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Collette Bromhead and colleagues from Aotea Pathology in Wellington present an overview of the state of Neisseria gonorrhoeae testing in New Zealand, and the challenges that are being faced. They state that molecular technologies are gradually taking over and ongoing monitoring of the specificity of methods in the face of high rates of genetic transfer in Neisseria as well as the increased cost of nucleic acid amplification tests (NAAT) testing, specialist facilities and technical skills required should be taken into account. Without a national consensus on gonorrhoea testing, concerns over false positive results from NAAT and the emergence of antibiotic resistance are being handled in different ways.

Laboratory-based screening for methicillin-resistant Staphylococcus aureus (MRSA) colonisation in patients and healthcare workers remains a cornerstone of infection control measures to limit the spread of MRSA. Aus Molan and colleagues determined if screening the throat increases the detection rate of MRSA colonisation; determined the performance of the Roche LightCycler MRSA advanced test in the detection of MRSA in comparison to routine culture on selective chromogenic agar; and determined MRSA isolation rates from swabs transported in semi-solid media. They found that the throat was an important habitat of MRSA; MRSA incidence among volunteers was 2.6% (culture) and 4.8% (PCR); and both swab types performed almost equally in maintaining the viability of MRSA during the study.

Mary Stevens from Canterbury Health Laboratories cultured and identified Clostridium difficile from EIA toxins A/B positive and equivocal faecal samples. Breakpoint susceptibilities were performed on isolates to determine a baseline for future monitoring of emerging antimicrobial resistance. She found that alcohol-shock treatment, combined with a selective agar, was an effective method of culture for C. difficile. MALDI-TOF was a reliable, rapid and inexpensive tool in the identification of this organism.

Of the isolates showed susceptibility to vancomycin, metronidazole, amoxycillin/clavulanic acid and meropenem, while most were resistant to ciprofloxacin. This work was presented at the NZIMLS ASM in Wellington last year and was the winner of the Hugh Bloore Memorial Prize for the best poster.

More patients with cystic fibrosis live into adulthood and since 2005 Canterbury Health Laboratories have noticed a significant increase in adult sweat test requests. Those who present as adults usually have had mild symptoms or lack the common symptoms of cystic fibrosis that may have made the disease apparent in youth. In this issue Sandy Woods looks at a period of 15 years of sweat testing in the laboratory, showing an increase in adult sweat test requests and compares sweat chloride and cystic fibrosis mutational analysis results and describes some atypical findings. This work was presented at the South Pacific Congress, Brisbane in 2011 by Sandy on a Barry Edwards/Rod Kennedy Scholarship of the NZIMLS.

Under current New Zealand guidelines a fasting sample is mandatory for lipid assessment due to effect of food intake on triglycerides which causes the equation to ‘overestimate’ VLDL and, therefore, artefactually ‘underestimate’ LDL. Internationally, measured LDL methods were introduced to overcome this shortcoming and Alan Simkins from Kaitaia Hospital laboratory decided to investigate whether this approach could further reduce the need for patient fasting. He compared calculated LDL levels by the Friedewald formula with measured LDL levels using a homogenous enzymatic cholesterol assay. He showed a strong correlation between the two methods and concludes that by measuring LDL, fasting is not necessary resulting in greater patient compliance with CVD screening programmes and benefits the laboratory’s phlebotomy services by reduce early morning phlebotomy queuing, reduces patient waiting times, increases customer satisfaction.
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Neisseria gonorrhoea testing in New Zealand, culture or PCR?
Collette Bromhead, Amanda Miller and Mark Jones

Abstract
There is change afoot for the diagnosis of Neisseria gonorrhoeae in New Zealand. As was the case for Chlamydia trachomatis in years past, molecular technologies are gradually taking over and it is important that laboratories do not expect the same smooth transition. Ongoing monitoring of the specificity of methods in the face of high rates of genetic transfer in Neisseria as well as the increased cost of nucleic acid amplification tests (NAAT) testing, specialist facilities and technical skills required should be taken into account. Without a national consensus on gonorrhoea testing, concerns over false positive results from NAAT and the emergence of antibiotic resistance are being handled in different ways. Following an open discussion at the 2012 NZIMLS Annual Scientific Meeting, it was our aim to present a high level overview of the state of N. gonorrhoeae testing in New Zealand, and the challenges that are being faced.

Key words: Neisseria gonorrhoeae, polymerase chain reaction, New Zealand, gonorrhoea, testing trends, Sputasol, porA/opa supplementary testing

N Z J Med Lab Sci 2013; 67: 4-6

Introduction
Neisseria gonorrhoeae was first described by Albert Neisser in 1879. Of the eleven species of Neisseria that colonise humans, only two are pathogens. N. gonorrhoeae is the causative agent of gonorrhoea, known colloquially as "The Clap," derived from the French word "clapier" (which has variously meant "brot hel" or "pustulant sore"), and is transmitted via sexual and perinatal contact (1). This gram negative diplococcus infects the human urogenital tract, eye, throat, and rectum, and can cause systemic infections. Most gonorrhoea infections in women go undetected as the patients are asymptomatic, although the long term sequelae can be significant (2,3). In males, the majority of patients experience symptoms; however there is an underestimate of prevalence.

N. gonorrhoeae bacteria are motile and possess type IV pilis to adhere to surfaces similarly to a grapping hook. Through cooperation, groups of N. gonorrhoeae are able to pull up to 100,000 times their own weight, the equivalent of a human pulling 2,000 African elephants (4). In 2011, researchers at Northwestern University found evidence of a human DNA fragment in a N. gonorrhoeae genome, the first example of horizontal gene transfer from humans to a bacterial pathogen (5).

For years, gonorrhoea has been easily treated with a single oral dose of antibiotics. However, N. gonorrhoeae has progressively acquired resistance to each new agent: sulphonamides in the 1940s, penicillins and tetracyclines in the 1970s and 1980s, and fluoroquinolones by 2007. Since then, 3rd generation cephalosporins have been the only antibiotics recommended for empiric treatment of gonorrhoea (6) but clinicians now face the emergence of 3rd generation cephalosporin- resistant N. gonorrhoeae without any well-studied, effective backup treatment options (7).

