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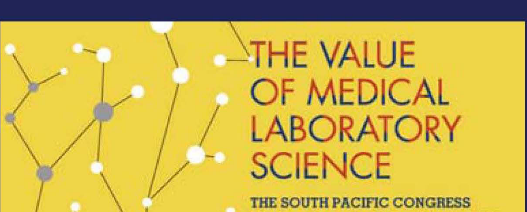

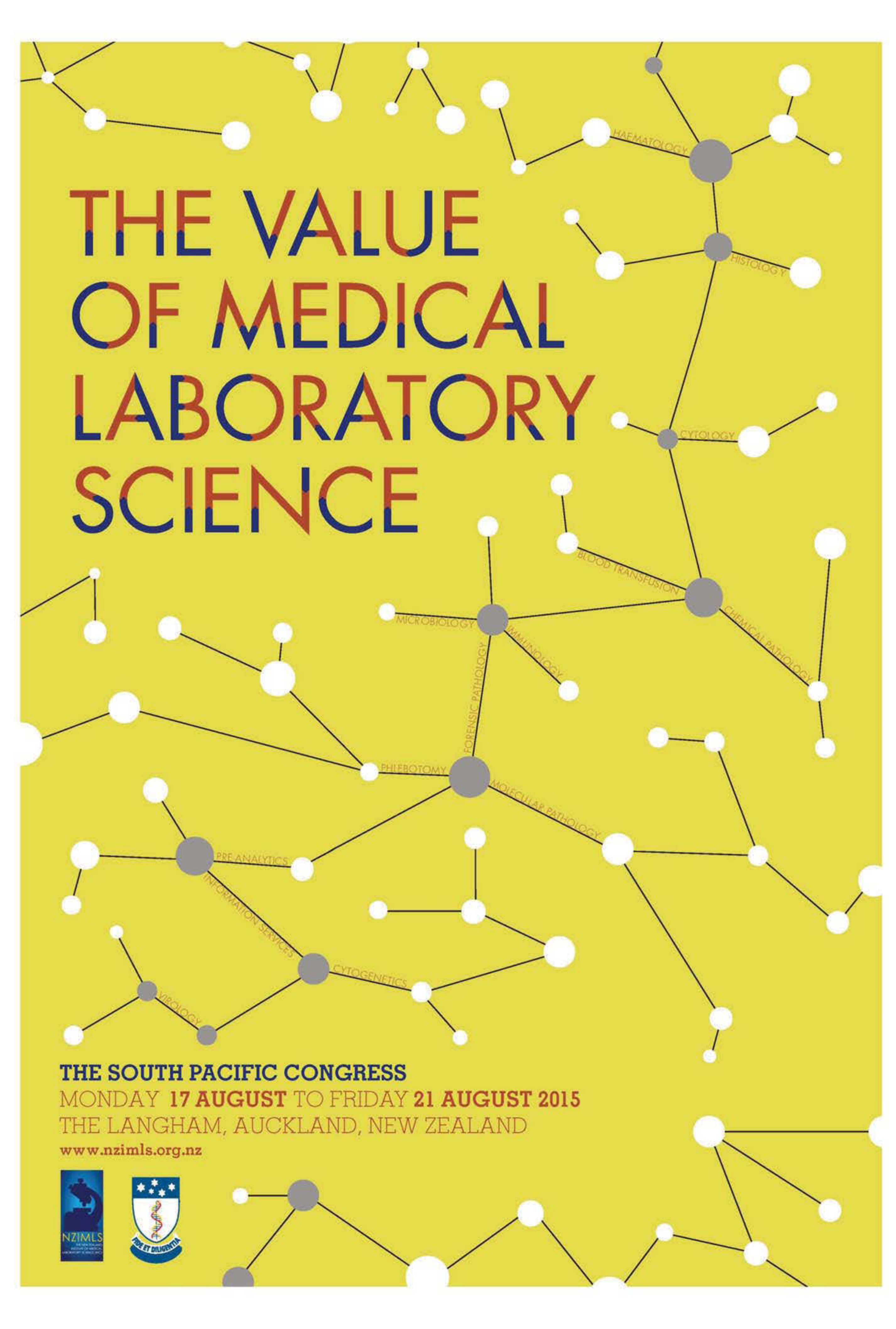
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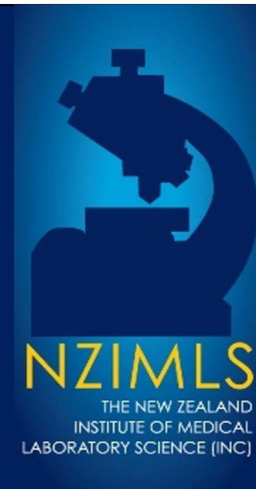

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In this issue

Rob Siebers, Editor

Giuseppe Lippi and colleagues from Italy and Australia in a review article provide a succinct update of the state-of-the-art of pre-analytical variability according to personal practice and experience, and then suggest some reliable strategies for improving awareness of the value chain of in-vitro diagnostics beyond the laboratory and into the broader system of health care. Professor Lippi will be a guest speaker at the upcoming South Pacific Congress of Medical Laboratory Science in Auckland this coming August.

Mona Scousboe and colleagues from Christchurch studied the contamination of the Waimakariri River in Canterbury and one of its contributing rivers by sampling water from the respective rivers at multiple points in 2004 and 2012, measuring the *Escherichia coli* count and testing *E. coli* isolates for antibiotic resistance. Their study demonstrated that *E. coli* contamination of the river has increased considerably in 2012 compared to 2004, both up and down river. They conclude that the Waimakariri River has become increasingly contaminated with antibiotic-resistant *Escherichia coli* over the 8 years period.

Staphylococcus saprophyticus is a common cause of urinary tract infection. Resistance to some of the agents commonly used to treat *S. saprophyticus* infections has been found, however, true resistance prevalence may be under estimated as susceptibility testing is not recommended by the Clinical and Laboratory Standards Institute. Julie Creighton from Christchurch compared Phoenix nitrocefin penicillinase detection, BBL chromogenic cefinase disk, Phoenix penicillin MIC and ampicillin 2µg disk diffusion for the detection of β-lactam resistance in 111 consecutive *S. saprophyticus* isolates. She found that the Phoenix nitrocefin test significantly classified more *S. saprophyticus* isolates as β-lactamase positive than the cefinase disk test. She concludes that there is a need to routinely perform antimicrobial susceptibility testing on *S. saprophyticus* isolates in order to detect emerging resistance and possible treatment failure.

Cryptococcus neoformans infection is a well-recognized AIDS defining illness among HIV patients. Due to lack of relevant data in Nigeria, Christopher Egbe and colleagues determined its prevalence among HIV patients on highly active antiretroviral therapy (HAART). They found an overall prevalence of 9.9% of *C. neoformans* infection among HIV patients on HAART. Additionally, HIV patients with a CD4 count of <200cell/µL had approximately a 2–9 fold increased risk of developing cryptococcal infection. Neither gender, nor age significantly affected the prevalence of cryptococcal infection.

Classical Hodgkin lymphoma (CHL) is a unique type of lymphoma because of the extraordinary and unexplained scarcity of its neoplastic Hodgkin Reed-Sternberg (HRS) cells that derived from clonal germinal center B cells with rearranged immunoglobulin genes bearing crippling mutations. The occurrence of these cells in the bone marrow aspirations are considered rare. Their presence is an expression of widely disseminated disease and it indicates poor prognosis. Bakheet and colleagues from Malaysia report a case of a 24-year-old female with relapsed Hodgkin lymphoma, after eleven years in remission with the standard chemotherapy regime. Repeated bone marrow aspiration revealed the presence of HRS cells. Immunophenotyping analysis by flow cytometry revealed a small population of cells expressing CD20, CD15, and CD30 that further supported the presence of HRS cells in the bone marrow aspirate.

MALDI-TOF MS has recently been introduced into clinical microbiology laboratories in New Zealand and has revolutionized identification of micro-organisms. In this issue, Michael Sun from Auckland presents a modification for the direct identification of positive blood cultures by MALDI-TOF MS and confirmed it is a promising approach to reduce laboratory turnaround time.

The CPD programme in New Zealand has two components: a compulsory competence sign off, and participation in professional development activities. The CPD programme in New Zealand stands for Competence and Professional Development. Competence is the most important component of our CPD programme. In an Editorial in this issue Jillian Broadbent, the Institute's CPD Co-ordinator, points out a number of competence activities that are considered to be part of your compulsory competence CPD claim and not part of your professional development component of the CPD programme.

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EDITORIAL

Medical laboratory science registration and competence

Jillian Broadbent
The New Zealand Institute of Medical Laboratory Science Inc. (NZIMLS), Rangiora

Recently, in an opinion piece in *Clinical Biochemistry*, Tony Badrick and Andrew StJohn from Australia stated that the key question is whether continuing professional development (CPD) and registration translates into competency and therefore improved patient safety. They raised the point that it would be possible for a medical laboratory scientist to be registered (through participation in a CPD scheme) yet not be fit to practice and that in New Zealand competency is considered separately from CPD (1). We responded and pointed out that in New Zealand the NZIMLS CPD programme stands for Competency and Professional Development rather than Continuing Professional Development and that all registered medical laboratory scientists (MLS) and medical laboratory technicians (MLT) practicing in New Zealand require an annual practicing certificate (APC) as well as registration (2). The APC is issued by the Medical Sciences Council of New Zealand (MSCNZ) and must be renewed on an annual basis whilst the MLS or MLT is still practicing. Issue of an APC is dependent upon demonstration of satisfactory participation and progress in the CPD programme as previously pointed out in the NZIMLS Journal (3).

The NZIMLS CPD programme has two components: a compulsory competence sign off, and participation in professional development activities. Competence is the most important component of our CPD programme and it is important that any person signing off the competence of a MLS or MLT hold those competencies themselves. A MLS or MLT will not receive an APC from the MSCNZ if this compulsory competence requirement is not fulfilled.

The MSCNZ has published a Code of Competencies and Standards for the Practice of Medical Laboratory Science (www.mscouncil.org.nz/assets_mlsb/Uploads/Code-of-Competencies-and-Standards3.pdf) with minimum standards outlined for both scientists and technicians. Maintaining high professional standards both ethically and legally are just as important as competence in the analysis and processing of samples, as is the demonstration of safe practice techniques. The ability to communicate, work with colleagues and to recognise the socio-cultural values of others are also components of competency.

Practitioners need to be aware that any activities relating to the competence standards described above are considered to be part of your compulsory competence CPD claim and not part of your professional development component of the CPD programme. Confusion often exists in the differentiation and separation of these competency components so consistency within the CPD categories is paramount. The booklets 'Competence and Professional Development Recertification Programme for Medical Laboratory Scientists' and 'Competence and Professional Recertification Programme for Medical Laboratory Technicians' (http://www.nzimls.org.nz/scientist-cpd-programme.html or http://www.nzimls.org.nz/technician-cpd-programme.html) should be consulted for clearer details on specific activities, and if doubt still exists then the CPD coordinator should be consulted for advice at cpd@nzimls.org.nz .

Thus, in New Zealand, the Competency and Professional Development programme leading to registration ensures improved patient safety.

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REVIEW ARTICLE

Pre-analytical variability and quality of diagnostic testing. Looking at the moon and gazing beyond the finger

Giuseppe Lippi[1], Camilla Mattiuzzi[2] and Emmanuel J Favaloro[3]

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ABSTRACT

Laboratory diagnostics develops through a closed loop, formerly defined as the "brain-to-brain turnaround time", which entails pre-analytical, analytical and post-analytical phases. After decades of research into total quality of laboratory testing, it is now undeniable that the pre-analytical phase is most vulnerable to a variety of errors, which may ultimately impair the reliability of test results and jeopardize patient safety. The leading problems seemingly emerging from mishandled or poorly standardised activities include patient preparation, blood drawing, sample handling, transportation and preparation. Therefore, the aim of this review article it to provide a succinct update of the state-of-the-art of pre-analytical variability according to personal practice and experience, and then to suggest some reliable strategies for improving awareness of the value chain of in-vitro diagnostics beyond the laboratory and into the broader system of health care.

Key words: Pre-analytical variability, laboratory errors, quality, harmonisation.

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INTRODUCTION

Laboratory diagnostics is commonly defined as the act or process of identifying the nature and cause of a disease by means of in-vitro diagnostic testing. Although it is generally agreed that 60 to 80% of clinical decisions may be influenced by results of laboratory testing as largely supported by anecdotal evidence (1), the value of laboratory medicine in patient care remains unquestionable.

According to the original model proposed by George D. Lundberg in the early 1980s, laboratory diagnostics develops through the so-called "brain-to-brain turnaround time" (2), which is conventionally articulated in three (to five) sequential parts,i.e., the (pre-)preanalytical, analytical and (post-) postanalyticalphases (3). This lucid interpretation depicts the testing process as a closed "loop", which starts from the physician's brain ('pre-preanalytical'; with test ordering) and eventually returns ('post-postanalytical'; with test result interpretation and resultant action) [Figure 1]. More specifically, the pre-analytical phase entails all those actions that are necessary in order to obtain diagnostic specimens, and these activities typically lie outside the traditional boundaries of clinical laboratories. These sequential actions include selection of tests to be ordered, tests prescription, patient preparation, followed by collection, handling, transportation, preparation and eventually storage of the specimen(s). The analytical phase is rather understandably limited to sample testing, whereas the post-analytical phase consists of results reporting, interpretation (and potentially resultant actions).

Exploring the iceberg of laboratory errors

As for any other medical discipline, laboratory diagnostics is vulnerable to errors. The warning that laboratory tests are not always foolproof was first raised by Richard J Henry and Sam Berkman in the early 1950s (4,5). These seminal reports paved the way to a subsequent series of studies aimed to identify the frequency and nature of laboratory errors. After more than 60 years of research in this field, we have now reached the conclusion that the pre-analytical phase is indeed the most vulnerable part of the "brain-to-brain turnaround time", with pre-analytical errors (i.e., 50-70% of total) being largely prevailing over those emerging from both the analytical (5-15% of total) and post-analytical phases (10-30% of total) (6). Unfortunately, the picture that still emerges from the collective imagination of most physicians and many laboratory professionals is dissimilar to the evidence, in that the concept of a "laboratory error" remains identified with that of an "analytical error", thus discounting the fact that most problems occurring in diagnostic testing are attributable to the analysis of poor quality specimens (7) [Figure 2]. The seminal concept that "anything that stands in the way of their prompt and perfect receiving of laboratory results for their patients is perceived as a laboratory problem or error" coined by George D Lundberg in 1981 is therefore as actual as ever (2), and the reiteration of this misconception does not help achieve a better quality of testing, thus concentrating on the finger pointing to the moon rather than on the moon itself. It seems thus reasonable to provide a succinct update of the state-of-the-art of pre-analytical variability according to personal practice and experience and then to suggest some reliable strategies for improving awareness of the value chain of in-vitro diagnostics beyond the laboratory and into the broader system of health care.

Patient preparation

Patient preparation before testing (i.e., fasting status, exercise and posture) is an often overlooked source of bias in laboratory diagnostics. Briefly, the current recommendations entail that the patient should abstain from foods and beverages for at least 6-12 hours before testing (8), in order to limit the unwanted effects of food ingestion and haemodilution on a number of laboratory analytes (9-11). Despite this standpoint being virtually incontestable, fasting requirement is still considered as unnecessary or "optional" in some (private) facilities worldwide (12). Physical activity is another important variable, wherein the performance of exercise before sample collection may impact on test results both directly, i.e., by release of muscle biomarkers

or acute phase proteins; or indirectly, i.e., for haemoconcentration (13). A number of studies have also shown that patient posture should be standardized during venipuncture, because sample collection in supine, sitting or standing position generates remarkable differences in a number of hematological and biochemical parameters (14,15).

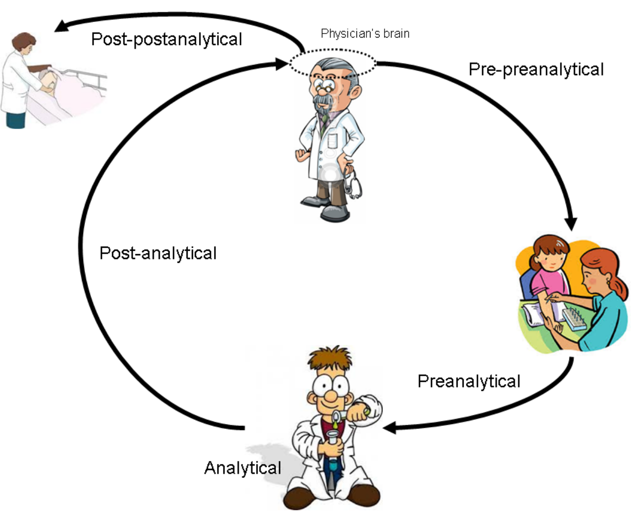

Figure 1. The "brain-to-brain turnaround time".

