolment due for renewal	sharon@nzimls.org.nz
31 January	CPD points for 2019 to be entered before 31 January	cpd@nzimls.org.nz
15 February	Material for the April issue of the Journal must be with the Editor	rob.siebers@otago.ac.nz
15 June	Material for the August Journal must be with the Editor	rob.siebers@otago.ac.nz
18 June	Nomination forms for election of Officers and Remits to be with the Membership (60 days prior to AGM)	fran@nzimls.org.nz
8 July	Nominations close for election of officers (40 days prior to AGM)	fran@nzimls.org.nz
26 July	Ballot papers to be with the membership (21 days prior to AGM)	fran@nzimls.org.nz
01 August	Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM)	sharon@nzimls.org.nz
09 August	Ballot papers and proxies to be with the Executive Officer (7 days prior to AGM)	fran@nzimls.org.nz
15 September	Material for the November Journal must be with the Editor	rob.siebers@otago.ac.nz
DATE	*NZIMLS EXAMINATIONS*	*CONTACT*
02 November	QMLT Examinations	fran@nzimls.org.nz

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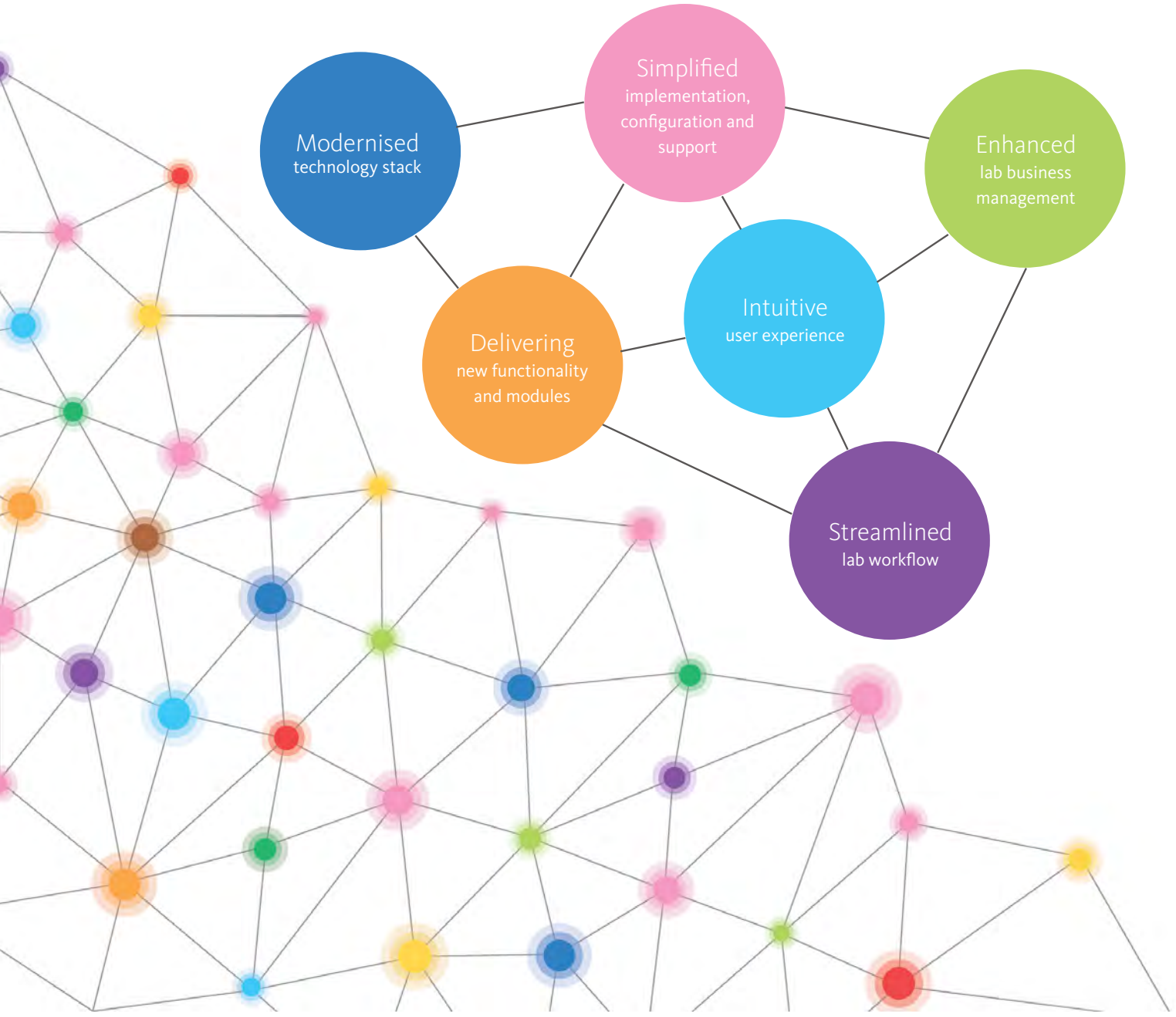
Delphic V10

Our continued focus at Sysmex is to provide expert solutions for patient data and diagnostic workflow, shaping the advancement of healthcare.

Version 10 of the Delphic Laboratory Information System is now ready for your lab to explore.

Look out for more details about our upcoming roadshows where we will be sharing the many new features and benefits of version 10 with our valued Delphic customers.



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www.sysmex.co.nz