Traditionally, N. gonorrhoeae detection has been performed by growing and identifying viable organisms by microbail culture before measuring antibiotic susceptibilities. Culture relies on organism viability, and detection of this is both time consuming and subjective. There has been a shift to NAAT for the diagnosis for infectious diseases, particularly for sexually transmitted infections (STIs), with detection of Chlamydia trachomatis by PCR recommended as best practice since 2008 in New Zealand (8).

However, when considering shifting to NAAT methods for detection of N. gonorrhoeae it is important that laboratories do not expect the relatively smooth transition seen previously when C. trachomatis moved from enzyme immunoassay to NAAT. N. gonorrhoeae is naturally competent for DNA transformation as well as being capable of conjugation. Both mechanisms allow the DNA of N. gonorrhoeae to undergo conformational changes, which can allow the bacteria to avoid detection if genetic changes occur in the gene targets of NAATs. The need for ongoing monitoring of the specificity of such methods, as well as the increased cost, specialist facilities and technical skills required, should be taken into account by laboratories.

New generation NAATs address some of these concerns by offering multiple genetic targets, reducing the chance of false negatives. Many molecular platforms now offer combined testing for C. trachomatis and N. gonorrhoeae. These next generation NAAT assays are more sensitive and specific than culture and predecessor PCR assays, and provide the option of screening using non-invasive specimens such as urine and self- taken vaginal swabs, although none are yet approved for use on non-genital sites. And none are yet able to offer reliable information on antibiotic susceptibilities.

National testing
During the 2012 NZIMLS ASM held in Wellington, a discussion about N. gonorrhoeae testing methods was opened to all attendees during our talk on “How do you like your gonorrhoea? Dead or alive?” There is little consensus between laboratories on how, when and whether to proceed to molecular testing for N. gonorrhoeae. The regions raised differing funding issues, instrumentation issues, laboratory space and the availability of technical skill as barriers to change. The NAAT instrumentation systems that are in use include the Roche cobas 4800 and the Roche cobas Amplicor platforms, BD Viper, and the Abbott M2000 platform. No attendees were using the Aptima Combo 2 system in October 2012. The majority of laboratories in New Zealand continue to perform culture based testing methods for routine N. gonorrhoeae detection, although many supplement this with some form of molecular testing.

Why didn’t we do it sooner?
Although dual target NAAT assays for Chlamydia and Gonorrhoea have been available in New Zealand since 1999, early versions of these assays were shown to lack specificity causing some hesitation in moving away from culture for N. gonorrhoeae. Two studies performed at Medical Laboratory Wellington did nothing to improve local confidence in the technology. In 2000, culture and Amplicor NG PCR (Roche) were compared for on 562 specimens from the Wellington Sexual Health Clinic. Positive PCR results were confirmed using the Amplicor NG 16s rRNA PCR (Roche). The positive predictive value of the confirmed cobas Amplicor NG test, however, was too low (83.3%) to consider replacing culture with PCR.
In 2006, following a case of PID in a patient post-termination of pregnancy in which \textit{N. gonorrhoeae} was later identified as the cause, the Amplicor NG test was revisited to answer the question: was culture missing \textit{N. gonorrhoeae} infections? (Personal Communication, Dr B. Lawton). After testing 300 self-taken vaginal swabs from patients having a termination of pregnancy not a single \textit{N. gonorrhoeae} positive PCR result was recorded (9) and the correlation of PCR with culture was 100%. So, still no benefit to stopping culture. However, culture itself could be made more accurate by restricting testing to cervical and urethral swabs. In 2008 we ceased \textit{N. gonorrhoeae} culture on vaginal swabs and urine, doubling the proportion of \textit{N. gonorrhoeae} identified per sample tested, but arguably reducing favourability for patients who were now accustomed to self-taken swabs and urine for Chlamydia PCR.

The arrival of the cobas 4800 CTNG test and its dual target approach to detecting both organisms caused a review. Nervous from previous data, it was decided to perform a large validation before considering shifting away from culture again. In 2011, over a 9 month period, we compared \textit{N. gonorrhoeae} culture with the cobas 4800 NG PCR in >18,000 urogenital and non genital samples and found that the critical specificity and positive predictive value parameters reached 99.9% and 94.9% in urogenital specimens, and 98.8% and 92.9% respectively in non-genital specimens. If PCR had been used for \textit{N. gonorrhoeae} culture on vaginal swabs and urine, the proportion of \textit{N. gonorrhoeae} identified per sample tested, but arguably reducing favourability for patients who were now accustomed to self-taken swabs and urine for Chlamydia PCR.

The results from patients whose cultures had grown \textit{N. gonorrhoeae} suggest some differences in assay sensitivity between \textit{N. gonorrhoeae} assays and PCR detection of \textit{C. trachomatis} by PCR than we tested for \textit{N. gonorrhoeae} by culture, indicating the higher acceptability of non-invasive urine and self taken vaginal swabs over cervical and urethral cultures. The validation data was reassuring enough for us to cease routine culture and move to cobas 4800 NG PCR in May 2012. Confirmatory testing for the \textit{porA} pseudogene and the \textit{opa} gene has been retained for non-genital positive results to acknowledge that the high rate of genetic exchange between \textit{Neisseria} in these sites may lead to false positive results. Recently this testing was used to identify one such organism in a throat swab from Auckland (10).

\textbf{Fishhooks}

Two issues were identified during the study that other laboratories should be aware of. Firstly, female urine specimens have been shown to have low sensitivity for Chlamydia PCR (11,12), and the data confirmed that this is also true for \textit{N. gonorrhoeae}, with only 86.7% sensitivity compared to a cervical or urethra culture test. Therefore urine cannot be recommended as a sole screening specimen for females.

Unexpectedly, we found a high incidence of ‘Failed’ cobas 4800 results from patients whose cultures had grown \textit{N. gonorrhoeae}. These errors are reported by the cobas instrument when the pipettor is blocked by material in the sample. It appears that the muco-purulent discharge, commonly associated with \textit{N. gonorrhoeae} infection, causes such interference. We eventually showed that 1:1 dilution of cobas collection buffer with Sputasol (1.4% DTT, Oxoid) could resolve a majority of muco-purulent errors. When unresolvable specimens should be reported with a comment highlighting the possibility of a masked infection (13).