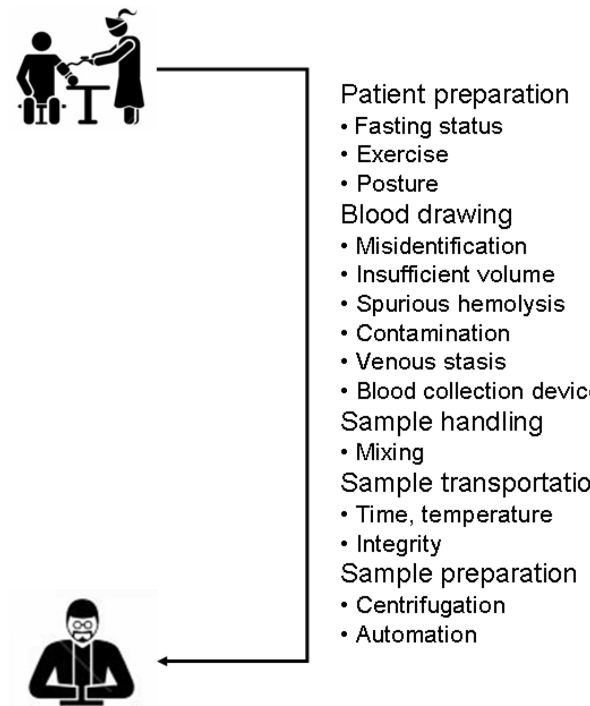

Figure 2. The leading causes of preanalytical variability.

Venous blood collection

The term phlebotomy originates from the Greek words "*phlebo*" (which means "pertaining to a blood vessel") and "*tomy*" (which means "producing an incision"). Therefore, this term currently identifies the process of making a vein incision with a needle, a procedure also conventionally known as "venipuncture", that is necessary and almost unavoidable for obtaining diagnostics blood specimens to be used for testing. In essence, phlebotomy should be regarded as an invasive medical procedure, which deserves specific training and deep acknowledgement regarding potential complications (16).

Immediately after collection, blood specimens are transported to the site of testing, and then subjected to centrifugation for obtaining serum or plasma in the case of clinical chemistry, immunochemistry and hemostasis testing, or analyzed as a whole in the case of blood cell enumeration, differentiation and sizing. It is hence obvious that the accurate performance of a venipuncture should be regarded as an essential criterion for quality of testing, since the analysis of unsuitable specimens would no longer mirror the condition of the patient in vivo, thus introducing undesired variability that may lead to inappropriate clinical decision making (17).

The leading causes of unsuitable specimens for testing can be typically classified into problems of volume or quality. The former condition is mainly due to drawing of a blood volume that is insufficient for completing the testing process, the so-called "insufficient sample" (18), or collection of a specimen with an inappropriate blood to additive ratio. The paradigmatic case is that of a hemostasis sample, in which the ratio between the anticoagulant – that is typically buffered sodium citrate - and the blood must fulfil a fixed ratio, that is one volume of anticoagulant and nine volumes of blood. When this fixed ratio is altered, the quality of testing may be substantially impaired. In particular, the excess of anticoagulant interferes with the normal development of clotting tests in "under-filled" tubes (19).

As regards the second condition, that is the quality of the specimen, this may be impaired when something goes wrong during sample collection. Errors of identification are globally rare compared to other more frequent mistakes, but should still be regarded as the most serious challenge to patients' safety (20). The more frequent problems are instead typically caused by a troublesome phlebotomy, in which the blood cells are subjected to injury, up to their full rupture. This would cause the release of a number of intracellular substances in the surrounding serum or plasma, especially hemoglobin, ions (i.e., potassium) and enzymes, which then generate an undesirable bias in test results (21,22). The leading causes of spurious haemolysis are represented by drawing blood from intravenous lines (23), and from small or fragile veins (24). Therefore, it is not surprising that the large majority of haemolysed specimens are generated in the emergency department, where the use of intravenous catheters is commonplace for collecting blood (25). A number of considerable technological advances have made it possible to accurately identify haemolysed specimens, and even to quantify the amount of cell-free hemoglobin present in serum and plasma samples (26,27), which would hence allow laboratories to suppress those test results that are selectively impaired according to the haemolysis degree. This would allow the establishment of a harmonised strategy for sample rejection in clinical laboratories, with little effect on laboratory workflow and turnaround time (28).

Beside the well-known problem of sample dilution or contamination by exogenous fluids (i.e., saline or glucose-containing solutions) (29), the cross contamination of "undesired" additives among tubes may also jeopardize the quality of testing (30,31). The establishment of a specific order of draw is a heritage of old and mostly anecdotal studies. Briefly, at the end of the 1970s, two independent groups observed that an incorrect order of draw was a cause of spurious hyperkalaemia and hypocalcaemia, which are two surrogate markers of in-vitro cross contamination of EDTA among tubes (32,33). This led to development of universal recommendations that a specific sequence of tubes should be followed during venipuncture. Recent evidence, mostly based on a more advanced generation of blood tubes, suggests that the use of such a specific sequence may no longer be justified, however (34). Even the collection of a discard tube seems now mostly unnecessary when drawing samples for hemostasis testing, except in the case where blood drawing is performed through catheters or butterfly devices (35).

Another important source of variability is represented by venous stasis. The application of the tourniquet is virtually unavoidable during routine venipuncture, since this practice allows a better identification of veins and puncturing sites. Several lines of evidence suggest, however, that the retention of the tourniquet for more than one to two minutes is a significant cause of haemoconcentration, which may also spuriously increase the concentration of several analytes (36,37), but which can be avoided by using transilluminator devices (38).

Interestingly, recent studies have shown that primary blood tubes with different brands are not alike, and this source of variability should be taken into account, especially when patient samples are collected with tubes produced by different manufacturers and when establishing the reference ranges of certain analytes (39).

Sample handling

As mentioned, venous blood specimens are typically collected in primary blood tubes containing additives, which can be either anticoagulants such as heparin, citrate or EDTA, when the biological matrix to test is plasma or whole blood; or different types of pro-coagulants, when the biological matrix to test is serum (40). Insufficient sample mixing may hence be a cause of inappropriate anticoagulation of plasma and whole blood specimens, whereas excessive shaking may generate blood cell injury (41). To avoid problems during sample mixing, it is universally recommended that samples should be gently inverted by four to six times, or following the specific indications provided by the different manufacturers of blood tubes (42).

Sample transportation

Once diagnostic samples have been collected, they should then be shipped to the laboratory; however, this can be located at variable distance from the site of collection. It is noteworthy that the recent trend towards consolidation of laboratory services into larger facilities driven by cost containment policies has contributed to amplify the challenges in sample transportation (43). As such, the quality of the specimens may be seriously jeopardized when these are shipped over long distances and under suboptimal conditions. To overcome this problem, the most critical phases of sample transportation should be accurately optimized and standardised, by adoption of stringent criteria of time and temperature, sample positioning, as well as establishment (and fulfilment) of rigid criteria of acceptability. Recent innovations in this area are represented by the development of integrated systems for transport monitoring, encompassing the use of appropriate containers, devices for time and temperature recording, and specific software that allow to readily identify when the established criteria of shipment have not been fulfilled (44). Recent evidence also suggests that serum samples should be preferred over lithium-heparin samples when long or problematic shipment of centrifuged specimens is expected. This consideration emerges from evidence that the presence of a higher number of blood elements in centrifuged plasma compared to serum may generate an analytical bias in the measurement of certain analytes such as potassium, glucose, aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) (45). Another related aspect is the management of centrifuged samples within the laboratory. Due to the presence of residual blood cells stratified at the upper layer of the gel or below the tube stopper in lithium-heparin specimens, which may then re-suspend in plasma during sample handling, plasma specimens should be handled with caution, avoiding mixing, inversion and inclination after centrifugation has been completed. Moreover, the use of serum should be preferred over lithium-heparin when a vertical, closure-up position of tubes cannot be guaranteed after separation (46).

Sample preparation

With the obvious exception of point of care testing or samples used for hematological testing, in which the analysis entails enumeration, identification and sizing of blood corpuscular elements, the specimens referred for clinical chemistry, immunochemistry and coagulation testing should be subjected to a process of separation of blood cells from serum or plasma. This is conventionally achieved by centrifugation. Specific indications about the issue of centrifugation are available from the manufacturers of blood tubes, as well as from the Clinical and Laboratory Standards Institute (CLSI), especially for clotting tests (47). Several lines of evidence now attest that the use of inappropriate conditions of centrifugation may impact on laboratory test results. In particular, shorter or longer times of centrifugation may be an important source of important bias in coagulation testing (48), whereas clinical chemistry tests are seemingly less vulnerable to the conditions of centrifugation (49,50). Interestingly, the use or not of the centrifuge brake appears to be another aspect that may have an impact on hemostasis testing (51). As regards laboratory automation, and specifically laboratory automation systems, sample transportation by means of belts or tracks seems to be a reliable mean for improving laboratory throughput and turnaround time, with minor effects on sample quality (52,53). Finally, the use of the last generation of pneumatic tube delivery systems seems another good opportunity to increase laboratory efficiency, with negligible impact on sample quality (54).

CONCLUSIONS

There is a widespread perception that the quality of diagnostic testing should be identified with the analytical quality (55). Although accuracy and precision cannot be discounted in the modern clinical laboratory (56), it is clear that extra-analytical variables currently exert a much greater influence on test results and patient safety (57). The results of a recent survey promoted by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) working group for the pre-analytical phase (WG-PA) showed that sample collection is still poorly standardised across as many as 28 European countries (58), which raises the question as to whether the quality of blood drawing should be regarded as an ongoing challenge for the quality of laboratory diagnostics. To see the moon (i.e., total quality), it is necessary to gaze beyond the finger (analytical quality), and recognize that a culture of pre-analytical quality should be further promoted and disseminated worldwide (Figure 3).

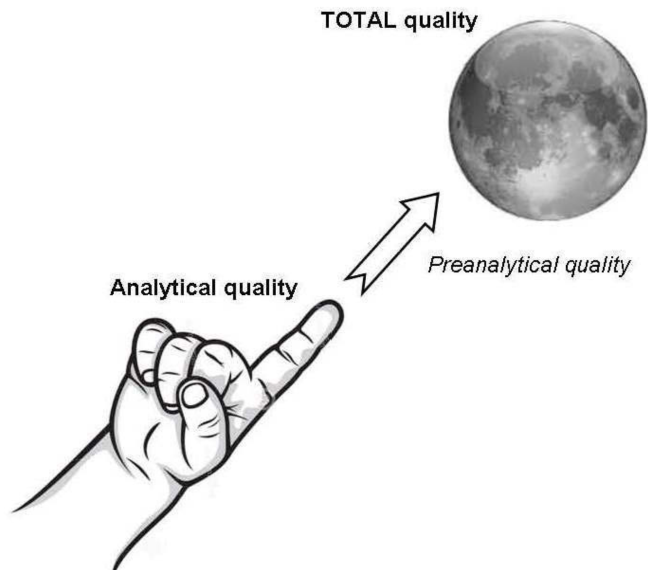

Figure 3. Analytical quality, preanalytical variability and total quality of laboratory testing: looking at the moon and gazing beyond the finger.

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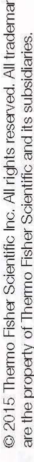
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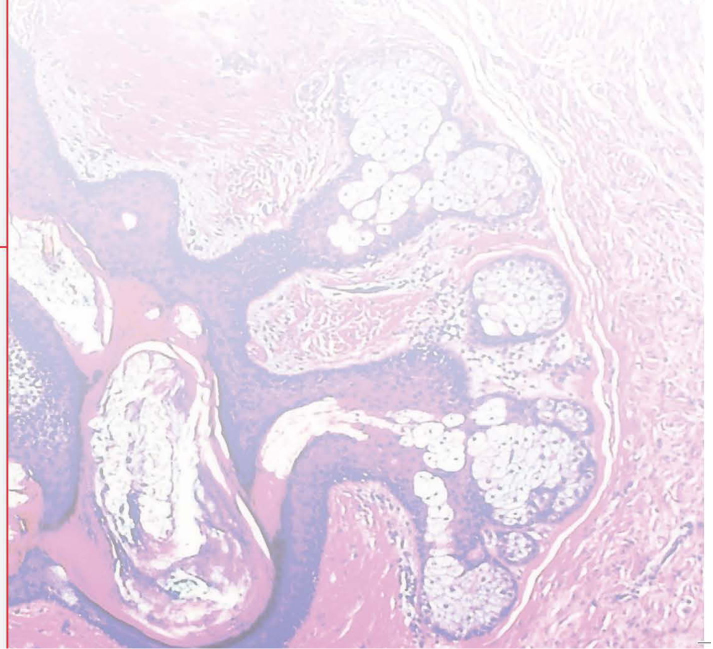

ORIGINAL ARTICLE

Increase in antibiotic resistant Escherichia coli in a major New Zealand river: comparison between 2004 and 2012- an interval of 8 years

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ABSTRACT

Background: Waimakariri River is one of the largest of the North Canterbury rivers in the South Island of New Zealand. It receives its water supply from the Southern Alps in the west and transverses the Canterbury plains to the Pacific Ocean in the east.

Methods: This study compared the contamination of the Waimakariri River and one of its contributing rivers by sampling water from the respective rivers at multiple points in 2004 and 2012, measuring the *Escherichia coli* count/100 mL and testing *Escherichia coli* isolates for antibiotic resistance.

Results: The study demonstrated that the *Escherichia coli* contamination of the river has increased considerably when comparing the results from 2012 with those obtained from samples in 2004 both up and down river. Multiply-antibiotic resistant *Escherichia.coli* was found in two sample sites in 2004 but this had increased to six sample sites in 2012.

Conclusions: The Waimakariri River has become increasingly contaminated with antibiotic resistant *Escherichia coli* over the 8 years period.

Keywords: New Zealand, North Canterbury, Waimakariri River, antibiotic resistance, *Escherichia coli* count, dairy farming.

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INTRODUCTION

Waimakariri River is one of the largest of the rivers in the Canterbury region of the South Island of New Zealand. It flows west/east from the Southern Alps through the Canterbury plain to the Pacific Ocean. The Waimakariri River provides water for the aquifers running under the plains which provide the artesian water supply for the second largest city in New Zealand, Christchurch. Historically much of Canterbury has been dry land and arable farming but there has been a huge increase in dairy farming in the region over the last 15-20 years which requires large amounts of irrigation, as the average rainfall is too low to support dairy cow pastures. The Waimakariri River has numerous contributory rivers of which Hawdon River is one. Most of the rivers originate in the Southern Alps. The Waimakariri River is of considerable cultural importance to the Ngai Tahu Iwi.

The increase in dairy farming has highlighted long-held concerns that waterways could become contaminated by spillage from fertilizer and animal waste. One of the measurements for such pollution is the measurement of coliform bacteria especially *Escherichia coli (E.coli)* in the river water. Though measuring the levels of *E.coli* can give us a clear indication of the levels of faecal contamination of a waterway, it cannot tell us anything about the source of the contamination. This is important as not all faecal contamination is created equal, with faeces sourced from humans, cattle and poultry being considered high risk due to the increased possibility of human pathogens being present (1,2). It is also a concern that antibiotics used in the treatment of the animals may promote antibiotic resistance in the faecal flora of the associated herd. Coliform bacteria, such as *E. coli,* are able to acquire these resistance factors via horizontal gene transfer and this resistance may subsequently be transferred into human bacterial flora.

The aim of our study was to compare *E. coli* counts in samples taken at predetermined sites in the Waimakariri River and one of its contributories, the Hawdon River, in 2004 and 2012 and to determine any difference in the *E. coli* count and antibiotic resistance of the *E. coli* isolates between the two periods.

METHODS

River water samples were obtained from the Hawdon River in February/March 2004 and December/January 2012 at seven locations between the Tarn and the point where it meets the Waimakariri River at Mt White Bridge. The sites are labelled: 1 (Tarn), 2 (above Hawdon Hut), 3 (below Hawdon Hut), 4 (Right Fork), 5 (Hawdon Shelter), and 6 (Mt White Bridge) [Figure 1].

The Waimakariri River samples were obtained in the same months as the Hawdon river samples from the following locations in 2012: 8 (Gorge Bridge), 9 (Pitts Intake), 10 (Cooks Road),11 (Intake), 12 (Willows /Courtney intake), 13 (Upstream South High-way (SH)1 bridge), 14 (Downstream SH1 Bridge), 15 (Stewarts Gully), and 16 (Brooklands Lagoon). The 2004 samples had five of the same sites but missed 9 (Pitts Intake), 11 (Intake), 13 (Upstream SH1 bridge), and 16 (Brooklands Lagoon). One of the authors supervised collection of samples both in 2004 and 2012.

E.coli levels were tested using the industry standard Colilert*-18/Quanti-Tray*/2000 for detection and enumeration of *E. coli*. Quanti-Tray*/2000 is designed to give quantitative bacteria counts of 100 mL samples using IDEXX reagent products. After the most probable number (MPN) of total coliforms and of *E. coli* counts were attained; an aliquot of broth was extracted from those cells in the Quanti-Tray®/2000 which tested positive for

E.coli, and E.coli was subsequently isolated from the positive wells. E.coli isolated from these cultures was further identified using an API rapid kitset (3) for the 2004 isolates. In 2012 the bacteria were visualized on Chromogenic media (Uriselect 4, Bio-Rad Laboratories Ltd.) and identification performed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF and FT-MS) technique (4).

Ten to thirteen isolates from each site were tested against a panel of antibiotics using the standard Clinical Laboratory Standards Institute (CLSI) disk diffusion method (2011). The antibiotic concentration in each disc and the range of antibiotics used for testing the susceptibility of *E coli* isolated from the river samples are summarised in Table 1.

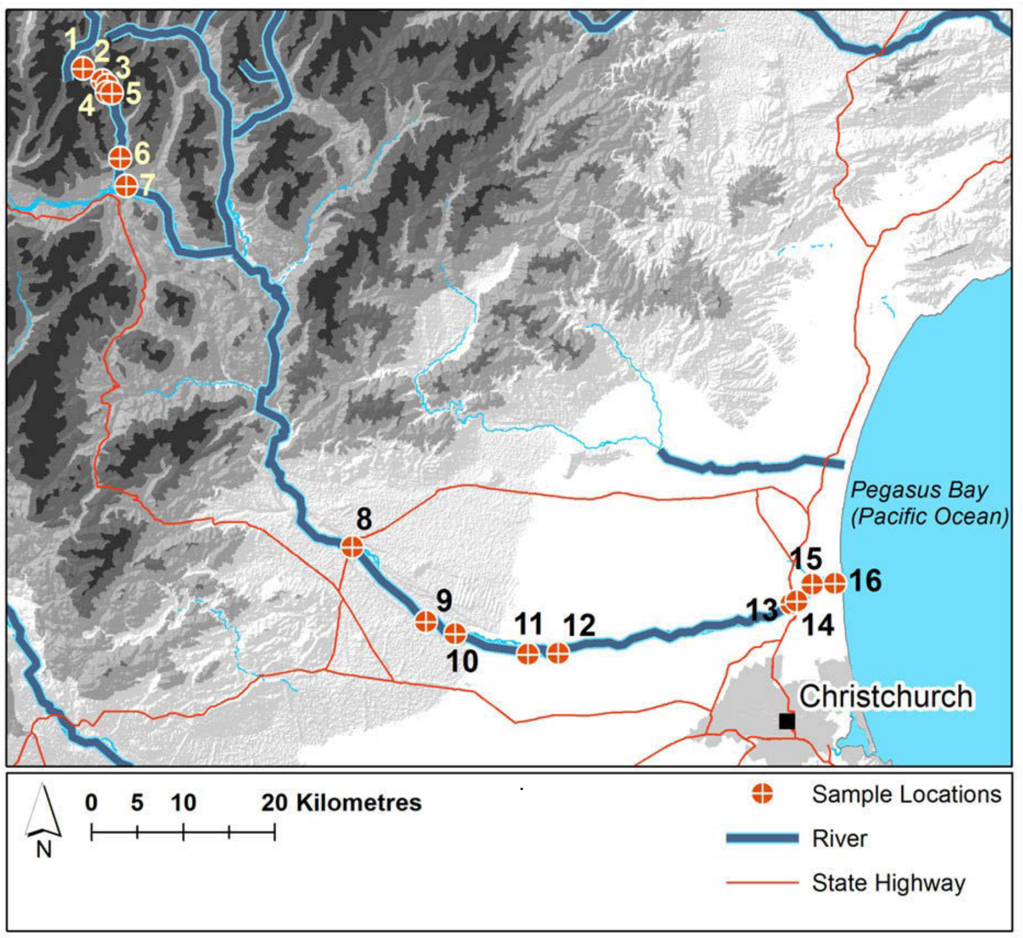

Figure 1. Sample locations.

Table 1. Antibiotics used in susceptibility testing at the recorded concentrations.

Antibiotic	Concentration	Antibiotic	Concentration
Apramycin	15 µg	Nalidixic acid	30 µg
Streptomycin	10 µg	Neomycin	30 µg
Sulphafurazole	300 µg	Nitrofurantoin	300 µg
Gentamicin	10 µg	Tobramycin	10 µg
Trimethoprim	5 µg	Tetracycline	30 µg
Chloramphenicol	30 µg	Cefoxitin	30 µg
Ampicillin	10 µg	Cefaclor	30 µg

Isolates were scored sensitive, intermediate, or resistant based on the level of growth around each antibiotic disk.

RESULTS

E.coli count per 100mL of river water from the sample locations of the Hawdon and Waimakariri rivers in 2004 and 2012 are shown in Tables 2 and 3.

Table 2. The most probable number (MPN) of *E coli* counts per 100mL of river water from the sample locations 1-7 on the Hawdon and upper Waimakariri Rivers in 2012 and 2004.

Site number	***E.coli* per 100mL of water 2012**		***E.coli* per 100mL of water 2004**	
	Location	**MPN count**	**Location**	**MPN count**
1	Tarn	<1	Tarn	<1
2	Above Hawdon Hut	<1	Above Hawdon Hut	<1
3	Below Hawdon Hut	<1	Below Hawdon Hut	<1
4	Left Fork	<1	Left Fork	2
5	Right Fork	<1	Right Fork	1
6	Hawdon Shelter	<1	Hawdon Shelter	2
7	Mt White bridge	<1	Mt White bridge	4

Table 3. *E. coli* counts per 100mL of river water from the sample locations on the Waimakariri River in 2012 and 2004.

Site number	***E.coli* per 100mL of water 2012**		***E.coli* per 100mL of water 2004**	
	Location	**MPN count**	**Location**	**MPN count**
8	Gorge bridge	201	Gorge bridge	8
9	Pitts Intake	25		
10	Cooks Road	125	Cooks Road	31
11	Intake	137		
12	Willows (Courtney intake)	167	Willows (Courtney intake)	68
13	Upstream of SH1 bridge	308		
14	Downstream of SH1 bridge	1,986	Downstream of SH1 bridge	70
15	Stewarts Gully	1,203	Stewarts Gully	57
16	Brooklands Lagoon	2,420		

Isolates were scored sensitive, intermediate, or resistant based on the level of growth around each antibiotic disk.

RESULTS

E.coli count per 100mL of river water from the sample locations of the Hawdon and Waimakariri rivers in 2004 and 2012 are shown in Tables 2 and 3.

Table 4. Sample location, site number, number of *E.coli* isolates tested for antimicrobial sensitivities, number resistant in and number in each resistance pattern (in bracket) from 2024 and 2012.

Site number		**Antibiotic resistance 2004**		**Antibiotic resistance 2012**	
	Location	**Number tested/ resistant**	**Resistance pattern**	**Number tested/ resistant**	**Resistance pattern**
8	Gorge bridge	13/0	-	10/0	
9	Pitts Intake			10/0	
10	Cooks Road	13/0	-	10/2	SF,Te,Amp (1) CEC (1)
11	Intake			10/4	S, SF, Te, Tri, Amp, C (1) Amp(2), Te (1)
12	Willows (Courtney intake)	13/4	S, SF, Te, Tri, Amp	10/3	SF, Tri, Amp, C, Na (1) S,SF,Te, Amp (2)
13	Upstream of SH1 bridge	13/4	S, SF, Te, Tri, Amp	10/3	S,SF,Te (1) Amp (1), Te (1)
14	Downstream of SH1 bridge		-		Amp (3) Intermediate sensitive F (1), CEC (2)
15	Stewarts Gully	11/1	Amp	10/4	F, Te (1) Amp (3)
16	Brooklands Lagoon			10/0	

S= streptomycin); SF= sulphafurazole; Tri= trimethoprim; C= chloramphenicol; Amp= ampicillin; Na= nalidixic acid; F= nitrofurantoin; Te= tetracycline; CEC= cefaclor.

DISCUSSION

The Waimakariri is well known for its braided appearance with wide shingle beds the length of the river from the source in the Southern Alps to the more eastern area where it flows through the Canterbury Plains. The braiding results from extensive shingle beds that shift with changes in flow and indirectly have a filtering effect on the water. A published league table of rivers from 2009 labelled the Waimakariri one of the ten most polluted rivers in New Zealand (5). In the past some of the pollution was caused by liquid waste from industries which discharged directly into the river mainly downstream of the State Highway1 bridge. From 2012 this waste was to be piped to the municipal sewage treatment plant, however, at times direct discharge to the river has still taken place (6).

The river water was sampled at 12 sites in spring/summer 2004 and at 17 sites in summer 2012. The sites sampled at the upper reaches show low or absent number of *E coli* per 100 mL of water in both 2004 and 2012. The river passes through a narrow gorge when leaving the mountain section before flowing onto the Canterbury Plains. The water sampling site just after the gorge is called the Gorge Bridge (sample site 8). Upstream from the Gorge Bridge there is little concentrated human habitation. An increase in *E. coli* per 100 ml sample is recorded at this site in 2012 compared with 2004. In both instances no antibiotic resistant *E.coli* were demonstrated. However, an increase in antibiotic resistant *E.coli* was noted downstream from the Cooks Road sampling site in 2012, where none were demonstrated in 2004. In 2004 antibiotic-resistant *E. coli* was not recorded before the Willows (Courtney intake) indicating that the contamination of the Waimakariri River with antibiotic resistant *E. coli* has spread further west towards the mountains during the eight year period.

Linkages between land management activities and stream water quality from dairy farming in Southland New Zealand have been reported. A computer model estimated that the *E. coli* emissions to streams from sheep farming was 70% of that from dairy farms, indicating that an increase in coliform contamination can be expected after conversion from sheep to dairy farming (7). Another concern is the reporting of Campylobacter species by polymerase chain reaction in a study of 42 river water samples (8). Campylobacter species were not looked for in this research but where faecal coliforms are found, it is possible that campylobacter and other faecal pathogens may also contaminate the water. The Ministry of the Environment (NZ) publishes a Microbial Assessment Category (MAC) with *E. coli* colonies/ 100 mL the indicator bacteria. The health risk in fresh waters (based on 95th percentile *E. coli* counts) is divided into tabular categories A to D (9). The table also gives a risk calculation for Campylobacter infection based on New Zealand rate. According to the MAC categories all the river samples in this work from 2004 would be category A with no calculated risk for campylobacter infection. From the 2012 samples Gorge Bridge and Courtney Intake would be category B, those sites in between those two categories A, Upstream of SH1 would be category C and the rest towards the ocean would be category D. Both category C and D indicate a *"substantial elevation in the probability of Campylobacter infection compared to the New Zealand background rate".* This raises a concern that the traditional seafood gathering practices of Maori may be threatened by increases in faecal pollution of the river. Shellfish, including mussels may concentrate and accumulate faecal pathogens. This possibility has been validated by the experimental immersion of mussels in rivers at sites impacted by faecal pollution. It was found that mussels acquired these organisms several kilometres from pollution (10). This suggests that harvesting mussel from rivers at sites with high coliform count could be a health risk.

The antibiotic panel chosen for testing included both those commonly used in human treatment and those known to be used for treatment of farm animals. Some have low use in human treatment (such as streptomycin and tetracycline), but are used in both horticulture and the promotion of animal health (11,12). It has been found that faecal coliforms are able to transfer streptomycin or tetracycline resistance determinants to an antibiotic sensitive strain of *E. coli* within hours (13). It is a concern that resistance to nalidixic acid, the first generation antibiotic in the quinolone group of antibiotics, was recorded. As only the river water was tested for the presence of coliforms, there is no evidence from the data that the deeper aquifers supplying Canterbury drinking water or local towns' water supplies are contaminated.

CONCLUSIONS

An increase in contamination with faecal coliforms represented by *E. coli* has been recorded in the Waimakariri River between 2004 and 2012. Multiple-antibiotic resistant *E. coli* were found on samples from only two sites in 2004 but by 2012 found on two further sites, both East and West of the 2004 sites. In 2004 antibiotic-resistant *E. coli* was not recorded before the Willows (Courtney intake) indicating that the contamination of the Waimakariri River with antibiotic resistant *E. coli* has spread upstream further west towards the Southern Alp mountains during the eight year period. It also indicates that the Waimakariri River can be a source of antibiotic-resistant coliforms from the sampling site of Cooks road and downstream eastward towards the Pacific Ocean. The Ministry of the Environment (NZ)'s Microbial Assessment Category should include assessment of antimicrobial resistance as well as *E. coli* count in the health risk assessment of the rivers.

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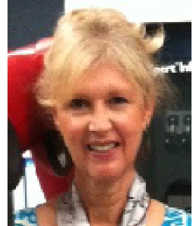

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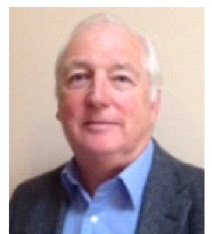

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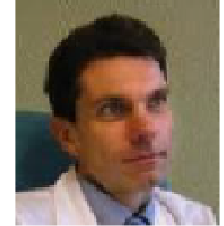

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ORIGINAL ARTICLE

Comparison of BD Phoenix nitrocefin, a cefinase disk test, and Phoenix MIC for the detection of hyper β-lactamase in *Staphylococcus saprophyticus*

JulieCreighton

Canterbury Health Laboratories, Christchurch, New Zealand

ABSTRACT

Background: Staphylococcus saprophyticus is a common cause of urinary tract infection. Resistance to some of the agents commonly used to treat infections has been found, including resistance to trimethoprim, amoxicillin and meth/flucloxacillin. However, true resistance prevalence may be under estimated as susceptibility testing is not recommended by the Clinical and Laboratory Standards Institute. In addition, testing and determination of resistance to β-lactams has not been clearly defined and in-house testing has revealed discrepancies between phenotypic methods for the detection of β-lactamase.The study objective was to compare Phoenix nitrocefin penicillinase detection, BBL chromogenic cefinase disk, Phoenix penicillin MIC, and ampicillin 2μg disk diffusion for the detection of β-lactam resistance in S. saprophyticus.

Methods: 111 consecutive S. saprophyticus isolates, collected between May to August 2013 at Canterbury Health Laboratories, New Zealand, were routinely analysed in BD Phoenix, using PMIC/ID-78 panels. Isolate identification was confirmed with MALDI-TOF and novobiocin disk diffusion. All study isolates were then repeated in Phoenix, using AST panel type PMIC-84. Chromogenic cefinase was performed using BBL discs, and ampicillin 2μg disk diffusion was performed according to EUCAST. On three isolates determined to be penicillinase hyper-producers, MICs to penicillin and ampicillin were performed by E-test, and an in-house PCR was used to determine blaZ and mecA status.

Results: 64/111 (57.7%) isolates were classified as β-lactamase positive by Phoenix nitrocefin, with only 28 of these isolates also positive by cefinase disk test. Three isolates were cefinase positive/Phoenix nitrocefin negative. 44/111 isolates were negative by both methods. Evaluation of Phoenix penicillin MIC values revealed three isolates with elevated MICs (≥2mg/L) and by E-test (≥1mg/L). Each of these 3 isolates had ampicillin disk diffusion zones of 14-15mm, which is below the EUCAST epidemiology cut-off limit of <18mm, and ampicillin MICs of 0.5mg/L. In addition, all three isolates were blaZ PCR positive but mecA negative. Resistance to other antimicrobial agents among all of the study isolates was found in trimethoprim (3.6%), trimethoprim/sulfamethoxazole (2.7%), tetracycline (12.6%) and erythromycin (17.1%). All isolates were susceptible to amoxicillin-clavulanate, ciprofloxacin, nitrofurantoin, and vancomycin.

Conclusions: Phoenix nitrocefin test classified 57.7% of S. saprophyticus isolates as β-lactamase positive, which was significantly more than the 27.9% positive by cefinase disk test. Whereas only three of these isolates showed penicillinase hyper-production, with penicillin MIC levels ≥2mg/L, ampicillin MICs of 0.5mg/L, ampicillin disk diffusion <18mm, and positive for the blaZ gene. This study has shown that penicillin MIC is a more reliable indicator of penicillinase hyper-production in S. saprophyticus than Phoenix nitrocefin or cefinase disk test, and that these isolates should be reported as resistant to penicillin and amoxicillin. Furthermore, resistance was found among other oral antimicrobials, indicating a need to routinely perform antimicrobial susceptibility testing on S. saprophyticus in order to detect emerging resistance and possible treatment failure.

Key words: Staphylococcus saprophyticus, urinary tract infection, β-lactamase, cefinase disc test, penicillinase, cefinase.

N Z J Med Lab Sci 2015; 69: 16-20

INTRODUCTION

In the clinical laboratory *Staphylococcus saprophyticus* is most frequently found as a uropathogen, being one of the leading causes of community acquired urinary tract infection (UTI) in young females (1,2). Most often the isolate will be susceptible to antibiotics commonly used to treat uncomplicated UTI, such as amoxicillin, trimethoprim, trimethoprim/sulfamethoxazole, nitrofurantoin, and meth/flucloxacillin (3). However, this does not tell the full story, in that serious infections such as bacteraemia have been reported to be caused by *S. saprophyticus* (1,4,5). In addition, isolates that are multi-drug resistant, including meth/flucloxacillin resistance due to *mec*A acquisition, have been reported in several studies (6-8).

Since 2001, the Clinical and Laboratory Standards Institute(CLSI) Guidelines has recommended that "*Routine testing of urine isolates of S. saprophyticus is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated urinary tract infections (eg. nitrofurantoin, trimethoprim ± sulfamethoxazole, or a fluoroquinolone)"* (9). Hence many laboratories may not routinely perform susceptibility testing on *S. saprophyticus*, perhaps under-estimating the true resistance prevalence. The testing and determination of resistance to β-lactam agents such as penicillin, amoxicillin and amoxicillin-clavulanate has not been clearly defined and these antibiotics are not mentioned in the CLSI statement. Yet they can be a common first line empirical choice for UTI treatment, particularly in a community setting. Furthermore, studies using different detection methods have resulted in varying levels of β-lactam resistance in *S. saprophyticus* as well as recognising problems with method accuracy (6,10-12).

In contrast to the CLSI, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints v3.1, 2013, included ampicillin disk diffusion (DD) breakpoints for S. saprophyticus, but with a resistant breakpoint of <15mm, and noted that S. saprophyticus was the exception as the majority of other staphylococci are penicillinase producers (13). EUCAST also noted that this breakpoint could be used to screen for mecA, in that any ampicillin-susceptible isolates were mecA negative. However, the breakpoints were not correlated to β-lactamase production.