Many local laboratories who are performing \textit{N. gonorrhoeae} NAAT routinely, confirm positive results by either in-house assay or another commercial testing platform. Supplementary confirmatory testing needs to be carefully considered as the results of the RCPA Microbiology \textit{C. trachomatis} / \textit{N. gonorrhoeae} panels from 2012 showed that 30-50% of participants missed the low load \textit{N. gonorrhoeae} samples in March 2012. The results also show that some laboratories are correctly obtaining a positive result for their screening method but a negative result using their supplementary method. This suggests some differences in assay sensitivity between laboratories which may be due to incompatibility between commercial specimen preparation systems, poor target choice or suboptimal performance of the supplementary assay (14).

\textbf{Emerging antibiotic resistance/how are we using/should we be using culture?}

The emergence of extensively drug resistant (XDR) strains of \textit{N. gonorrhoeae} that display high level resistance to third generation cephalosporins (ceftixime and cefixime) is a major public health concern, as well as a concern for laboratories providing diagnostic testing. These organisms pose a problem for empirical treatment of patients not tested by microbial culture for \textit{N. gonorrhoeae}. Recommendations from the CDC include outlines for enhanced antibiotic susceptibility surveillance to monitor the extent of the problem globally (15,16). These concerns have generated much debate over the wisdom of transferring \textit{N. gonorrhoeae} diagnosis to NAAT methods. The problem for NAAT based testing methods is that to provide susceptibilities, a viable organism is required. The lysis buffers utilised for many commercial \textit{N. gonorrhoeae} assays destroy the organism in order to gain access to the nucleic material of the cell.

Our current approach is to culture symptomatic patients selected by the Wellington Sexual Health Clinic and to monitor Ceftriaxone and Ciprofloxacin susceptibility. ESR has indicated enhanced STI surveillance to include \textit{N. gonorrhoeae} antibiotic susceptibility results will commence in 2013. Whilst this type of survey will decrease the risk of a ‘surprise’ 3rd-generation cephalosporin resistant organism emerging, there are no moves to develop a plan for patient management after it arrives.

A solution for the future may be in the development of molecular methods for the detection of sequences responsible for resistance. Until such tests are available for routine laboratory use, a duplication of testing facilities for both culture and PCR detection of \textit{N. gonorrhoeae} is required in order to continue surveillance of antibiotic susceptibility (17). The conundrum of a diagnostic laboratory arises when it is not economically viable to provide both tests. In this situation we pose the question, is sensitivity subordinate to surveillance?

\textbf{Best practice guidelines for testing and treatment}

At the 2012 New Zealand Sexual Health Society (NZSHS) gonorrhoea workshop, it was agreed that standardisation of testing and treatment was needed in New Zealand, and a panel of experts was convened to tackle this task on behalf of the NZSHS. The group includes sexual health physicians, technical laboratory specialists in both molecular and microbiology, and representatives from ESR.

The aim of this ‘Gonorrhoea Working Group’ is to develop a best-practice guideline document to advise diagnostic testing and clinical management of \textit{N. gonorrhoeae} infection in New Zealand. In particular:

- To standardise testing methods for detection and reporting of infection and antimicrobial susceptibility.
- To develop an action plan for management of cases with suspected treatment failure, enhance ESR surveillance of resistance within the community and provide recommendations for outbreak management at a regional level.

Consultation with stakeholders is planned as part of this process. We look forward to the development of this guideline in 2013.

\textbf{Summary}

\textit{N. gonorrhoeae} is an intriguing bacteria, with the strength to move elephants (relatively), change genes faster than Clarke Kent turning into Superman, and it can even use human DNA to its own advantage. Finally, we are nearing the end of the line for empiric treatment with reports of rising MIC’s for 3rd generation Cephalosporins, the last group of antibiotics known to be effective at clearing \textit{N. gonorrhoeae} infection.
The use of NAATs to screen for infection increases the accessibility of testing for those who would otherwise refuse an invasive swab sample. Other advantages include optimal sensitivity and detection of non-viable organisms allowing for less stringent requirements for the collection and storage of specimens. However NAATs are currently unable to provide antibiotic susceptibility information data (to referring clinicians or for monitoring of resistance patterns). Gonorrhoea control relies on early detection and treatment. Consensus needs to be developed about best practice testing for infection and how to monitor the incursion of ceftriaxone resistance strains into New Zealand. Even more important is to develop an action plan for when this unwelcome visitor does arrive.

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**References**


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Methicillin-resistant *Staphylococcus aureus* (MRSA): isolation from nasal and throat swabs transported in liquid or semisolid media; identification by PCR compared with culture

**Aus Molan, Mary Nulsen and Gavin Thomas**

**Abstract**

**Objectives:** A multi-site study evaluated the clinical performance of the Roche LightCycler PCR assay in the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in comparison to routine chromogenic agar culture method (BD BBL CHROMagar II). Two other variables were also investigated: to establish if screening the throat increases the detection rate of MRSA colonisation; and, to determine MRSA isolation rates from swabs transported in semi-solid media (Copan Venturi Transystem) and liquid-based media (Copan ESwab).

**Methods:** 810 swab specimens were collected from the anterior nares and throat to determine the site specific isolation of MRSA. Swabs were transported in liquid (Copan Elution swab) and semi-solid (Copan Venturi Transystem without charcoal) transport media to assess the effect of collection swab type on the viability of MRSA. Swabs specimens were screened for MRSA using chromogenic (direct and broth enriched) and PCR techniques.

**Results:** MRSA incidence among volunteers was 2.6% (culture) and 4.8% (PCR). The 2.2% higher yield obtained by the PCR method was statistically significant (*P* = 0.04). This increase in sensitivity incurs an additional cost (per test, the PCR assay was 2.5 times more expensive). However, the PCR method had a much faster result turnaround time (2-3 hours) compared to culture (48 hours) while both methods had comparable sample hands on time (1.65 min and 1.20 min, respectively).

**Conclusions:** The throat was found to be an important habitat of MRSA. In this study, if only the nares were tested, 38.5% (PCR) to 42.8% (culture) of the total MRSA carriers would have been missed. Thus, any screening program for MRSA should include swab specimens from the throat. Both swab types performed almost equally in maintaining the viability of MRSA during the study. While the elution swab was approximately double the price of the Venturi counterpart, the liquid phase allows the pooling of samples and multiple testing to be performed from one swab specimen.

**Key words:** methicillin-resistant *Staphylococcus aureus*, MRSA, polymerase chain reaction, chromogenic culture, mass screening, infection control, microbial viability.

**N Z J Med Lab Sci 2013; 67: 8-16**

**Introduction**

*Staphylococcus aureus* is an opportunistic pathogen carried as a commensal organism on the skin and in nares of approximately 30% of the normal population (1). *S. aureus* is responsible for a broad range of clinical infections, most notable of which are cases of bacteraemia and endocarditis (2). Methicillin-resistant *S. aureus* (MRSA) was first described in 1961, with the first hospital outbreak reported in 1963 (3). Healthcare associated infections caused by MRSA have become an important issue for healthcare facilities worldwide due to high rates of infection, mortality, and high costs of treatment (4). During 2005, it was estimated that invasive MRSA infections in the United States resulted in almost 19,000 deaths, a figure higher than that for HIV/AIDS (5).

Laboratory-based screening for MRSA colonisation in patients and healthcare workers remains a cornerstone of infection control measures to limit the spread of MRSA (6). Factors that determine the success of a screening program include: the efficiency of a collection systems to maintain viability and recovery of organisms (7); the sensitivity and speed of laboratory testing (8,9); the speed of result determination (8-10); and sampling site or sites (11,12). To rapidly detect MRSA colonisation in patients, laboratories must choose between molecular methods and selective agar-based methods (13). In high-risk patients, the rapid detection of MRSA can be of double value: not only are they in the interests of patients infected with MRSA (in order to start adequate treatment as early as possible), but they also help to protect other patients from potentially spreading the pathogen.

The use of chromogenic media in recent years has become an important method for the rapid identification of microorganisms in clinical samples (14). In comparison to conventional culture media, chromogenic media allows direct colony colour-based identification of the organism from the primary culture. This in turn, reduces the need to subculture for further biochemical testing and hence the time until a result is obtained (15). The advantages of using BBL CHROMagar II media include: short hands on time, minimal personnel time required for reading cultures, and the low cost of the test in comparison to PCR methods. The disadvantage with using this media is the long turnaround times. Several chromogenic and differential MRSA media have been shown to yield results within 18 to 48 hours (16-18). In contrast, PCR methods can yield results within two to three hours (8,11,13).

In 2008, Roche Molecular Systems (Switzerland) introduced the LightCycler (LC) MRSA advanced test, an *in vitro* diagnostic PCR method for the rapid detection of MRSA colonisation to aid in the prevention and management of MRSA infections in healthcare settings. The test targets the integration site of the SCCmec cassette into the *S. aureus* chromosome with melting point analysis of the PCR product (8,11). This PCR assay is performed on the LC 2.0 (Roche Applied Science) instrument, an extremely fast thermocycler with on-line fluorescence detection. The shortfall with PCR techniques however, is that the emergence of new MRSA strains with diverse genetic background also means that the capability of PCR testing will need to be constantly updated in order to detect SCCmec cassettes with novel sequences (19).
For MRSA detection, swabs are the most commonly used sampling device. These systems must maintain organism viability during transit while allowing maximal organism recovery during laboratory testing. Given the increasing frequency of transport delays due to cost containment measures, consolidations, and services being shifted to centralised or reference laboratories, robust transport systems are becoming increasingly important. The vast majority of swab transport systems used in New Zealand and the rest of the world contain a semi-solid medium which is reported to give better survival of organisms, particularly of anaerobes (20).

A new type of swab system has recently been introduced in a growing number of laboratories (21). The Copan Elution Swab (ESwab) consists of a sterile package containing two components: a pre-labelled polypropylene screw-cap tube with conical shaped bottom filled with 1mL of liquid Amies transport medium and a specimen collection swab which has a tip flocked with soft nylon fibre (22). This swab is prepared by spray-on flocked fibre technology which provides stronger capillary action and strong hydraulic uptake of liquids, which should result in better specimen collection (23). Organisms present in the specimen remain close to the surface and when placed in transport medium, elute completely and immediately (24). An extra advantage of the liquid medium over conventional swabs is that every inoculated plate receives a similar inoculum (20).

To date, current New Zealand MRSA screening programmes do not include throat swabs as specimen of choice. Such samples are thought to add discomfort to the patient during collection with little anticipated gain. This belief is based on the idea that throat carriers of *S. aureus* are likely to carry *S. aureus* in the nares as well. However, several studies have shown individuals may have colonisation exclusively in the throat that would be missed on screening limited to the anterior nares only (25-29). Additionally, the existence of different clones of *S. aureus* in the nares and in the throat has been confirmed (25). This supports the notion that staphylococcal flora in the nose and the throat are independently formed and that attention should also be directed to the carriers of *S. aureus* in the throat for the control of nosocomial infection (30). Unrecognised carriers may spread MRSA and render infection control programmes futile; therefore one must question the practice of screening of the anterior nares alone and evaluate the additional benefit of screening both the nares and the throat.

The purposes of this multi-site study were to: determine if screening the throat increases the detection rate of MRSA colonisation; to determine the performance of the Roche LightCycler MRSA advanced test in the detection of MRSA in comparison to routine culture on selective chromogen agar (BD BBL CHROMagar II); and, to determine MRSA isolation rates from swabs transported in semi-solid media (Copan Venturi Transystem) and liquid-based media (Copan ESwab). The spa type and MRSA strain (if the spa type indicated that the MRSA isolate belonged to a named strain) were reported. spa typing could only be performed on cultured isolates.