The presence of β-lactamase confers resistance to penicillinase -labile penicillins (e.g. penicillin and amoxicillin); whereas the presence of *mec*A expands resistance to also include flucloxacillin, β-lactamase inhibitor combinations (e.g. amoxicillin-clavulanate), and most other β-lactam antibiotics. A recent study at our institution revealed discrepancies between Becton Dickinson (BD) Phoenix penicillinase detection and a chromogenic cefinase disk method for the determination of penicillin resistance (14). In order to resolve these discrepancies, the objective of this study was to compare detection of penicillinase in clinical isolates of *S. saprophyticus* by the Phoenix nitrocefin test and the BD-BBL chromogenic cefinase disk test methods, and to correlate those results with Phoenix penicillin MIC and ampicillin 2μg DD. Production of clinically significant levels of penicillinase was confirmed with *bla*Z PCR. We aimed to provide a practical method for Phoenix users to enable the interpretation of β-lactam results and enhance the reporting of antimicrobial susceptibilities that could guide treatment outcomes.

METHODS AND MATERIALS

Bacterial isolates

A total of 111 consecutive, non-duplicate *S. saprophyticus* isolates were collected between May to August 2013 at Canterbury Health Laboratories (Christchurch Hospital) and stored at -80°C in PROTECT cryopreservative fluid. Fifty of these isolates were referred from Christchurch Southern Community Laboratories (CSCL). All isolates originated from urine cultures. The majority of isolates were from females (104/111; 93.7%), with only seven (6.3%) from male patients.

Susceptibility methods

Study isolates were routinely analysed in BD Phoenix™ (Becton Dickinson Diagnostic Systems, Australia), using PMIC/ID-78 Combo panels which include a nitrocefin test to determine β-lactamase production. Results were stored on BD EpiCenter software database. Organism identification was confirmed with duplicate spots on a Bruker MALDI-TOF (Science Directions Limited, New Zealand) as well as novobiocin resistance by disk diffusion. All study isolates were retrospectively repeated in Phoenix, using AST panel type PMIC-84. Chromogenic cefinase was performed using BD-BBL discs (Fort Richard Laboratories Ltd, Auckland). Ampicillin 2μg DD was performed in duplicate, with interpretation according to EUCAST criteria, breakpoint table v3.1. Etest (bioMérieux) was also used to determine the minimum inhibitory concentration (MIC) of penicillin and ampicillin on three isolates classified as penicillinase hyper-producers. The PCR method of Kaase *et al.* was used to determine *bla*Z status and an in house PCR was used to detect *mec*A (15). *S. aureus* ATCC 25923 (β-lactamase negative) and ATCC 29213 (weak β-lactamase positive) were used as controls.

RESULTS

Overall, 67 (60.4%) of the 111 *S. saprophyticus* isolates were classified as β-lactamase positive by either Phoenix nitrocefin or BBL cefinase tests. Sixty-four isolates were β-lactamase positive by nitrocefin test, with only 28 of these 64 also positive by cefinase disk test. A positive nitrocefin test means that the Phoenix BDXpert rules override any results of penicillinase-labile penicillins, hence potentially changing a susceptible MIC for penicillin to an interpretation category of resistant. Three isolates were cefinase positive/nitrocefin negative. Fourty-four out of 111 isolates were negative by both methods. Results are summarised in Table 1.

Table 1. Comparison of Phoenix nitrocefin and BBL cefinase tests for β-lactamase production in 111 *S. saprophyticus* isolates.

	BBL cefinase positive	BBL cefinase negative	Total
Phoenix nitrocefin positive	28	36	64 (57.7%)
Phoenix nitrocefin negative	3	44	47 (42.3%)
Total	31 (27.9%)	80 (72.1%)	

In comparison, evaluation of Phoenix penicillin MIC values showed that the majority of *S. saprophyticus* isolates had low penicillin MICs, with 84 (75.7%) having an MIC of 0.125mg/L and 24 (21.6%) having an MIC of 0.25mg/L. The remaining three isolates had elevated Phoenix penicillin MICs of 2, 4 and 8mg/L respectively, and were termed 'hyper-penicillinase' producers (Figure 1). Similarly, 108 (97.3%) isolates gave ampicillin MIC values of ≤0.25mg/L, with only the three hyper-penicillinase producers having a value of 0.5mg/L.

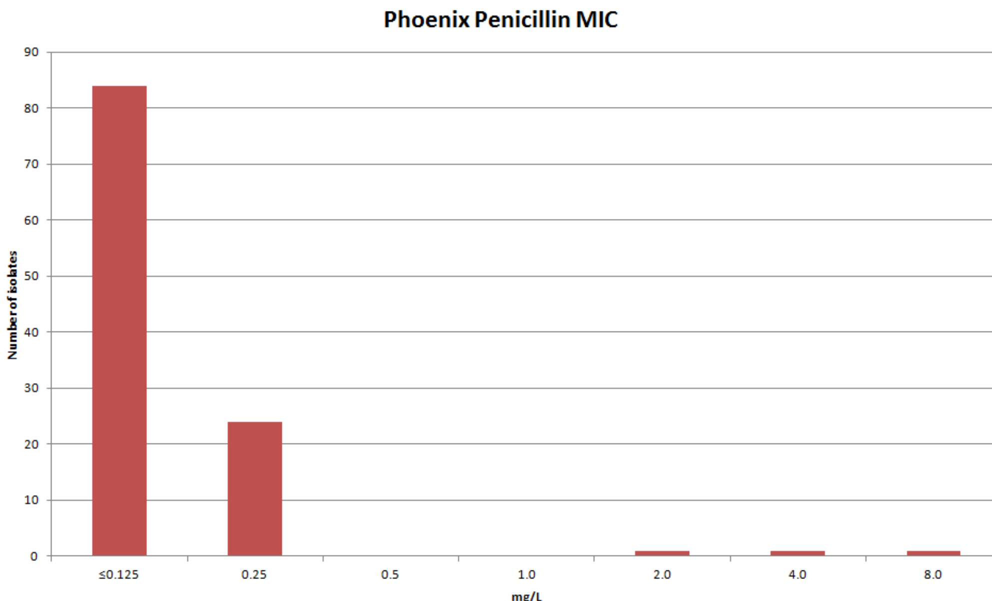

Figure 1. Frequency of penicillin MIC as determined by Phoenix.

For ampicillin DD, zones ranged between 14 and 31mm, with 85% of zones lying between 22 to 28mm. Three outlying strains, with zones of 14-15mm were the same three strains that gave elevated penicillin and ampicillin MICs. A graph of the zone size distribution is shown in Figure 2.

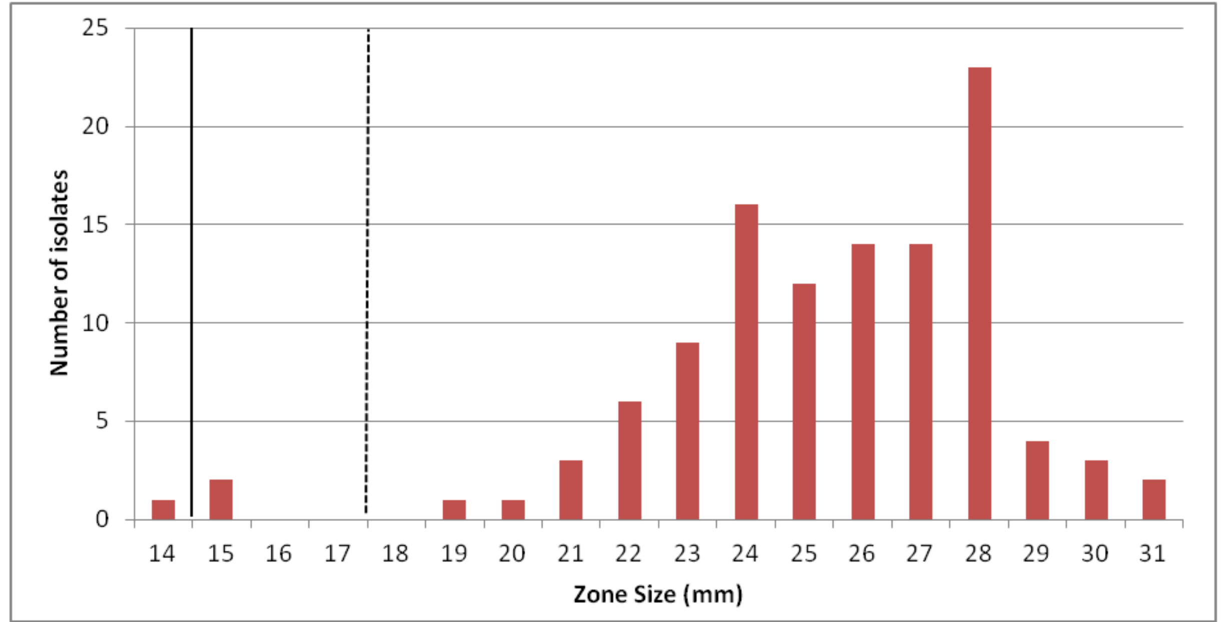

Figure 2. Ampicillin 2µg disk diffusion zone size distribution of 111 *S. saprophyticus* study isolates. The vertical straight line at <15mm represents the resistance breakpoint for EUCAST v3.1 2013. The dashed line at <18mm represents the current EUCAST v4.1 2014 table.

Etest evaluation of the three hyper-penicillinase strains produced penicillin MIC levels of 1mg/L and an ampicillin MIC of 1mg/L for two isolates and a value of 2mg/L for the remaining isolate. *bla*Z PCR was performed on these three isolates as well as a selection of nine study isolates that were positive by both nitrocefin and cefinase and had various resistances to other antibiotics. Only the three hyper-penicillinase producers were *bla*Z positive. *mec*A PCR was negative for all three strains. The data from this part of the study are shown in Table 2.

Table 2. Phenotypic and PCR results for three *S. saprophyticus* isolates determined to be hyper-penicillinase producers.

	Phoenix MIC (mg/L)		E-Test MIC (mg/L)		Disk diffusion (mm)	PCR	
Lab No.	**PENI**	**AMPI**	**PENI**	**AMPI**	**AMPI 2**	***bla*Z**	***mec*A**
SCL034	8.0	0.5	1.0	1.0	15	Pos	Neg
SCL041	2.0	0.5	1.0	2.0	15	Pos	Neg
SCL048	4.0	0.5	1.0	1.0	14	Pos	Neg

PENI = penicillin; AMPI = ampicillin.

The three *bla*Z positive isolates were also sent for analysis to the EUCAST Laboratory for Antimicrobial Susceptibility Testing, Sweden. The results from their investigations are detailed in Table 3.

Table 3. Phenotypic and PCR results as determined by the EUCAST laboratory for AST, Sweden.

ID	MALDI-TOF ID	Score	FOX30	FOX MIC	AMP2	AMP MIC	*mecA*	PCG1	PCG MIC
SCL034	*S. saprophyticus*	2.190	28	4	16	1	Neg	12	1
SCL041	*S. saprophyticus*	2.095	28	4	16	1	Neg	12	1
SCL048	*S. saprophyticus*	2.103	28	4	16	1	Neg	12	1

FOX30 = cefoxitin 30µg; FOXMIC = cefoxitin Etest; AMP2 = ampicillin 2 µg; AMP MIC = ampicillin Etest; *mec*A = *mec*A status according to PCR; PCG1 = benzylpenicillin 1 unit; PCG MIC = benzylpenicillin Etest.

Resistance to other antimicrobial agents among all of the study isolates was also evaluated, revealing low level resistance to trimethoprim (3.6%) and trimethoprim/sulfamethoxazole (2.7%), but higher levels of resistance to tetracycline (12.6%) and erythromycin (17.1%). All study isolates were susceptible to amoxicillin-clavulanate, ciprofloxacin, nitrofurantoin, and vancomycin.

DISCUSSION

Many clinical laboratories use automated susceptibility testing analysers such as Phoenix, Vitek, and MicroScan for reliable, standardised and rapid throughput of common clinical pathogens. While there are many publications evaluating the performance of these analysers, limited information is available on how they perform with *S. saprophyticus*. A previous study at our institution found discrepancies between Phoenix nitrocefin and a chromogenic cephalosporin test in the determination of penicillinase activity (14), which led us to prospectively investigate these differences and to compare results with Phoenix MIC values for penicillin and ampicillin and to compare results with ampicillin disk diffusion.

Our study of 111 *S. saprophyticus* clinical isolates suggests that the determination of penicillin resistance can be overestimated by the Phoenix nitrocefin test and the BBL cefinase test, with β-lactamase positive classifications of 57.7% and 27.9% respectively. However, only three of these isolates were positive by *blaZ* PCR for presence of the penicillinase enzyme, indicating that most of the nitrocefin and cefinase tests were false positive. Chromogenic cephalosporin tests can be difficult to read with the 1 hour reaction time required with staphylococci, and their use is not recommended by EUCAST. A similar finding with a chromogenic cephalosporin test was reported in the study by Ferreira *et al.,* who found that 52/57 57 (91.2%) of *S. saprophyticus* isolates were classified as β-lactamase positive by the Vitek, compared to only 5.3% positive using a nitrocefin disk test (6). *blaZ* PCR was not performed to resolve discrepancies in their study. Johnson *et al.* revealed problems with the Vitek 2 system erroneously reporting *S. saprophyticus* as methicillin resistant (16). They suggested not using Vitek 2 to perform susceptibility testing on *S. saprophyticus*, but instead to consider the detection of *mecA* by PCR. However, using this approach does not detect resistance to other antimicrobials.

Problems with the reliability of phenotypic and chromogenic cefinase detection methods for resistance to β-lactam agents in *S. saprophyticus* have also been recognised by other studies and is part of the reason why both CLSI and EUCAST have changed their guidelines over the years (9-11,13,17). *S. saprophyticus* may produce a low level penicillinase that is weak and difficult to reliably detect and is not picked up by the *blaZ* assay. Stratton *et al*. found the presence of penicillin binding protein (PBP 2a) in three *S. saprophyticus* strains that had high level methicillin resistance, but the authors demonstrated only negligible β-lactamase activity, despite using induction techniques (18). Alternatively, there may be other undefined mechanisms at play. For instance, Malyszko *et al.* recently published a report of a newly discovered *mec* variant, *mecC2*, which conferred methicillin resistance to *S. saprophyticus* strains recovered from animals (19). It is possible that these strains could be passed onto humans through the food chain.

Our study also looked at penicillin and ampicillin MIC values on Phoenix and ampicillin DD in order to assess their ability to determine an interpretation for β-lactams. The use of ampicillin 2µg was recommended by EUCAST Clinical Breakpoint Table v3.1 as a way of screening for methicillin resistance in *S. saprophyticus*, with zones of <15mm indicating further investigation for the presence of *mecA*. EUCAST Note 1/A stated that "*With the exception of Staphylococcus saprophyticus, most staphylococci are penicillinase producers*". This would imply that only *mecA* is responsible for ampicillin resistance in *S. saprophyticus*, rather than significant penicillinase production. At the beginning of 2014, EUCAST table v4.1 was updated to include a breakpoint interpretation for ampicillin of resistance at <18mm and the statement regarding *S. saprophyticus* not being a penicillinase producer was removed. Zones ≥18mm can be regarded as susceptible to both ampicillin and methicillin. No MIC breakpoint values were included.

The data from our study, shown in Figure 1, would indicate that a penicillin MIC value of ≥0.5mg/L could be used as a suitable screening method for the detection of hyper penicillinase, with values below these limits considered susceptible to penicillin, ampicillin, and methicillin. Raw MIC data can be obtained in Phoenix by temporarily changing the organism identification to "unidentified organism". Once the MIC values for penicillin are obtained then the isolate identification can be corrected back to *S. saprophyticus*, with the penicillin and ampicillin category interpretation manually entered according to the penicillin MIC values obtained.

Our results from the ampicillin 2µg DD method would concur with the current EUCAST cut off and it is a useful method to confirm Phoenix results or as a standalone test for laboratories without automation. It is probably not necessary to confirm the ampicillin resistant isolates with *blaZ*, but it is necessary to check for *mecA*. Data (not shown) from our study agrees with previous investigations in that MIC values for cefoxitin and oxacillin are not specific for the detection of *mecA* with 3.6% of our isolates having a cefoxitin MIC of 4mg/L and 84.7% of isolates having an oxacillin MIC >0.25mg/L (considered resistant for coagulase-negative Staphylococci). Yet all of these isolates, except for the three hyper-penicillinase producers, were susceptible to penicillin. The methicillin-resistant, *mecA* positive *S. saprophyticus* strains isolated so far (albeit infrequently) at our institution have all been highly resistant to all β-lactams as well as amoxicillin-clavulanate.

The clinical relevance of antibiotic resistance in *S. saprophyticus* has not been fully defined. It could be argued that antibiotics commonly used to treat UTI are concentrated in the urine at such high levels that most infections are treated despite *in-vitro* resistance. In addition, many healthy adult females can sometimes clear a UTI in several days without antibiotic intervention. On the other hand, we have had anecdotal reports of treatment failure in patients with cultures growing *S. saprophyticus* that are resistant to the prescribed antibiotic, including amoxicillin and trimethoprim. In our opinion these hyper-penicillinase producing isolates should be reported as resistant to penicillin and amoxicillin as these first line antibiotics may be clinically ineffective. Furthermore, *S. saprophyticus* UTI can lead to serious complications such as septicaemia and this pathogen has been isolated from sites outside of the uro-genital tract. Surely it is the duty of a clinical laboratory to use the most reliable and accurate means in order to provide suitable treatment choices to clinicians and also to detect emerging resistance.

Limitations of this study include the inability to rule out duplicate isolates. Of the 50 isolates collected from CSCL, only gender and age were recorded, thus some of the strains could possibly be from the same patient who had a recurrent or relapsed infection.