**Materials and methods**

Swab specimens were collected from volunteers between June and December 2011. Subject samples were collected at nine sites across New Zealand. Specimens were tested at one site in Palmerston North, New Zealand. The volunteers consisted of: patients from hospital non-intensive units; medical laboratory staff; and hospital medical staff including nurses, physicians, infection control and administration staff. All volunteers were ≥16 years of age and provided written informed consent to participate in the study. Exclusion criteria included (i) antibiotic therapy, either topical or systemic that is active against MRSA up to three months prior to sample collection (ii) previous enrolment in the study (iii) contraindications to nasal and throat sampling according to the institution's policy.

This study was conducted in accordance with the New Zealand Health and Disability Ethics Committee multiple-site guidelines (Ethics reference number: CEN/11/EXP/030).

**Specimen collection**

Three swabs were collected from each subject, two nasal and one throat swabs. Nasal specimens were collected using two swab types: (i) Venturi Transystem rayon-tipped swab with Amies gel transport media (Copan, Italy), and (ii) Elution Swab (ESwab), a nylon-tipped flocked swab with 1mL of liquid Amies transport media (Copan, Italy). For throat specimens, only the ESwab was used. For collection, the anterior nares were sampled by insertion of the swab tip approximately one inch into the nostril and rotated against the mucosa five times. The same procedure was then repeated for the second nostril using the same swab. The throat was sampled by rotating the swab tip on both tonsils. Swabs of the anterior nares were collected first and then, using a different swab, the throat was sampled. The swabs were transported to the laboratory in their respective transport containers.

**Experimental design**

Nasal and throat ESwab specimens were tested by the Roche LC MRSA advanced PCR test, and the results were compared to those obtained by direct culture on BD BBL CHROMagar II MRSA medium (Fort Richard, Auckland) and broth-enriched culture containing 6.5% NaCl (salt/enrichment broth, Fort Richard, Auckland) followed by culture on BD BBL CHROMagar II MRSA media (referred to as CHROMagar from here on in). The nasal Venturi swabs were not tested on the LC. Swabs were cultured on the day of collection and batched in a run of 30 specimens for the LC PCR test. Swab samples were frozen (-20 ±2°C) after direct culture if a batch of 30 specimens was not available.

Electronic timers were used to monitor the start and finish times for sample preparation, working master-mix preparation, and the PCR preparation for the LC PCR test. Similarly, for CHROMagar culture, electronic timers were used to document the times for media inoculation, streaking, broth sub-culture of the samples and the final reading for directly plated and enrichment cultures. Times to result for all samples included in the study were also documented and are presented as mean times.

**Culture methods**

All three swab heads were cultured by rolling the swab head directly onto CHROMagar MRSA medium. The plates were incubated for a total of 48 ±4 hours (35 ± 2°C), with examinations at 24 ±4 hours and 48 ±4 hours of incubation. The ESwab heads were then processed using the LC PCR test.

Subsequently, the Venturi Transystem swab head and a 0.5 mL aliquot of the Liquid Amies transport media from each ESwab were individually inoculated into a vial containing 1mL 6.5% salt broth. Following incubation for 18-24 hours (35 ± 2°C), the broth was sub-cultured into another CHROMagar MRSA plate, and the plate was incubated for 24 ±4 hours as described above.

**Molecular methods**

The Roche LightCycler MRSA advanced test was performed according to the manufacturer's instructions (Roche Diagnostics, Switzerland). This assay targets the integration site of the SCCmec cassette into the *S. aureus* chromosome. Essentially, the LC PCR assay is performed on the LightCycler 2.0 instrument and has been validated with three transport media (Liquid Stuart, and Amies gel with or without charcoal).

**MRSA isolation and confirmation**

After incubation, growth of mauve colonies were considered positive (indicating MRSA) and no growth or colonies with other colours were considered negative.
The positive isolates were inoculated onto nutrient agar slopes (Fort Richard, Auckland), incubated at 35 ±2°C for 24 ±4 hours, and stored at room temperature for additional testing. To confirm MRSA, suspected isolates underwent a tube coagulase test (Remel) and cefoxitin resistance screening using the Clinical Laboratory Standards Institute (CLSI) disk diffusion method (31). Colonies were suspended in tryptic soy broth (TSB, Fort Richard, Auckland) to a turbidity of 0.5 McFarland standard, plated on Mueller-Hinton agar (Fort Richard, Auckland), and a 30 µg cefoxitin disc (Thermo Fisher Scientific, Auckland) was placed within the inoculum. Inhibition zone diameters were measured and recorded after a 24 ±4 hours incubation at 35-37°C (susceptible, ≥22mm; and resistant, ≤21mm).

PCR positive swab specimens that were culture negative were confirmed using the Qiagen DNAeasy Blood & Tissue Kit (Qiagen Sciences, MD). This assay tests for the presence of mecA using a method proposed by Huletsky et al. (32). A set of PCR primers specific to the different SCCmec right extremity sequences (meci354, mecii519, meciv511, meciv492, and meciv512) as well as a primer specific to S. aureus orfX (Xsaau325) were used in combination with three molecular beacon probes (XAsauB5-FAM, XAsauB8-FAM, and XAsauB9-FAM) targeting orfX’s sequences (32). The PCR reaction mix for this assay contained the following: 1x PCR buffer (minus Mg), 0.2 µM dNTP, 1.5 mM MgCl₂, 0.2 µM of each primer 1.0 unit of Platinum Taq DNA polymerase, template DNA and sterile MQ water (to make up to 50 µl). The PCR products were run on a 1.5% agarose gel (containing ethidium bromide) in 0.5X TBE buffer at 100 Volts for 75 minutes. The bands were visualised using the Multi Doc-It Digital Imaging System (UVP, CA).