In summary, the results of this study would suggest that the Phoenix nitrocefin and chromogenic cephinase tests should be discouraged for the determination of β-lactamase in *S. saprophyticus*. We have shown that penicillin MIC is a practical and more reliable indicator of penicillin hyper-production and that these isolates should be reported as resistant to penicillin and amoxicillin. Routine susceptibility testing of *S. saprophyticus* should be performed in order to enhance the reporting of antimicrobial susceptibilities that could guide successful treatment outcomes.

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Grateful thanks to Erika Matuschek, EUCAST Laboratory for Antimicrobial Susceptibility Testing, Sweden, for analysing the three *blaZ* positive *S. saprophyticus* isolates and for her helpful review of the manuscript. Also thank you to CSCL for providing some of the study strains; to Trevor Anderson, CHL, for his

assistance with the *blaZ* PCR; to Julian Flamank, CHL laboratory intern 2013, for performing some of the manual phenotypic testing; and to BD for supplying the Phoenix PMIC-84 AST panels used in this study. Part of this work was presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC, USA, 2014, poster number D-868.

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Hugh Bloore Memorial Poster Award available at SPC 2015

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ORIGINAL ARTICLE

Cryptococcus neoformans infection among human immunodeficiency virus patients on highly active antiretroviral therapy in Benin City, Nigeria

Christopher A Egbe, Richard Omoregie and Otibhor Alex-Ighodalo

University of Benin Teaching Hospital, Benin City, Nigeria

ABSTRACT

Objective: *Cryptococcus neoformans* infection is a well-recognized AIDS defining illness among HIV patients. Against the background of no data on *C. neoformans* infection among HIV patients in Nigeria, we determined its prevalence among HIV patients on highly active antiretroviral therapy (HAART).
Methods: Blood samples were collected from 333 HIV patients on HAART (156 males; 177 females) and analysed for cryptococcal capsular antigen and CD4 count using standard techniques.
Results: Gender was not a significant risk factor for acquiring cryptococcal infection (OR = 1.40; 95%CI: 0.67-2.92, P = 0.471). The prevalence of cryptococcal infection was not significantly affected by age (P = 0.302). A CD4 count of <200 cell/µL was a significant risk factor for acquiring cryptococcal infection among HIV patients on HAART (OR = 4.06; 95%CI: 1.89-8.71, P = 0.0003).
Conclusion: An overall prevalence of 9.91% of *C. neoformans* infection among HIV patients on HAART was observed. HIV patients with a CD4 count of <200cell/µL had approximately a 2–9 fold increased risk of developing cryptococcal infection. The data presented will be useful in the epidemiology and management of *C. neoformans* infections among HIV patients on HAART.
Keyword: *Cryptococcus neoformans,* HIV, HAART, Nigeria.

N Z J Med Lab Sci 2015; 69: 21-23

INTRODUCTION

Infection with the pathogenic yeast *Cryptoccocus neoformans* is a well-recognized complication of immunosuppression (1). *C. neoformans* meningitis occurs in 30% - 50% of human immunodeficiency virus (HIV) infected individuals in sub-Saharan Africa and the developing world (2). A recent analysis estimated that each year there are >900,000 cases of and 600,000 deaths due to cryptoccocal meningitis globally, with most cases occurring in sub-Saharan Africa (2).

Cryptococcal meningitis is a common opportunistic infection and AIDS–defining illness in patients with late stage HIV infection, particularly in Southeast Asia and Southern and East Africa. Cryptococcal meningitis also occurs in patients with other forms of immunosuppression and in apparently immunocompetent individuals. In parts of sub-Sahara Africa with the highest HIV prevalence, cryptococcal meningitis is now the leading cause of community–acquired meningitis ahead of *Streptococcus pneumoniae* and *Neisseria* meningitis (3).

In HIV infected patients, cryptococcosis is associated with CD4 counts of <200 cell/µL (2,3). In the highly active antiretroviral therapy (HAART) era, the prevalence of *C. neoformans* infection had been reported to decrease (4). To our knowledge, there is no report on *C. neoformans* infection in our Institution and records of cerebrospinal fluid among non–HIV individuals in the last 5 years has not reported *C. neoformans* as being the cause of meningitis. Although HAART has been reported to decrease the prevalence of *C. neoformans* infections, it carries the additional burden of immune-reconstitution inflammatory syndrome in patients with *C. neoformans* infections (4). To our knowledge there are no reports of *C. neoformans* infections among HIV patients, especially those on HAART. Therefore, we determined the prevalence of *C. neoformans* infections among HIV patients on HAART, as well as the effect of age, gender and CD4 count on this prevalence.

MATERIALS AND METHODS

Study area

This study was carried out between 1st February 2010 to 31st January 2011, at the University of Benin Teaching Hospital. The hospital is a tertiary hospital with a referral status and centre for the United States President's Emergency Plan for AIDS Relief (PEPFAR) and Institute of Human Virology, Nigeria (IHVN) HIV/AIDS intervention. The intervention involves free HIV screening, treatment and management of HIV patients.

Study population

A total of 333 HIV patients on HAART for 3 – 6 months were recruited for this study. The age range of the patients was 21 – 60 years and consists of 156 males and 177 females. The HAART regimen included zidovudine, nevirapine and stavudine. All patients had signs and symptoms of *C. neoformans* infections such as fever, headache, photophobia, phonophobia, cough, and altered mental status including personality changes. Informed consent was obtained from all patients prior to specimen collection. The Ethical Committee of the University of Benin Teaching Hospital approved the protocol for this study.

CD4 and cryptococcal capsular antigen testing

Ten ml of blood was aseptically collected from each patient and dispensed into ethylenediamine tetracetic acid (EDTA) and plain vacutainers. The EDTA sample was used to determine CD4 cell counts by flow cytometry (Partec, Germany) following the manufacturer's instruction. Briefly, 20 µl of whole blood was placed in a Partec tube, and 20 µl of CD4+ T cell monoclonal antibodies was added. The mixture was then incubated in the dark for 15 minutes at room temperature after which 800µl of buffer was added. The tube was then placed in the flow cytometer for counting and the CD4+ T cells value obtained from a programmed computer connected to the instrument.

Sera obtained from the clotted samples in plain containers were used to detect cryptococcal capsular antigen using the latex-cryptococcus antigen detection system (Immuno-Mycologic, Inc, Norman, USA) following the manufacturer's instructions.

Statistical analysis

The data were analyzed with Chi square (X^2) or Fisher's exact test and odds ratio analysis using the statistical software, INSTAT® (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

The prevalence of cryptococcal infection was higher in females (11.3%) than in males (8.33%), Although female gender appeared to be a risk factor for cryptococcal infection, this failed to reach statistical significance (Table 1). The prevalence of cryptococcal infection decreased with age from 14.04% in the 21 – 30 years age group to 6.90% in the 41 – 50 years age group, rising slightly to 7.02% in the 51 – 60 years age group. Age did not significantly affect the prevalence of cryptococcosis. Patients with CD4 cell counts of < 200 cell/µL had a significantly higher prevalence of cryptococcal infection than those with CD4 cell counts of ≥ 200 cell/µl, and CD4 counts of < 200 cell/µL was associated with cryptococcal infections among HIV patients on HAART (Table 1).

Table 1. Effect of gender, age and CD4 count on the prevalence of cryptococcosis among HIV patients on HAART

Characteristics	No. tested	No. positive (%)	OR	95% CI	P
Gender					
Male	156	13 (8.3)	0.714	0.342,1.487	0.471
Female	177	20(11.3)	1.401	0.672,2.920	
Age (years)					
21 – 30	57	8 (14.0)			0.302
31 – 40	103	13 (12.6)			
41 – 50	116	8 (6.9)			
51 – 60	57	4 (7.0)			
CD4 count (cell/µL)					
< 200	121	22 (18.2)	4.06	1.893,8.708	0.0003
≥ 200	212	11 (5.2)	0.246	0.115,0.528	

OR = odds ratio; CI = confidence interval.

DISCUSSION

The incidence of infections caused by the encapsulated yeast *C. neoformans* has risen markedly over the past 20 years as a result of the HIV epidemic and increasing use of immunosuppressive therapies (3). Although incidence and mortality have decreased in the era of HAART, cryptococcal meningitis remains an important cause of mobility and mortality in the AIDS population especially in the developing world (4).

An overall prevalence of 9.91% of cryptococcal infection among HIV patients on HAART was observed in this study. This is higher than 3.2% observed among HIV patients from sub-Sahara Africa (5). This is the first report of HIV–associated cryptococcosis in our Institution and perhaps in Nigeria. It has been reported that in countries with a large access to anti-retroviral therapy, overall mortality and incidence of AIDS-defining opportunistic infections have been reduced dramatically (6), and indeed a 46% reduction in cyptococcosis in the HAART era was reported in France (7). The prevalence of 9.9% observed in this study is, however, lower than a prevalence of 30% in individuals of African origin in France (7). Although being of African origin has been reported to be associated with an increased risk of cryptococcosis in the HAART era (7), geographical location and time of analysis may play a more important role on the prevalence of cryptococcosis among HIV patients as the prevalence obtained in our study was lower than previously reported (2).

Age and gender did not significantly affect the prevalence of cryptococcosis among HIV patients on HAART. Older age (35 – 45 years) was reported to associated with cryptococcosis among HIV patients on HAART (7). This was not observed in our study. However, CD4 counts of < 200 cells/µL was significantly associated with cryptococcosis. This agrees with previous reports (1,2). It is important to note that those studies (1,2) did not state whether their patients were on HAART. HAART has been reported to improved CD4 counts (8).However, patients on HAART that still have low CD4 counts (< 200 cell/µL) are at an increased risk of developing opportunistic infections and in our study *C. neoformans* infections.

It has been reported that some of the antiretrovirals used in the HAART regime interact with antifungal agents used to treat cryptococcosis and HIV patients with cryptococcal meningitis who initiate antiretroviral therapy are at particularly high risk for the immuno-reconstitution inflammatory syndrome (IRIS) (4). Physicians should bear these in mind as they manage cryptococcal infections in HIV patients and indeed some have suggested that HAART should be initiated 2 – 6 weeks after commencement of antifungal therapy in an effort to reduce the occurrence of IRIS (4).

Our study underscores the importance of cryptococcal infections among HIV patients on HAART where an overall prevalence of 9.9% was observed and HIV patients on HAART with CD4 counts of < 200 cells/µL had a 2 to 9 fold increased risk of developing cryptococcal infections.

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South Pacific Congress 2015
Social Programme

Icebreaker / Opening of the Exhibition	
The welcome function will give you the opportunity to catch up with friends and colleagues whilst enjoying drinks and nibbles. It is also a special time for our exhibitors to host this function around their exhibits.	
Date	Tuesday 18 August 2015
Time	6.30pm to 8.30pm
Cost	Complimentary to full delegates, sponsors and exhibitors $56 per additional ticket
Poster Session	
Date	Wednesday 19 August 2015
Time	5.00pm to 6.00pm
Cost	Complimentary to full delegates, Wednesday delegates, sponsors and exhibitors $35 per additional ticket
Authors	Poster authors will be available to speak about their work
Congress Dinner	
Date	Thursday 20 August 2015
Time	7.00pm – midnight
Venue	The Langham
Entertainment	To be advised
Theme	Art Deco 1930s
Cost	Included within the cost of registration for full delegates only $120 per additional ticket

CASE STUDY

Hodgkin and Reed-Sternberg cells in bone marrow aspirations of a patient with advanced classical Hodgkin lymphoma

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ABSTRACT

Classical Hodgkin lymphoma (CHL) is a unique type of lymphoma because of the extraordinary and unexplained scarcity of its neoplastic Hodgkin Reed-Sternberg (HRS) cells that derived from clonal germinal center B cells with rearranged immunoglobulin genes bearing crippling mutations. The occurrence of these cells in the bone marrow aspirations are considered rare. Their presence is an expression of widely disseminated disease and it indicates poor prognosis. We report a case of a 24-year-old female with relapsed Hodgkin lymphoma, after eleven years in remission with the standard chemotherapy regime. She was initially diagnosed during childhood with CHL stage IIIB. On this current presentation, she was noted to have cervical lymphadenopathy during her antenatal check-up. The lymph nodes biopsy confirmed relapse of the disease; however, there was no evidence of bone marrow infiltration. She was given various chemotherapy regimes in which she was refractory to. At this point, repeated bone marrow aspiration interestingly revealed the presence of HRS cells. The immunophenotyping analysis by flow cytometry revealed a small population of cells expressing CD20, CD15, and CD30 that further supported the presence of HRS cells in the bone marrow aspirate. The bone marrow biopsy confirmed infiltration of scattered Reed-Sternberg cells and mononuclear Hodgkin cells in a reactive background. In conclusion, we highlight the presence of the rare HRS cells in this patient with advanced relapsed Hodgkin lymphoma.

Keywords: Hodgkin Reed-Sternberg cells, classical Hodgkin lymphoma, bone marrow aspirates, flow cytometry.

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INTRODUCTION

Hodgkin Lymphoma (HL) is a group of lymphomas of mainly nodal origin with similar clinical and histological features (1). HL encompasses 95% of classical Hodgkin lymphomas (CHL) and the rest are of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). These two entities differ in their aetiology, clinical features, histopathological features, immunophenotype and molecular genetic features. NLPHL is an uncommon monoclonal B cell neoplasm characterized by large neoplastic cells known as popcorn or lymphocyte predominant (LP) cells in an inflammatory background. NLPHL is considered to be of germinal centre B-cell origin. It is managed differently from classical HL. The prognosis is good; however, late relapses and transformation to high grade non-Hodgkin lymphoma can occur (1,2).

Classical Hodgkin lymphoma (CHL) is a monoclonal lymphoid neoplasm representing less than 1% of all *de novo* neoplasms occurring each year worldwide (2). It is unique among lymphomas due to the distinctive malignant multinucleated Hodgkin Reed-Sternberg (HRS) cells as well as the mononuclear Hodgkin cells (2,3). Generally, there are four subtypes of CHL; nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted. These subtypes may differ in the clinicopathological aspects, however, the immunophenotype of the tumor cells remain the same. The distinctive Reed-Sternberg and Hodgkin cells are nearly always CD30 positive. Most of the CHL cases are also CD15 positive whilst CD20 is positive with varied intensity in 30-40% of cases.

Hodgkin cells and Reed-Sternberg (HRS) cells are derived from the same clonal B cells population. The rearranged immunoglobulin (Ig) genes of the tumor cells harbor a high load of somatic hypermutations in the variable region of the Ig heavy chain genes usually without signs of ongoing mutations. These clonal rearrangements are usually detectable only in the DNA of isolated single HRS cells and not in whole tissue DNA (2).

Hodgkin lymphoma (HL) is a curable disease in adults. With modern treatment strategies majority of the HL patients with various anatomical stages and histological subtypes can be cured (4). Improvement in its diagnosis and staging, treatment of the disease, as well as its supportive management, has contributed in the outcome of the disease (5). Nevertheless, relapse or progression of the disease after the initial treatment still occurs in 20-30% of the patients. The pathogenesis and mechanism of treatment failure are still not well understood partly due to the difficulties in studying the molecular phenotype of the rare malignant HRS cells (3).

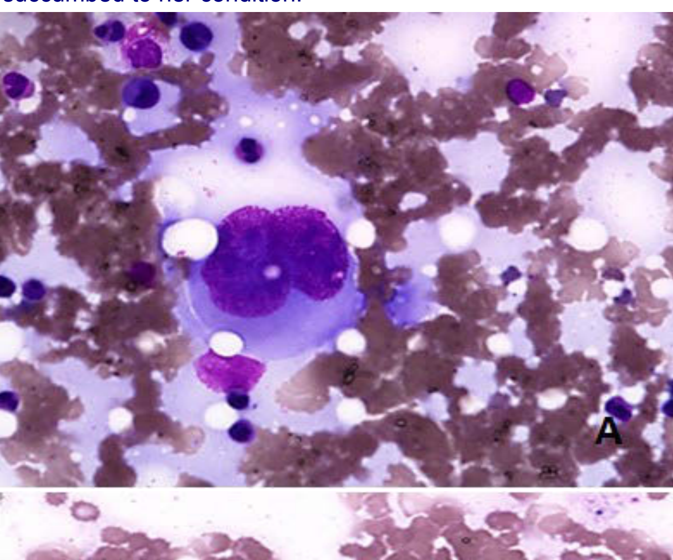
We report a case of advanced CHL with presence of Reed-Sternberg cells in the bone marrow where the diagnosis of CHL stage IIIB was made during childhood period. She was treated successively with chemotherapy. However, the disease relapsed and these cells were demonstrated in her bone marrow samples only after the disease had disseminated and became refractory to chemotherapy.

CASE REPORT

A 24-year-old Chinese female was first diagnosed with classical Hodgkin lymphoma at the age of 11 years. She was treated with the standard ABVD regime (Adriamycin, bleomycin, vinblastine, dacarbazine) and achieved remission for 11 years until she presented to us again with a mass in the neck during pregnancy. One month post-partum, a biopsy from the cervical lymph node was performed to reveal relapsed CHL, mixed cellularity subtype. The computed tomography (CT) scan demonstrated extensive cervical, mediastinal, axillary, abdominal, and inguinal lymphadenopathy. At this point of time, bone marrow examination showed no evidence of lymphomatous infiltration. The patient was treated with five cycles of BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) chemotherapy. Mid-cycle assessment with PET scan 5 months later showed evidence of active lymphoma in the chest node and bone involvement. In view of these findings, the chemotherapy regimen was changed to ICE (ifosfamide, carboplatin, etoposide) and the patient was planned for autologous stem cell transplantation (ASCT). Following completing two cycles of ICE, stem cells harvesting was attempted twice but unfortunately failed. In view of disease progression, as evidenced by CT scan findings that demonstrated worsening of the mediastinal, intra-abdominal lymphadenopathy with lung, spleen, liver and urinary bladder infiltration, chemotherapy regimen was escalated to modified IGEV (ifosfamide, gemcitabine, vinorelbine).

Half way through this chemotherapy regime, her full blood count exhibited mild anemia, mild leukocytosis, and severe thrombocytopenia (haemoglobin: 111.0 g/L; MCV: 87.6 fl; MCH: 30.1 pg; white cells count: 10.1x 10^9/L; platelet count: 4 x 10^9/L). The full blood picture showed occasional nucleated red cells and a few atypical lymphocytes but no abnormal lymphoid cells. There was no leukoerythroblastic picture noted. However, the bone marrow aspirates demonstrated a normocellular marrow but there was an occasional presence of very large cells. These cells exhibited binuclearity with inclusion-like nucleoli and abundant cytoplasm resembling the typical Reed-Sternberg (RS) cells (Figure 1 A & B). There was also the presence of mononuclear Hodgkin cells, which are large cells of a similar appearance but with a single nucleus. Interestingly, the immunophenotyping analysis demonstrated a small abnormal population (2.39%) gated at the CD19 positive area which showed positivity for CD45 (dim), CD20 (dim), CD15, and CD30. This small population consisted of large cells with complex characteristics as evidenced from the forward and side scatter gating (Figure 2). The bone marrow biopsy showed infiltration by scattered Reed-Sternberg cells and mononuclear Hodgkin cells in reactive background consisting of small lymphocytes, epithelioid histiocytes, and occasional neutrophils and eosinophils (Figure 3). The immunohistochemistry staining results showed these cells to be CD30+ (strong and diffuse); CD15 and PAX5 were positive in a few of these cells. However, they were negative for CD20. The reticulin stain exhibited coarse fibre network. These findings were concluded as bone marrow infiltration by classical Hodgkin lymphoma.

Her condition took a downfall when she developed sepsis secondary to the infected chemoport site, thus chemotherapy was delayed. Furthermore, her serum alkaline phosphatase and bilirubin levels were increasing in trend. Urgent ultrasound revealed hepatosplenomegaly and multiple ill-defined hypoechoic lesions within the spleen, which may represent multiple abscesses or lymphomatous infiltration. Later, she developed acute renal failure, which resulted in alteration in her level of consciousness and a few episodes of seizures. Owing to the clinical features coupled with the full blood picture findings of microangiopathic haemolytic anaemia (MAHA), she was treated for thrombotic thrombocytopenic purpura (TTP) and underwent three cycles of plasmapheresis. A CT brain scan was done and demonstrated left frontal infarct with haemorrhagic transformation as well as marked perilesional oedema and mass effect. However, lymphatic tumoral infiltration could not be excluded. Eventually, she developed multiorgan failure and succumbed to her condition.



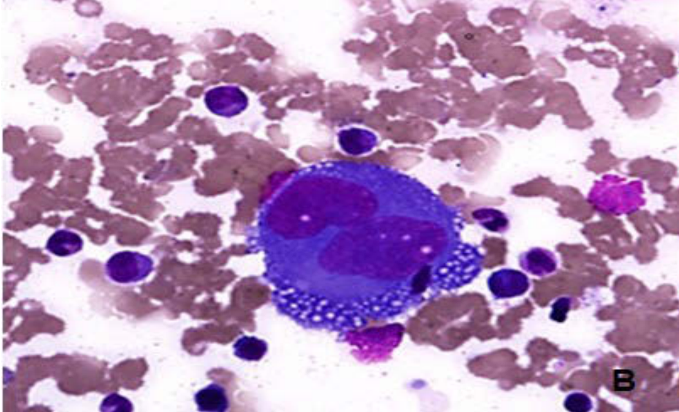


Figure 1. Bone marrow aspirates (A & B) showing Reed-Sternberg cells- very large cells that exhibit paired nuclei with inclusion-like nucleoli and abundant cytoplasm. MGG x600.

DISCUSSION

Neoplastic cells, either the classical Reed-Sternberg (RS) cells or the Hodgkin cells characteristically represent only a minority of the cellular infiltrate with an incidence ranging from 0.1- 10% (2). Nevertheless, the confirmation of HL requires morphologic diagnosis of the neoplastic cells with the appropriate cellular background along with the result of immunophenotyping as cells resembling Reed-Sternberg cells can be found in cases of B and T lymphomas, melanomas, sarcoma, and in some reactive conditions such as infectious mononucleosis, which are common in populations across the globe (6).

In 1998, Küppers *et al*. performed molecular studies in a single cell of HRS cells in HL (7). Their study showed that HRS cells in CHL, as well as NLPHL, originate from the germinal center (GC) B cells in most cases, if not all. HRS cells in NLPHL represents transformed antigen-selected GC B cells with evidence of ongoing immunoglobulin (Ig) V gene mutation. Whereas HRS cells in CHL appear to often or always derive from GC B cells that have lost the capacity to express a functional antigen receptor. Piccaluga *et al*. (6) also mentioned that HRS cells are sustained by an autocrine and/or paracrine production of several cytokines, including IL-5, IL-8, IL-9, CCL- 5, and CCL-28. The release of these molecules is also accountable for most of the symptoms observed in patients with HL, in addition to the ability of the neoplastic cells to escape from growth controls and immunosurveillance.

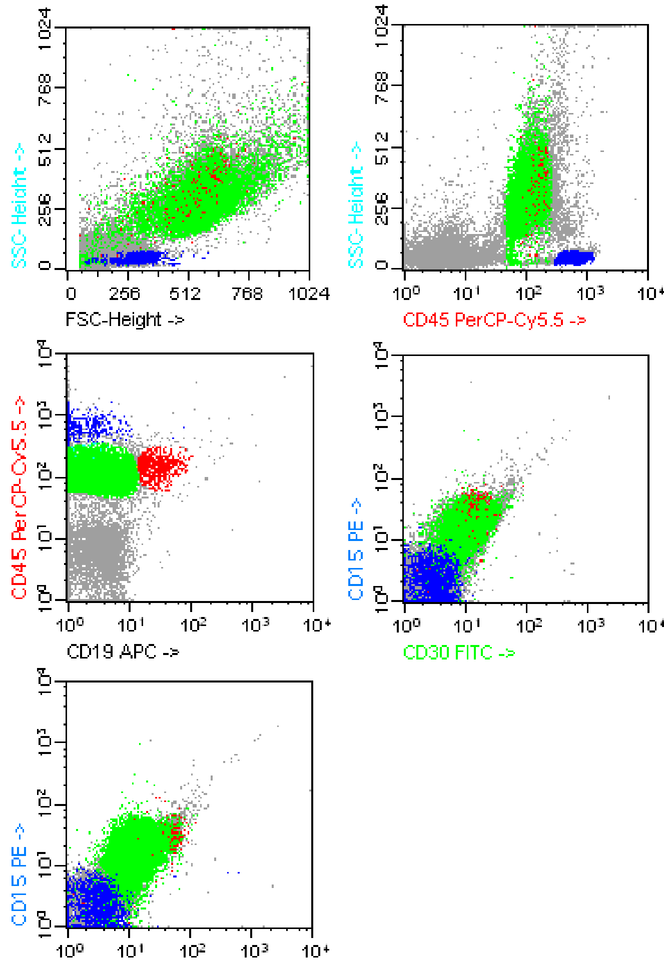


Figure 2. Flow cytometry immunophenotyping analysis showing the small (2.39%) abnormal populations (highlight-red). These cells expressed CD45 (dim), CD19, CD20, CD15, and CD30.

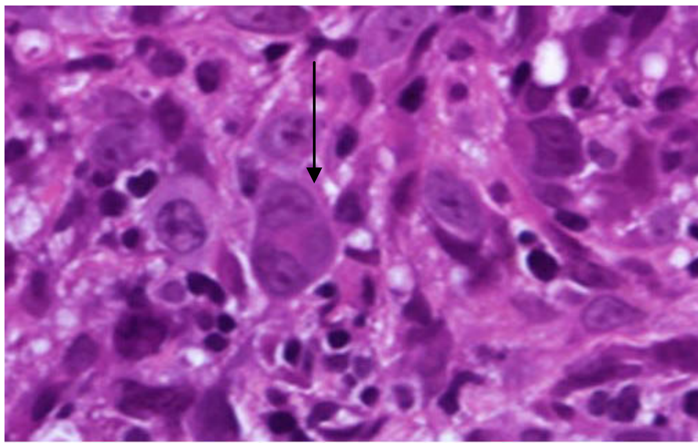


Figure 3. Bone marrow biopsy section showing focally hypercellular marrow space displaying infiltration by scattered Hodgkin cells and Reed-Sternberg cells (arrow). H&E x 600.

Bone marrow involvement is rare in patients with HL. Its incidence varies between 4% and 14% in the series reported during the past 20 years (8). As the bone marrow lacks lymphatics, infiltration of the bone marrow by Hodgkin's lymphoma indicates vascular dissemination of the disease (stage IV). The incidence of bone marrow involvement in Hodgkin's lymphoma varies with the histologic subtype: 10% in classical Hodgkin's mixed cellularity, approximately 1% in lymphocyte predominant and lymphocyte rich CHL, and 3% in nodular sclerosis subtype (9). The reason for this rarity can be due to the scattered focal lesions which may not be aspirated by the bone marrow aspirate needle, and also in HL the bone marrow tends to have fibrous tissue, making their aspiration more difficult (10). Fibrosis is a common finding in Hodgkin's lesions in the bone marrow and is not limited to nodular sclerosis or lymphocyte depletion variants (9). RS cells are found mainly in bone marrow of patients with generalized advanced stages of HL (10). Thus, the presence of RS cells in bone marrow is an expression of widely disseminating disease (11). Therefore, features of diffuse fibrosis associated with polyploid RS cell variants or large abnormal mononuclear cells with huge nucleoli are sufficient evidence for the determination of marrow involvement on random biopsy (11).

Morphologic findings and immunohistochemical stains are vital in the diagnosis of CHL (12). In the majority of cases, flow cytometry analysis is of little or no value to the detection of HRS and the diagnosis of CHL, as neoplastic cells are rarely seen in the cytological preparations (1), and it usefulness is limited to tissue samples such as lymph nodes (12). Recently, Fromm and Wood (13) described a method of identifying HRS cells in lymph nodes by flow cytometry using a single-tube (6-colour assay) with high sensitivity and specificity. Also, they proposed that this method might obviate the need for immunohistochemistry in many cases. Furthermore flow cytometry offered several potential benefits in the diagnosis of CHL in which it is more sensitive in equivocal or possibly negative cases by morphology. It has a rapid turnaround time with significant cost effectiveness compared to the elaborate immunohistochemical panels. In our present case we have concluded that there was a presence of HRS cells in our analysis using a 4-colour assay flow cytometry in the aspirate samples.

According to the WHO classification (2), Hodgkin and Reed-Sternberg cells are almost always positive for CD30 and positive for CD15 in 75-85% of cases. Both CD30 and CD15 are typically present in a membrane pattern increasing in the Golgi area. For B-cell markers, HRS cells are positive for CD20 on a minority of the neoplastic cells with varied intensity in 30-40% of cases, CD79a is less often expressed. The B-cell nature of HRS cells can be demonstrable in approximately 95% of cases by their expression of the B-cell specific activator protein PAX5 and it is usually weaker than that of reactive B cells. Even though HRS cells are usually negative for CD45 (2), the total absence of CD45 is unlikely (14).

CD20 is important in the regulation of human B-cell growth and differentiation. It is present on most mature normal and neoplastic B lymphocytes (15). The prognostic significance of CD20 expression in CHL is controversial and a matter of ongoing debate. A review of the literature showed that the expression of CD20 was not associated with different clinical and laboratory features among equivalently treated patients (15) and has no prognostic significance for the failure-free survival and overall survival in CHL patients (15,16). One study on 248 CHL patients found that failure-free and overall survival were reduced considerably in CD20-positive patients as compared with CD20-negative patients (17). Whereas in another study of 119 CHL patients showed a significantly higher frequency of disease relapses in the CD20-negative group and a better failure-free and overall survival in the CD20-positive group (18).

Previously, bone marrow biopsy (BMB) was the recommended approach for staging in newly diagnosed patients with HL. Recently, positron emission tomography/computed tomography (PET/CT) is required for staging and response assessment in lymphoma according to the recommendations whenever it is available (19-21). Studies have shown that conventional staging can detect bone marrow involvement in only 5–8% of patients, whereas PET/CT staging can detect bone marrow involvement in up to 18% of the cases (21).

Moreover, HL is [18F] fluorodeoxyglucose - avid almost all, thus PET-CT using FDG is more accurate than CT for staging in HL and NHL with increased sensitivity, particularly for extranodal disease (20).

Furthermore, PET/CT leads to change in stage in 10% to 30% of patients, more often upstaging, although alteration in management occurs in fewer patients, with no demonstrated impact on overall outcome (19). El-Galaly *et al.* (22), in a cohort study, investigated whether BMB can add useful information to FDG-PET/CT staging in patients with HL. They concluded that PET/CT can accurately detect marrow involvement therefore the evaluation by BMB is often unnecessary. Likewise, Adams *et al.* made the same observation for the appropriate use of FDG-PET/CT to replace BMB in newly diagnosed HL (23). They also mentioned that the major advantages of FDG-PET/CT over BMB are the fact that it is non invasive and able to visualize the entire bone marrow therefore eliminating any sampling errors. Nevertheless, biopsy is recommended to confirm residual disease and to exclude false-positive uptake with FDG before starting second-line therapy (21).

We reported a case of refractory classical Hodgkin lymphoma in which Hodgkin Reed-Sternberg (HRS) cells were only demonstrated in bone marrow aspirations and trephine biopsy sections only when the disease disseminated. The patient's bone marrow aspirations revealed the typical morphologic characteristics of Reed-Sternberg cells. Whereas, the trephine biopsy sections showed infiltration by scattered Reed-Sternberg cells and mononuclear Hodgkin cells in reactive background. Immunophenotyping analysis by flow cytometry and immunohistochemistry confirmed the presence of HRS cells.

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SCIENTIFIC LETTER

A modified protocol for the direct identification of positive blood cultures by MALDI-TOF MS

Michael Sun
Labtests, Auckland

Rapid identification of organisms in positive blood cultures reported with appropriate antibiotic susceptibility testing is an important function of the clinical microbiology laboratory (1). Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has recently been introduced into clinical microbiology laboratories in New Zealand and overseas. MALDI-TOF uses soft ionisation technology to generate single-charged protein ions, which pass a high vacuum electromagnetic field with different flight times that are captured by a detector to produce an organism's proteomic fingerprint profile. This innovative technique has been called revolutionary in microbiology, being simple, fast, accurate and cost effective (2).

MALDI-TOF is primarily used to identify colonies growing on agar media. However, its ability to identify organisms from broth, such as blood cultures, can lead to a reduction in the turn -around time for targeted treatment of blood stream infection. Several protocols have been reported for the rapid direct detection of microbes from positive blood culture vials, including a centrifugation/washing method, a lysis solution method and a gel-based tube method (3,4,5). The aim of this study was to compare direct MALDI-TOF MS identification of positive blood cultures using an optimised dual protocol, with the conventional method.

From August to November 2014, 52 positive blood culture samples (26 BACTEC plus Aerobic/F blood culture vials, 26 BACTEC lytic 10 Anaerobic/F blood culture vials) were sub-cultured onto agar plates and incubated overnight to obtain bacterial colonies. The colonies were identified by Bruker microflex™ LT MALDI-TOF (Bruker Daltonics, Bremen, Germany). Additionally, all blood culture broths were processed directly by the following modified in-house protocol.

Flagged positive blood culture broths (6ml from aerobic vials, 10ml from anaerobic vials) were injected into BD Vacutainer tubes (SSTII gel tube for aerobic vials, non-additive Z tube for anaerobic vial), followed by centrifugation (2,000g and 10 min for aerobic vials; 4,000g and 10 min for anaerobic vials). The supernatant was carefully pipetted off without disturbing the sediment layer below, 2ml distilled water added and gently mixed until cloudy; and 1.5ml of this suspension was then transferred to a micro centrifuge tube and spun at 13,000rpm for two minutes. After decanting off the supernatant, 250 µl of distilled water was added, mixed well and 750 µl of 100% ethanol was then added. After 10 minutes the mixture was centrifuged twice for two minutes at 13,000rpm. The supernatant was again discarded and the pellet dried in a biohazard-cabinet for five minutes until all the ethanol had evaporated. Then, 50 µl 70% formic acid was added, mixed well, and left for five minutes. This was followed by adding 50 µl of acetonitrile, thoroughly mixed and then centrifuged at 13,000rpm for two minutes. Finally, 1 µl of supernatant from the upper part of extraction was spotted onto a target plate in duplicate, air dried for five minutes, then 1 µl matrix HCCA (α-cyano-4- hydroxycinnamic acid with 50% acetonitrile and 2.5% trifluoroacetic acid) was overlaid and left to dry for 20 minutes. The target plate was then inserted in to the Bruker microflex LT for analysis. (Ion source voltages: 20kv and 18kv; laser shoots: 240 measurements per well; m/z range: 2,000 to 20,000; linear positive mode with delayed extraction).