Antimicrobial susceptibility testing
To characterise MRSA strains, coagulase positive isolates determined to be resistant to cefoxitin (i.e., MRSA) underwent antibiotic susceptibility testing using the CLSI method described above, for the following antibiotics (Thermo Fisher Scientific, Auckland): penicillin (10 µg), rifampicin (5 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), erythromycin (15 µg), clindamycin (10 µg) fusidic acid (10 µg), gentamicin (10 µg), mupirocin (5 µg), and tetracycline (30 µg). Except for fusidic acid and mupirocin, zones of inhibition were interpreted according to CLSI criteria (33). Fusidic acid zones of inhibition were interpreted as: ≥21 mm susceptible; 20 mm intermediate; and ≤19 mm resistant (34). Mupirocin zones of inhibition were determined with a 5 µg disc and interpreted as: ≥14 mm susceptible, and ≤13 mm resistant (35). Additional testing included the determination of oxacillin minimum inhibitory concentration (MIC, 256-0.015 µg/mL) and the detection of inducible clindamycin resistance using the CLSI reference methods (31). S. aureus ATCC 29213 was the positive control. The isolates were then inoculated onto nutrient agar slopes, incubated (35°C for 24 h) and sent to the Institute of Environmental Science and Research, Porirua, New Zealand (ESR) for staphyloccocal protein A gene (spa) typing.

Staphylococcal protein A (spa) typing
ESR sequence the spa gene using a method adapted from that described by Strommenger et al. (36). spa sequences were analysed using Ridom StaphType software version 2.0.3 (Ridom GmbH, Würzburg, Germany).

Statistical analysis
Statistical significance was determined by the McNemar’s test. A P value of ≤0.05 was considered statistically significant.

Results
During the study period from June to December 2011, 810 specimens were collected from 270 eligible volunteers enrolled at the nine clinical study sites. In total, 91% of volunteers were aged 16-60 years, and 9% were aged >60.

The median age group was 40-49 years. Females were over-represented, comprising 73% of the total volunteers. The majority of specimens were collected from medical laboratory workers (89%), followed by specimens from medical staff (9%), and hospital patients (2%).

Comparison of culture and PCR for the detection of MRSA
From a total of 270 volunteers screened, the CHROMagar culture method (direct and broth enriched) detected seven (2.6%) MRSA positive volunteers and the Roche LC PCR test detected 13 (4.8%) MRSA positive volunteers (Figure 1). The 2.2% higher yield obtained by the PCR method was statistically significant (P = 0.04). MRSA isolation via broth enriched culture was considerably higher (2.6%) than direct culture (1.5%). While females were over-represented in all groups except the hospital patient group, no significant difference in isolation rates was observed between males and females (data not shown).

MRSA was isolated from one hospital patient. The remaining MRSA positive samples were isolated from laboratory workers.

Figure 1. MRSA positivity rate among the 270 volunteers screened for MRSA using: the Roche LC test (PCR); culture on BD CHROMagar (direct culture); and culture in salt broth followed by subculture onto BD CHROMagar (broth culture). Among the 540 pairs of swabs tested by PCR and culture, 10 were positive by culture and 16 were positive by the LC assay. The 10 specimens positive by culture were from seven volunteers, and the 16 MRSA PCR positive specimens were from 13 volunteers, all of whom had two swab specimens tested with each method (one nose and one throat swab). Table 1 summarises the efficiency of the LC assay in comparison with culture for the detection of MRSA. Of the six specimens from six volunteers that were PCR positive but culture negative, none were culture-positive after subculture from the enrichment broth, but
Site-specific isolation of MRSA
Of the 270 volunteers studied, the percentage of MRSA carriers considering the nares, the throat and both sites, using both the PCR and culture methods, was 4.8% (13 volunteers). The incidence of MRSA colonisation from each site is displayed in Table 2.

Table 2. Swabs from the anterior nares and throats of 270 volunteers were screened for MRSA by the Roche LC test (PCR) and by culture on BD CHROMagar (direct and salt broth culture combined).

<table>
<thead>
<tr>
<th>Types of carrier</th>
<th>PCR method</th>
<th>Culture method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior nares</td>
<td>Anterior nares</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>257</td>
</tr>
</tbody>
</table>

The Roche LC PCR assay displayed superior performance compared to the culture method in the detection of MRSA from volunteers with single site colonisation, especially the anterior nares. However, the PCR method had equal performance to the culture method in detecting MRSA from volunteers with both nose and throat colonisation of MRSA (Figure 2).

When the PCR assay was used, throat only MRSA carriers accounted for 38.5% of the total MRSA isolations, equal to that isolated from nasal-only carriers. Dual site colonization accounted for the remaining 23%. However, when culture was employed, 42.8% of the total MRSA positive volunteers were throat only carriers, equal to dual site carriers. The remaining 14.4% were nose only carriers, less than half the figure detected using the PCR assay (Table 3).

Table 3. MRSA isolation from the different sampling sites (nose and/or throat) among the MRSA positive volunteers using the Roche LC test (PCR) and the BD CHROMagar culture method (direct and salt broth culture combined).

<table>
<thead>
<tr>
<th>Type of carrier</th>
<th>% (#) of total MRSA carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR</td>
</tr>
<tr>
<td>Throat only</td>
<td>38.5 (5)</td>
</tr>
<tr>
<td>Nasal only</td>
<td>38.5 (5)</td>
</tr>
<tr>
<td>Nasal and throat</td>
<td>23.0 (3)</td>
</tr>
</tbody>
</table>

Comparison of swab type on the viability of MRSA
From the seven MRSA culture positive volunteers, one tested positive using the ESWab that was missed using the Venturi swab. This was re-tested and confirmed. In regards to the remaining negative MRSA culture results, the ESWab and the Venturi swab systems were 100% concordant.

Turn-around time performance
The technical hands on time as well as the mean time to result for the Roche LightCycler MRSA advanced test and CHROMagar culture are presented in Table 4. For both methods, the labour involved was documented for 30 specimens and then averaged for a per sample value. The culture labour calculations included: inoculation and streaking of media; enrichment broth inoculation; 24-hour culture interpretation; broth sub-culture; and 48-hour culture interpretation. Time for confirmatory testing was not included. For the LC PCR assay, calculations included: lysate preparation; master mix preparation; and specimen and control PCR preparations.