According to the Bruker direct blood culture ID biotyper score system (species level ID ≥ 1.8; genus level ID ≥ 1.6) (4), when comparing direct MALDI-TOF identification from blood culture broth with routine MALDI on colonies, the overall concordance in this trial was 69% at the species level and 78% at genus level. 10 of 11 (91%) *Staphylococcus aureus* and 9 of 9 (100%) *Escherichia coli* were accurately identified (Table 1). No misidentification was observed.

A correlation was found between the microorganisms' physical characteristics and the optimal protocol parameters. Numerous studies have shown that Gram-negative bacilli ID scores are superior to Gram-positive cocci ID scores, particularly problematic streptococci (3). However, these studies did not use "flexible" assay conditions for those different organisms. With our optimised dual protocol, 6ml broth was used for Gram -negative bacteria in aerobic vials as with other protocols, but 10ml broth was used for Gram-positive cocci in anaerobic vials (6). In contrast to 10 minutes at 2,000g centrifugation for Gram-negative bacteria in other studies, in order to harvest enough bacterial pellet for MALDI-TOF we applied 10 minutes at 4,000g to anaerobic vials with Gram-positive cocci, as they have a smaller size and different biomass (7,8). As shown in the table, six *Streptococcus pneumoniae* and two α-haemolytic *Streptococcus spp.* Isolates scored over 2.0 by applying this protocol.

Another important part of the protocol is the optimisation of the manufacturer's recommended ethanol FA/ACN full extraction method. To date, previous studies followed the manufacturer's recommended method, i.e. without an incubation time for ethanol or the FA extraction steps (3,5). However, an additional 10 minute incubation for ethanol protein precipitation and an additional five minutes incubation time for FA cell lysis were found to improve the test performance. Extended conditions, i.e. 20 minutes of 75% ethanol and 10 minutes of FA incubation, may be useful for Gram-positive bacilli which have thick and tough cell walls, such as *Clostridium perfringens* (See Table).

This study was limited by the small numbers of positive blood cultures. The optimized procedure only requires one to two hours, and can be done the same day the bottles signal as positive. In addition, with the ID, AST testing can occur earlier, which facilitates correct antibiotic selection, particularly where resistance is a concern.

In summary, this pilot study investigated a modified rapid direct identification protocol for positive blood cultures and confirmed it is a promising approach to reduce laboratory turnaround time and consequently to improve blood stream infection management.

Table 1. ID results by direct ID protocol and routine culture protocol using MALDI-TOF

MALDI-TOF direct blood culture ID results and Biotyper score						Routine MALDI-TOF Colony ID (number of isolates)
	Species ID Score ≥2	Species ID Score 1.9-1.8	Genus ID Score 1.7-1.6	Unreliable ID (<1.5)	No Peaks	
Staphylococcus aureus	8	2	1			*Staphylococcus aureus* (11)
*Staphylococcus epidermidis**		2	1			*Staphylococcus epidermidis* (3)
*Staphylococcus capitis**	1					*Staphylococcus capitis* (1)
*Staphylococcus hominis**	1	1	1			*Staphylococcus hominis* (3)
Streptococcus dysgalactiae	1					*Streptococcus Dysgalactiae* (1)
Streptococcus pneumoniae	6			4		*Streptococcus Pneumoniae* (10)
a-haemolytic *streptococcus spp.*	2	1	1	3	3	a-haemolytic *streptococcus spp.* (10)
Enterococcus faecalis			1	1		*Enterococcus faecalis* (2)
Escherichia coli	9					*Escherichia coli* (9)
Clostridium perfringens	1					*Clostridium perfringens* (1)
Polymicrobial contaminated sample†					1	Mixed organisms in plates, No process (1)
Total	29	6	5	8	4	52

*Coagulase Negative Staphylococcus group. † Not included in study statistics.

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News from the universities

MASSEY UNIVERSITY

NZIMLS 2014 BMLSc Top Student

Ashleigh Renwick was the top third year BMLSc student last year so has won the NZIMLS Prize for 2015. She is a most deserving recipient since she scored a perfect eight A+ for her eight subjects, the first BMLSc student to achieve this at Massey!

Ashleigh Renwick

I am Ashleigh Renwick and I'm a fourth year medical laboratory student at Massey University. I started my schooling at Pahiatua Primary School where I received my first Team CREST award after completing a group project in 2004. I then went on to attend Tararua College, also in Pahiatua, where I studied a wide range of subjects including accounting, art, biology, chemistry, and physics. I completed NCEA Level 1 and 2 with Merit and Level 3 and received the Massey University Vice Chancellor's High Achievers Scholarship (Academic) in 2013. I also completed Team Bronze, Silver, and Gold CREST projects and became the first person in New Zealand to receive all four team CREST awards. I have always been interested in science, in particular biology and chemistry, so after finishing college I wanted to continue studying science at Massey University. I heard about the Bachelor of Medical Laboratory Science after attending a careers exhibition and found it intriguing. I wish to complete my fourth year placement in the haematology and microbiology fields at Medlab Central in Palmerston North. After completing my degree, I wish to find a position in one of these areas before considering any further study. My other interests include supporting my brothers when they are racing stockcars at speedway, drawing, reading, and photography.

UNIVERSITY OF OTAGO

The BMLSc class of 2014 graduated on 6th December, and this year saw a record number of students graduating with distinction or credit. They were:

BMLSc with Distinction

Nicola Collins
Avnish Goyani
Isuri Hapuarchchi
Natasha Henden
Suzanne Kwok
Jordyn Lamont
Jaqy Lim
Aimee Sanders

BMLSc with Credit

Katie Anderson
Sally Annan
Jessica Carmichael
Mathew Duggan
Amanda Fraser
Tian Gao
Alexandra George
Joel Harris
Philip Ibrahim
Michael Kwong
Maia Marino
Jessica Olsen
Farah Rashid
Emi Sekine
Russell Stirling
Madeleine Ruscoe

Prize winners

Prize	Winner
Roche Diagnostics Haematology Prize:	Avnish Goyani
AACB Chemical Pathology Prize:	Suzanne Kwok
NZIMLS Prize for top 4th year student:	Jordyn Lamont
Colin Watts Prize for top student overall (over 3 years):	Jordyn Lamont
Prince of Wales Nomination:	Suzanne Kwok and Jordyn Lamont

Interview with Jordyn Lamont, NZIMLS prize winner for top 4th year student

What made you decide to become a medical laboratory scientist?

I have always wanted to be able to do something medical related and I loved the laboratory, so it made sense. It was the perfect combination of the two! Also my high school had a career day that took us through the laboratories and I remember going through and thinking that it would be an awesome job!

You are originally from Nelson, what attracted you to Otago University?

I wanted to start studying broadly in the Medical field just to make sure I liked it, so I needed to do Health Science. Health Sciences down in Otago seemed like the best option for me, I also had the added bonus of Matt (my boyfriend) being already down there.

Which aspects of the course did you like best? Which aspects did you find most challenging?

I love haematology. The most challenging part would have been fighting the cold Dunedin weather to make it to lectures and labs!

What do you like about medical laboratory science? And haematology and histology in particular?

I love how medical laboratory scientists are "behind the scenes" in helping to find the presence or absence of disease in patients. I always wanted to be able to help in people's lives and I feel like it is a rewarding job in knowing that I am.

I love love looooove Haematology! It really clicked with me and it never felt like a chore studying. I am always fascinated at how so much about a person's health can be determined by the shape, colour, type and quantity of blood cells. As for histology, I found it rather therapeutic cutting sections and staining the slides. There are some really beautiful stains that can be done.

Your clinical placements were in Nelson. How did you find that experience, in a smaller lab?

I loved working in Nelson. I was able to get to know everyone in the lab across all the disciplines in a short time period. I particularly enjoyed seeing how all the disciplines can work together.

What are you up to now?

Since graduating my boyfriend and I set off on a journey to South America to do amazing things like walk the Inca Trail to Machu Picchu in Peru and bike Death Road in Bolivia. However, I am now back in New Zealand and applying for jobs all over the place hoping to start my working career soon.

What are your plans for the future?

I would love to work preferably in haematology reading blood films and sending out the reports. However, for now I would be happy with securing any scientist role so I can start saving for my next adventure!

New Director for Medical Laboratory Science

Dr Heather Brooks was welcomed as the new director for Medical Laboratory Science, on the 26th of January 2015. Dr Brooks is a medical microbiologist with both professional and academic training. She is a graduate of The University of Surrey, UK (BSc Hons) and St. Bartholomew's Hospital Medical School, University of London, UK (PhD). She is also an Associate of the Institute of Medical Laboratory Scientists (UK), gaining experience in diagnostic microbiology at Barts and Dulwich Hospitals. Dr Brooks is passionate about student education and is an award-winning teacher. She has taught at two other New Zealand Universities (Massey and Auckland) and has lectured in the University of Otago Medical Laboratory Science programme since 1993. She currently convenes the medical microbiology courses in years 3 and 4 of the BMLSc degree and in year 5 the Postgraduate Diploma in Medical Laboratory Science. Dr Brooks' research interests are in the area of applied medical microbiology and she works collaboratively with the Department of Chemistry at the University of Otago and the Dunedin School of Medicine. She is looking forward to the challenges that her new role as Director of the Medical Laboratory Science programme will bring.

The top graduates in BMLSc for 2014 were Tony Cole and Samantha Smith. It was unusual to have two winners of this prize, but it proved impossible to separate Tony and Samantha in their outstanding academic performance throughout their degrees. The NZIMLS sponsored prizes were awarded at the AUT prize giving on 18th December by Ailsa Bunker. Tony is employed by LabPlus and Samantha by Pathlab Waikato. We wish Tony and Samantha well in their respective careers as medical laboratory scientists.

AUCKLAND UNIVERSITY OF TECHNOLOGY

AUT opened its Roche laboratory in June 2014. This laboratory is generously sponsored by Roche Diagnostics and houses two Cobas modular analysers, a Sysmex XN and real-time PCR suite. The equipment is in a new, purpose-built laboratory on AUT's city campus. Third year BMLSc students specialising in Chemistry, Immunology, Haematology and Molecular Diagnostics now have some of their classes in the Roche lab, training on this equipment. Training includes machine start-up, QC, running of routine assays, and trouble-shooting. The director of the laboratory is Dr Fabrice Merien; AUT Senior Lecturer in Immunology. Fabrice says *"When I started looking into a partnership with the industry, I thought AUT and Roche Diagnostics would be a perfect team because we want to give the best to our students."*

The University believes the experience gained in the laboratory will help the students with clinical placement and making the transition to employment upon graduation. They will be able to tell potential employers which machines they have worked with, and which assays they have run. AUT is very research-active, and the instruments in the Roche laboratory are also being extensively used by staff and graduate students in their various projects. AUT would like to thank Roche for its continuing generous support.

Below are the photos of Dr Fabrice Merien, and the students in the Roche laboratory at AUT.

NZIMLS Journal prize

The 2014 winner of the NZIMLS Journal Prize is Julie Creighton, Senior Medical Laboratory Scientist Microbiology Department, Canterbury Health Laboratories, Christchurch.

Julie's article, "Susceptibility testing of extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae against oral antimicrobials, including fosfomycin and mecillinam", was published in the April 2014 journal issue, vol. 68; pp. 19-23.

Council of the NZIMLS approve an annual Journal prize to the value of NZ$300 for the best article published in the Journal during the calendar year. The article can be a review article, original article, case study, research letter, or technical communication (excluded are Fellowship dissertations).

Many studies are presented at the Annual Scientific Meeting, SIG meetings, and the North and South Island Seminars, yet are rarely submitted to the Journal for wider dissemination to the profession.

Consider submitting your presentation to the Journal. If accepted, you are in consideration for the NZIMLS Journal prize and it will also earn you valuable CPD points.

Please contact the Editor or any Editorial Board Member for advice and help. Contact details are on the NZIMLS web site (www.nzimls.org.nz) as are instructions to authors.

No formal application is necessary but you must be a financial member of the NZIMLS to be eligible. All articles accepted and published during the calendar year (April, August and November issues) will be considered. The Editor, Deputy Editor and the President of the NZIMLS will judge all eligible articles in December each calendar year. Their decision will be final and no correspondence will be entered into.

NZIMLS NICE WEEKEND MAY 2014

The 25th National Immunohaematology Continuing Education (NICE) weekend was held over the 23rd to the 25th of May at the Bayview Wairakei Resort in Taupo. Over the weekend we were treated to 48 fantastic presentations and were able to view and discuss 14 poster presentations. All presenters did a fantastic job and the general consensus was that the standard of presentations delivered over the weekend was very high and a wide variety of presentation topics were covered.

Congratulations to all those attendees who took away awards for their presentations/poster

- The Abbot Award for best overall presenter went to Juliet Jaggard (NZBS Waikato) for her talk – No Blood Required
- The Ortho Clinical Diagnostics Award for Most Promising Transfusion Scientist went to Belinda Reilly (NZBS Waikato) – Rabies Immunoglobulin
- The Thermo Fisher Award for best poster went to Jovelyn Iringan (Middlemore Hospital) for her poster A Case of severe haemolysis due to Paroxysmal Cold Haemoglobinuria (PCH) requiring Plasma Exchange Therapy
- The CSL Biotherapies award for a NZ attendee to attend NICE Australia was this year won by Muni Knacker (NZBS Auckland)
- The award sponsored by Grifols for the best first time speaker. This is a presenter who has never attended NICE weekend before, giving them the title of NICEst Virgin went to Jane Phillips (Northland DHB) for her talk Hereditary Angioedema and Berinert
- Congratulations must also go to Ellen Jones (NZBS Wellington), Melissa May (NZBS Technical Services), Jessie Hugget (NZBS Dunedin) Kirsty Imazu (NZBS Wellington), Ruth Brookes (NZBS Palmerston North), Geri Dimitrova (NZBS Auckland), Lee Neale (Waitemata DHB), Leon Griner (NZBS Auckland), Stacey Lucas (Waitemata DHB) and Anastasia Osadchuk (ACT Pathology) who received special mention for their presentations

Celebrating 25 years of being NICE

Dianne Whitehead, Natalie Fletcher, Raewyn Cameron, Grant Bush

The statistics – NICE 2014 was attended by 79 people in total including 14 trade representatives, one sponsored convener, 64 delegates including one participating TMS, one TNS and one Australian visitor. Of these 49 were NZIMLS members and 28 where non-NZIMLS members.

The theme for this year's NICE weekend was Silver – 25 in acknowledgement of NICE's 25th Anniversary, all attendees looked fantastic in a wide variety of Silver themed costumes and a fun evening was had by all.

A special mention must go to Holly Perry who handed the role of TSSIG convenor over to Lorna Wall over the weekend. Thank you Holly, for all your years of outstanding leadership and support. Another big thank you to the judges who had a very tough role.

A huge thank you on behalf of all must be extended to our amazing NICE Convenors – Grace Agustin and Raewyn Cameron. They delivered yet another spectacular educational weekend, so on behalf of the TSSIG and the wider NICE group I would like to extend a huge Thank You. We look forward to NICE 2015 15th- 17th May 2015 with its Do-Re-Mi Musicals theme.

NICE 2015 prizewinners:
(Left to right) Juliet Jaggard, Muni Knacker, Jane Phillips, Jovelin Iringan and Belinda Reilly

Melissa May,TSSIG Committee member, June 2014

NZIMLS Barrie Edwards & Rod Kennedy Scholarship

The Barrie Edwards & Rod Kennedy scholarship is one of the most significant awards offered by the NZIMLS. The scholarship provides the winner with support to attend an international or national scientific meeting up to a maximum value of $7,500.00.

Application for this prestigious scholarship is invited from Fellows, Members and Associate Members of the NZIMLS. Applicants must be a current financial member of the NZIMLS and have been a financial member for at least two concurrent years prior to application. To be eligible applicants must make an oral presentation or present a poster as first author at their nominated scientific meeting.

All applications will be considered by a panel consisting of the President and Vice-President of the NZIMLS and the Editor of the New Zealand Journal of Medical Laboratory Science (who are ineligible to apply for the scholarships). The applications will be judged on your professional and academic abilities together with your participation in the profession. The panel's decision is final and no correspondence will be entered into.

Barrie Edwards

Application is by letter. Please address all correspondence to:

NZIMLS Executive Officer PO Box 505 Rangiora 7440

Rod Kennedy

There is one scholarship awarded in each calendar year. Closing date is December 20th in any given year.

In your application letter please provide the following details:

- Full name, position, work address, email address and contact phone number
- The length of time you have been a financial member of the NZIMLS
- The conference you wish to attend – please provide dates
- A budget comprising airfares, conference registration and accommodation costs
- The abstract of your intended oral or poster presentation and whether it has been accepted for presentation (proof required)
- Your intentions to publish your results
- State briefly your history of participation in the profession over the last 5 years
- State the reasons why you wish to attend your nominated scientific meeting

Successful applicants will be required to provide a full written report on return which will be published in the Journal. If not intended to publish elsewhere, successful applicants will be required to submit their study results for consideration by the New Zealand Journal of Medical Laboratory Science.

The winner of the 2014 Barry Edwards & Rod Kennedy Scholarship is Maxine Reed from Aotea Pathology, Wellington. She will be attending and presenting at the AACC Conference in Atlanta, Georgia, USA in July 2015.

Fellowship of the New Zealand Institute of Medical Laboratory Science

The NZIMLS encourages members to consider Fellowship as an option for advancing their knowledge and career prospects. Fellowship provides an attractive option to academic postgraduate degrees at a fraction of the cost.

Fellowship of the NZIMLS may be gained by examination, by thesis or by peer-reviewed publications.