Table 4. Mean processing times and time to result of the PCR assay and culture for a batch of 30 samples. 27 batches were tested by the culture method using the 810 swab samples collected from 270 volunteers. 18 batches were tested by the PCR method using the 540 swab samples collected from 270 volunteers.

<table>
<thead>
<tr>
<th>Time for assay/test</th>
<th>Mean time ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche LightCycler MRSA Advanced Test (PCR)</td>
<td></td>
</tr>
<tr>
<td>Lysate preparation</td>
<td>27 ± 3.7 min</td>
</tr>
<tr>
<td>Master mix preparation</td>
<td>2.6 ± 0.3 min</td>
</tr>
<tr>
<td>PCR preparation</td>
<td>20 ± 3.0 min</td>
</tr>
<tr>
<td>Amplification and detection</td>
<td>79.0 ± 2.4 min</td>
</tr>
<tr>
<td>Total hands on time per run</td>
<td>49.4 ± 5.2 min</td>
</tr>
<tr>
<td>Total time per run (time to result)</td>
<td>2.14 ± 0.1 hours</td>
</tr>
<tr>
<td>Average hands on time per specimen</td>
<td>1.65 ± 0.17 min</td>
</tr>
<tr>
<td>BD BBL CHROMagar II culture</td>
<td></td>
</tr>
<tr>
<td>Swabbing, streaking, and broth inoculation</td>
<td>36.7 ± 3.4 min</td>
</tr>
<tr>
<td>Direct culture- 24-h reading (time to result)</td>
<td>24 ± 4 hours</td>
</tr>
<tr>
<td>Salt broth culture- 48-h reading (time to result)</td>
<td>48 ± 4 hours</td>
</tr>
<tr>
<td>Average hands on time per specimen</td>
<td>1.20 ± 0.1 min</td>
</tr>
</tbody>
</table>

Regardless of the result, the average personnel hands-on-time was 1.65 min per sample for the LC PCR assay, which was comparable to that of culture value of 1.20 min per sample. However, the average time to get the result for the PCR technique was considerably shorter than that of culture (2.14 and 48 hours respectively).

Antimicrobial resistance and strain variation
MRSA isolates were penicillin (100%), erythromycin (10%), clindamycin (10%), ciprofloxacin (0%), mupirocin (20%), rifampicin (0%), gentamicin (0%), tetracycline (0%), fusidic acid (20%), and co-trimoxazole (0%) resistant. Table 5 summarises the antimicrobial resistance, melting temperatures (Tm), and strain variation between the MRSA isolates.
**Table 5.** Antimicrobial susceptibility patterns, melting temperatures (Tm, °C), spa types, MRSA strains, and SCCmec types of the 10 MRSA cultures isolated by the culture method (direct and salt broth culture combined).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Site</th>
<th>Fox</th>
<th>Ox MIC*</th>
<th>E</th>
<th>DA</th>
<th>CIP</th>
<th>FD</th>
<th>MUP</th>
<th>PEN</th>
<th>SXT</th>
<th>RD</th>
<th>TET</th>
<th>CN</th>
<th>Tm (°C)</th>
<th>spa type</th>
<th>Strain</th>
<th>SCCmec type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Nose</td>
<td>R</td>
<td>32</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>58.1</td>
<td>1002</td>
<td>AK3</td>
<td>IV</td>
</tr>
<tr>
<td>A2</td>
<td>Throat</td>
<td>R</td>
<td>32</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>58.1</td>
<td>1002</td>
<td>AK3</td>
<td>IV</td>
</tr>
<tr>
<td>B</td>
<td>Throat</td>
<td>R</td>
<td>4</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>58.7</td>
<td>15677</td>
<td>No strain</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Throat</td>
<td>R</td>
<td>4</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>59.6</td>
<td>1019</td>
<td>WSPP</td>
<td>IV</td>
</tr>
<tr>
<td>D</td>
<td>Nose</td>
<td>R</td>
<td>4</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>58.9</td>
<td>15677</td>
<td>No strain</td>
<td>-</td>
</tr>
<tr>
<td>E1</td>
<td>Nose</td>
<td>R</td>
<td>64</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>59.6</td>
<td>1019</td>
<td>WSPP</td>
<td>IV</td>
</tr>
<tr>
<td>E2</td>
<td>Throat</td>
<td>R</td>
<td>64</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>59.6</td>
<td>1019</td>
<td>WSPP</td>
<td>IV</td>
</tr>
<tr>
<td>F</td>
<td>Throat</td>
<td>R</td>
<td>64</td>
<td>S</td>
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<td>No strain</td>
<td>-</td>
</tr>
<tr>
<td>G1</td>
<td>Nose</td>
<td>R</td>
<td>32</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>57.7</td>
<td>1127</td>
<td>AK1</td>
<td>IV</td>
</tr>
<tr>
<td>G2</td>
<td>Throat</td>
<td>R</td>
<td>32</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>57.7</td>
<td>1127</td>
<td>AK1</td>
<td>IV</td>
</tr>
</tbody>
</table>

*Oxacillin minimum inhibitory concentration (ug/mL)

Fox, cefoxitin; E, erythromycin; DA, clindamycin; CIP, ciprofloxacin; FD, fusidic acid; MUP, mupirocin; PEN, penicillin; SXT, co-trimoxazole; RD, rifampicin; TET, tetracycline; CN, gentamicin.

Three volunteers who were nose and throat MRSA positive carried the same MRSA strain in both sites. The only discrepancy was that in volunteer A, the throat strain was resistant to both erythromycin and clindamycin (induced by erythromycin, as was shown by a positive induction test result, i.e. a “D-shaped” zone was observed between the erythromycin and clindamycin discs), while the nasal counterpart was sensitive to both antibiotics. No pattern was evident to suggest that certain strains inhabit certain sites. However, a link between the MRSA strain and the melting temperature was apparent: the WSPP (Western Samoan Phage Pattern) strain had the highest Tm, followed by the AK3 strain, and lastly, the WK/AK1 strain has the lowest Tm. Additionally, the WSPP strain was the most frequently isolated strain, followed equally by the AK3 and WK/AK1 strains. Three spa patterns were not related to any identifiable spa types.