Examination

Consists of two parts:

a) Part 1: Two written papers each of three hours duration.
b) Part 2: Upon successful completion of Part 1 a dissertation of 3000 - 5000 words.

The dissertation may take the form of a review, development of a hypothesis or any other presentation that meets with the approval of the Fellowship Committee.

Thesis

The thesis must be based on the style of Master of Science by Thesis requirements of New Zealand Universities and not exceed 20,000 words.

Publications

A minimum of seven peer-reviewed publications, of which the candidate must be first author of at least four, may be submitted for consideration. These need to have been published in international or discipline acknowledged scientific journals. A review of the submitted articles of 3000 – 5000 words must also be submitted. The candidate must state the significant contributions he or she has made to the publications.

Exemption from Part 1

Candidates who are holders of postgraduate or professional qualifications in Medical Laboratory Science may be exempt from the Part 1 examinations but are still required to submit a dissertation for Fellowship.

Qualifications recognised by the NZIMLS for the purpose of exemption to sit the Part 1 examinations are:

- Fellowship of the Australian Institute of Medical Scientists (FAIMS), the Institute of Biomedical Science (FIBMS) the Australasian Association of Clinical Biochemists (FAACB), and the Institute of Biology, London (FSB).
- An academic postgraduate qualification, normally at least a postgraduate diploma, in medical laboratory science or closely related subject. The course of study must meet the minimum requirement of one year's full-time university study.

For full Fellowship regulations and the application process visit the NZIMLS web site: www.nzimls.org.nz or contact the Fellowship Committee Chair: Associate Professor Rob Siebers at rob.siebers@otago.ac.nz

NZIMLS presents the 2015 North Island Seminar

Join us at

The Distinction Hotel

Rotorua

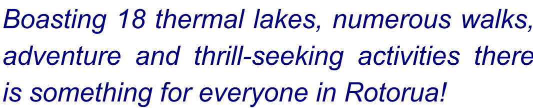
9 May 2015

Presentations welcome!

Contact:

Mary-Ann Janssen

mary-ann.janssen@waikatodhb.health.nz



Boasting 18 thermal lakes, numerous walks, adventure and thrill-seeking activities there is something for everyone in Rotorua!

Take some time after the Seminar to visit the beautiful Museum, go for a bike ride, or for the more adventurous, how about a spin in an Ogo? Just wanting to relax? Then a soak in a geothermal pool will do the trick!

NZIMLS presents MICROBIOLOGY SIG 2015

Join us at

The Novotel Tainui Hotel

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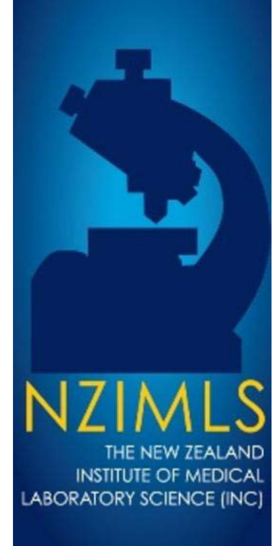
13 June 2015



Presentations welcome!

Contact:

Sean Munroe

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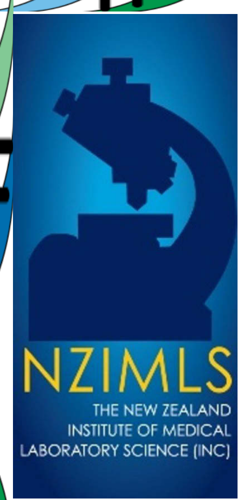
"Hamilton - where it's Happening"

Spend a weekend with us and Discover the real Middle-Earth at Hobbiton; Enjoy scrumptious High tea at Zealong Camellia Tea House; Explore theme gardens at Hamilton Gardens



NZIMLS presents the 2015

Molelcular Diagnostics

Special Interest Group Seminar

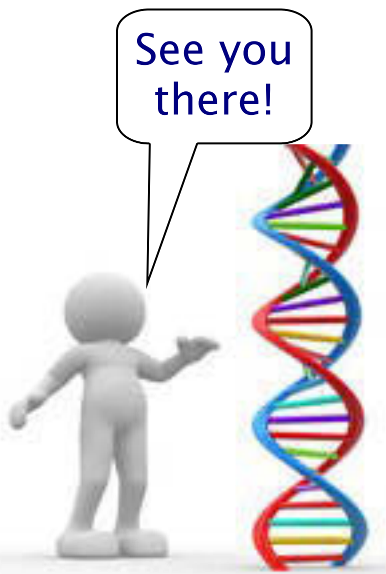
Saturday 20 June

The Hunter Centre

Dunedin

Contact Ngaire Monk with your presentation:

ngaire.monk@sclabs.co.nz



Dunedin, the home of Otago University, Giant Albatross, The Chinese Tea House, Cadbury's and much more!

GREETINGS TO YOU ALL!

Wellington based training courses 2015

This year the PPTC is offering 7 courses and these include:

- Haematology and Blood Cell Morphology
  2 March – 27 March 2015
- Health and Safety and Infectious Diseases
  13 April – 8 May 2015
- Laboratory Quality Management Systems
  25 May – 19 June 2015
- Biochemistry 13 July – 7 August 2015
- Microbiology 31 August – 25 September 2015
- Phlebotomy 5 October – 23 October 2015
- Blood Transfusion Science 2 November – 27 November 2015

New Centre Based courses for 2015

Health and Safety and Infectious Diseases

a) **Health and Safety Component:**
Implementing a laboratory health and safety programme, hazard identification, risk assessment, laboratory premises and design, biohazard waste and disposal of contaminated material; personal protection; code of conduct; staff health and medical surveillance; administrative procedures; chemical and dangerous goods storage; material data safety sheets; major incident response; llaboratory emergency planning; and laboratory biosecurity concepts.

b) **Infectious Diseases Component:**
Notifiable diseases; role of public health; surveillance and monitoring disease outbreak; workforce occupational exposure; communicable diseases; what makes an epidemic; vaccine availability; bacterial causes of infectious diseases: isolation procedures to identify and confirm major bacterial pathogens associated with gastrointestinal infections, seafood poisoning and marine environments, food and water contaminating organisms; bacterial agents of pneumonia, septicaemia and meningitis; agents of sexually transmitted infections. viral causes of infectious diseases: a selective summary of disease specific viruses such as gastrointestinal viruses; respiratory viruses; parasites of infectious disease and mycology review.

Phlebotomy

The PPTC is offering a three week training course on Phlebotomy. The course contents will address the following topics:
the role of a phlebotomist in the medical laboratory diagnostic process; anatomy and physiology of specific human tissue (this will take into consideration sites of blood collection, and overall discussion with reference to the circulatory system and normal haemostasis/blood coagulation); patient safety and safety of procedure; the practise of infection control; adverse incidents with the application of first aid; the laboratory and its sample collection policy with reference to laboratory collection forms, patient identification, post collection requirements, tests profiles and test requirements; medical abbreviations and medical terminology; pre analytical variables such as patient status, collection techniques, sample storage and transportation; blood collection (venous and capillary); patient assessment and preparation including considerations for both elderly patients and neonatal patients; site preparation; sample collection devices and equipment in terms of antiseptics, dressings, evacuated blood collection equipment's, needles and syringes, tourniquets and sample collection tubes; the collection process and post collection process for both venous and capillary samples; blood culture collection; specialised collection procedures, collection and handling of non-blood specimens (Urines, faeces, sputum, saliva, swabs, mycological samples and seminal fluid, in terms of equipment, collection, specimen stability, sterility, storage, transportation and handling; the handling and transportation of aspirates, histology and cytology samples; specialised test procedures such as bleeding time, mantoux test and skin prick testing.

In country teaching and training

Short term in-country teaching workshops have proven extremely valuable and have made an immediate and significant impact in terms of capacity expansion and up skilling personnel, however on-going consolidation programmes are essential if such a difference in the development or enhancement of skill is to be further advanced and maintained.

The PPTC is currently compiling a potential list of technical consultants who would be prepared to travel the Pacific region on request to assist with capacity building and strengthening of Pacific Island laboratories.

The PPTC has labelled the situation in the Pacific in terms of haematology and biochemistry as requiring intense corrective action and therefore it plans to intensify its presence through in - country training in 2015 recruiting additional haematology and biochemistry expert consultants to increase both frequency and coverage of visits across the Pacific. Consultancies are generally 1 to 2 weeks in duration and dependent on PPTC funding availability.

If you are a qualified NZ registered Medical Laboratory Scientist with at least 15 years experience in haematology, biochemistry or laboratory management and would like to be added to our list of consultants we would like to hear from you. Should you be interested, please forward a copy of your current CV to the PPTC .

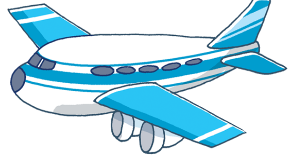
Teaching resources

Depending on the availability of funding, the PPTC intends to supply appropriate learning material (text books, journals, etc) to Pacific laboratories that are desperately in need of educational resources so a reference centre can be established within the laboratory as a promotional way forward in terms of professional development.

Can you help?

If any New Zealand medical laboratories have a surplus of teaching resources in terms of wall charts, haematology stained blood films, projector slides, textbooks, journals etc, the PPTC would be most grateful if they could be donated through its centre to Pacific Island laboratories where there is an exceptional need.

Overseas Travel



January 2015: Phil Wakem and Navin Karan both attended the PIHOA (Pacific Island Health Officers Association) meeting in Guam. The theme of this meeting focused on the enhancement of quality with reference to the public health laboratory services in the US affiliated Pacific Islands through regional capacity building.

Russell Cole, the PPTC'S Laboratory Quality Manager, carried out his first LQMs visit to Kiribati and during his stay also visited the Marine Training Centre to assess the laboratory's quality programme.

Pacific Infectious Substances Shipping Training (ISST) Nadi, Fiji , 9- 11 February 2015

Navin attended the above training workshop provided by WHO in Nadi, Fiji . The training addressed shipping requirements for all potentially infectious substances, focusing particularly on highly infectious materials.

Farewell to Clare Murphy

Clare is a NZ graduate from the Australian National University. She started her career in Laboratory work at what was then Gisborne Hospital, becoming a specialist in clinical chemistry at Wellington Hospital working mostly in Quality, initially in quality control, and latterly in quality management where she became the Laboratory Services Quality Manager for Capital and Coast DHB. Clare performed this role for many years.

Since 1987 and for nearly three decades on, Clare pioneered and managed the PPTC Clinical Chemistry External Quality Assessment Programme for laboratories across the Pacific and South East Asia. Clare initially began as a volunteer and took responsibility for 12 Pacific participating laboratories using Wellington Hospital QC material. From this, the programme has now expanded to 58 participants which now utilises RCPA QAP material and statistics allowing the programme to monitor a large variety of analytes. Clare has worked closely with the RCPA QAP, and their support and generosity is greatly appreciated by the PPTC.

It is with great sadness that we are to say farewell to Clare as the PPTC Regional External Quality Assurance Co-ordinator for biochemistry. She has given total dedication and commitment to this programme and has contributed greatly to the education and capacity strengthening of clinical laboratories throughout the Pacific and South East Asian regions. The PPTC is very fortunate however to retain Clare as a consultant and as such, she will continue to offer support to the centre and to Pacific laboratories in terms of guidance and expertise in such areas as laboratory quality management and REQA.

Apart from PPTC responsibilities, Clare continues to hold the position of Point of Care Testing Co-ordinator at Wellington Hospital, New Zealand.

CONTACT US

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Manager

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Journal questionnaire

Below are 10 questions based on articles in the April 2015 Journal issue. Read the articles carefully as most questions require more than one answer.

Answers are to be submitted through the NZIMLS web site. 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.

The site has been developed for use with Microsoft's Internet Explorer web browser. If you are having problems submitting your questionnaire and you are using the Firefox web browser, try resubmitting from a computer or system using Microsoft's Internet Explorer.

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 17th July, 2015. 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. Please direct any queries to her at cpd@nzimls.org.nz.

APRIL 2015 JOURNAL QUESTIONS

1. Of total laboratory errors, what percentages are attributed to the pre-analytical, analytical and post-analytical phases?
2. What three factors are involved in patient preparation before laboratory testing?
3. On how many sites on the Waimakariri river were antibiotic resistant *Escherichia coli* found in both sampling years?
4. What is recommended by the EUCAST Clinical Breakpoint Table v3.1 for screening for methicillin resistance in *S. saprophyticus*, and what criteria for further investigation for the presence of *mecA*?
5. What are the main causes of community acquired meningitis in sub-Sahara Africa?
6. In the *Cryptococcus neoformans* infection article, what signs and symptoms did all studied patients have?
7. What is nodular lymphocyte predominant Hodgkin lymphoma characterised by?
8. Why is classical Hodgkin lymphoma unique among lymphomas?
9. Name three protocols for the rapid direct detection of microbes from positive blood culture vials by MALDI-TOF.
10. List 5 activities apart from the analysis and processing of samples which are part of your competency.

NOVEMBER 2014 JOURNAL QUESTIONS AND ANSWERS

1. What is the empirical treatment and alternative treatment of gonorrhoea in New Zealand?
500 mg IM ceftriaxone plus 1g azithromycin. Ciprofloxacin is an alternative treatment option.

2. Penicillin resistance of *N. gonorrhoea* may be due to what?
Either mutations in chromosomal genes encoding penicillin-binding proteins and/or affecting outer membrane permeability, or by acquisition of plasmids encoding production of a beta lactamase.

3. What is the major limitation when developing possible molecular solutions to address a lack of antimicrobial susceptibility information?
In contrast to phenotypic methods, genotypic assays will not detect novel, uncharacterized mechanisms of resistance to antimicrobial agents.

4. What has been reported to predict reduced susceptibility to ciprofloxacin in the treatment of *N. gonorrhoea* infected patients?
gyrA mutation at codon 91, from serine to phenylalanine.

5. What fears have been voiced by health professionals regarding breast feeding for pain relief in infants undergoing heelstick or venepuncture?
A mother may drop her infant, the infant may choke, and the infant may associate pain with breastfeeding.

6. What substance in breast milk may procure pain relief in infants and what does this substance increase?**Tryptophan. Increases beta endorphins.**

7. Der p 1 is the major group one allergen of which mite species, what does it induce, and what does it contribute to?
***Dermatophagoides pteronyssinus*. Induces an IgE response in susceptible individuals. Contributes towards the prevalence and severity of asthma.**

8. Acaroid mites can survive in many environments. Name six such environments.
Storehouse. Farmhouse. Stored food stuff. Various drugs. Packing material. Household objects. Human and animal bodies.

9. The possibility of a true mite infection should only be considered when?
In presence of a considerable number of adult mites with inflammatory reaction and after repeated identification of mites in consecutive samples from a symptomatic patient, and that the clinical findings are compatible with such an infestation.

10. Which immunochemistry test confirms diagnosis of neuroendocrine carcinoma?
Positive chromogranin and synaptophysin and negative TTF1 staining.

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2015 NZIMLS CALENDAR

Dates maybe subject to change

Date	Seminars	Contact
7 March	South Island Seminar, Opera House, Oamaru	erolia.rooney@nzblood.co.nz
9 May	North Island Seminar, Distinction Rotorua Hotel	Mary-ann.Janssen@waikato.health.nz
15-17 May	NICE Weekend, Wairakei Resort	raewyn.cameron@lsr.net.nz natalie.fletcher@sclabs.co.nz
13 June	Biochemistry SIG Seminar, Palmerston North	
13 June	Microbiology SIG Seminar, Hamilton	SeanEdward.Munroe@waikatodhb.health.nz
20 June	Molecular Diagnostics SIG Seminar, Dunedin	Ngaire.Monk@sclabs.co.nz
October	Histology SIG Seminar	
17 October	Haematology SIG Seminar, Rydges, Queenstown	Leigh-Ann.Aitcheson@sclabs.co.nz
November	Immunology SIG Seminar	
November	Mortuary SIG Seminar	
Date	**NZIMLS Examinations**	**Contact**
30 April	Applications close for Fellowship Examinations	fran@nzimls.org.nz
20 May	Applications close for QMLT/QSST Examinations	fran@nzimls.org.nz
04 November	QMLT and QSST Examinations	fran@nzimls.org.nz
10-11 November	Fellowship Examinations	fran@nzimls.org.nz
Date	**Council**	**Contact**
5-6 March	Council Meeting, Christchurch	fran@nzimls.org.nz
7-8 May	Council Meeting, Rotorua	fran@nzimls.org.nz
16 August	Council Meeting, Auckland	fran@nzimls.org.nz
20 August	Annual General Meeting, Auckland	fran@nzimls.org.nz
November	Council Meeting	fran@nzimls.org.nz
Date	**Events**	**Contact**
17-21 August	South Pacific Congress, Auckland	rossh@adhb.govt.nz fran@nzimls.org.nz
Date	**Membership Information**	**Contact**
January	Membership and CPD enrolment due for renewal by 28 February	sharon@nzimls.org.nz
January	CPD points for 2015 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
19 June	Nomination forms for election of Officers and Remits to be with the Membership (60 days prior to AGM)	fran@nzimls.org.nz
15 June	Material for the August Journal must be with the Editor	rob.siebers@otago.ac.nz
9 July	Nominations close for election of officers (40 days prior to AGM)	fran@nzimls.org.nz
29 July	Ballot papers to be with the membership (21 days prior to AGM)	fran@nzimls.org.nz
5 August	Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM)	sharon@nzimls.org.nz
12 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

NZIMLS

THE NEW ZEALAND
INSTITUTE OF MEDICAL
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