In total, six swabs were MRSA PCR positive/culture negative. The Qiagen DNAeasy PCR assay was used to confirm the LC PCR assay. All of the six samples were positive for the mecA gene and generated fragment lengths of approximately 278 base pairs (Figure 3 and Table 6). mecA positive PCR results can have band sizes of 176, 223, or 278 bp (32).

**Figure 3.** Qiagen DNAeasy PCR assay gel electrophoresis results

**Discussion**

A principal finding of the present study is that the MRSA incidence of 4.8% among the volunteers investigated is higher than the reported national incidence of 0.017% (37), but compares with a previous study conducted on New Zealand health workers in which 4% of samples tested were MRSA positive (38). While females were over-represented in all except the hospital patient groups, no significant difference in isolation rates was observed between the sexes.

**Culture vs. PCR**

The Roche PCR method was significantly more sensitive than the chromogenic agar method, detecting 2.2% more volunteers colonised with MRSA. In the current study, the PCR assay had a sensitivity of 100%, a figure previously reported by Boyce and Havill (13) but marginally higher than that reported in other studies in which nasal swab specimens were directly inoculated onto agar (range 90% to 98.5%; ref. 39-43) and similar to or better than in four other studies in which agar based cultures included a broth enrichment stage (range, 81% to 92%; ref. 41,43-45).
The specificity and PPV of the Roche LightCycler MRSA assay in the present study were 98.9% and 62.5%, respectively. Numerous studies have reported PPVs ranging from 63% to 95.8% which most likely reflects the differences in the predominant circulating MRSA types and by the respective study populations (13,48). High sensitivity of any MRSA test is necessary because the objective of a MRSA screening program is to swiftly identify all those colonised with this bacterium, even if detection results from identifying DNA no longer associated with viable organisms (8).

Possible explanations for the observed differences in the detection rates between the culture method and the PCR assay could be: i) false positive PCR tests due to mecA loss from SCCmec cassettes, ii) MRSA being below the limit of detection of culture and/or the presence of substances in the specimen, such as antibiotics, which could inhibit the growth of MRSA but not interfere with PCR amplification of the organism and, iii) the ability of the PCR method to detect dead organisms. While false positive PCR tests that occur in the presence of "empty cassettes" resulting from the specific loss of mecA have been described previously (32,49,50), it is not something that happens very often (F. Merien, personal communication, March 17, 2012). However, this was a limitation in the present study that we can not investigate further. A possible solution would have been to use non-selective agar to allow growth of MSSA to examine the likelihood of this organism having a residual SCCmec right-extremity fragment following the deletion of a chromosomal segment containing mecA (32).

A previous study by Malhotra-Kumar et al. (15) found that chromogenic media tend to show reduced sensitivity at low MRSA bacterial loads. They concluded that this is due to some MRSA strains harbouring the SCCmec types III or IV which did not yield characteristic colonies at 24 h of incubation at residual MRSA loads of 1 and 10 colony forming units, or that the colony colour was not specific enough, causing some investigators to misinterpret these samples as MRSA negative. These conclusions may explain the inconsistency in medium performance seen between studies, which may be influenced by both, the predominant circulating MRSA types and by the differences in their colonisation potential (51).

Another major difference between the MRSA detection rates of PCR and culture is the ability of the latter to detect dead bacteria. PCR methods are able to detect organisms as long as the target DNA sequence is not injured, no matter whether the cell is viable, inactive or dead (52). Consequently, PCR techniques are evidently more sensitive than culture; nonetheless, they lack the ability to distinguish active cells from dead cells unless supplementary methods, such as viability assays, are used (53).

Interestingly enough, when we compared the isolation rate of MRSA from the nares and throat, via PCR and culture, the most noticeable increase in detection rate was from the anterior nares when using the PCR assay. PCR more than doubled the number of MRSA from the anterior nares when compared to culture (1.85% and 0.40%, respectively). This shows that even if PCR is applied to current MRSA screening practices (nasal only screening) current chromogenic methods may be missing potential MRSA colonisation, high test specificity can be important when undertaking a large number of tests to minimise false positive test results which can lead to inappropriate patient isolation (8).

Test result turnaround time and cost
In the present study, the personnel hands on time per sample for the culture and PCR method were comparable (1.20 and 1.65 min, respectively). The significant difference was the time to result for each method. By using the Roche LC PCR assay, laboratories have the ability to obtain results within two to three hours. Realistically, in a clinical setting, where batch processing of samples for PCR assays is performed once a day, the turnaround time for the PCR method may equate to 10-15 hours, a figure previously reported by Boyce and Havill (13). Even if this was the case, the PCR method is still much faster. This allows earlier isolation and treatment of MRSA affected patients and hence is critical for the prevention of MRSA outbreaks. A study by Cunningham et al. demonstrated a 50% decrease in the MRSA prevalence from 13.9/1,000 patient days (under culture method) to 4.9/1,000 patient days under PCR screening (10). Additionally, the PCR method used in this study required only four hours of staff training with minimal expertise required to perform the assay. One other major factor that all health institutions consider when implementing a new method is the cost of the test. Varying costs for chromogenic media and molecular methods have been previously reported: $7.52 and $25.50 per test, respectively (13), and € 40 versus € 4 per test, respectively (54). From our study, we have estimated that the Roche PCR assay costs 2.5 times more that the BBL CHROMagar method (data not shown). However, we believe that this additional cost may be offset by cost reductions due to pre-emptive isolation of patients. The objective of pre-emptive isolation is to prevent secondary MRSA cases while waiting for screening results. Specifically, this comes down to result turnaround times and test sensitivity, features that favour PCR methods over chromogenic methods. On the other hand, the cost efficacy of the direct PCR depends on the local infection control algorithms employed and the local epidemiology of MRSA. In patient populations with low MRSA incidence, the broad use of PCR probably is not cost-effective (55). As such, in countries like New Zealand where a MRSA incidence is modest, further local studies are required to assess the cost saving by employing a PCR method to replace or be run in parallel to current culture methods.

Transport media and organism viability
The present study suggests that the ESwab had a superior ability to maintain MRSA viability when compared to the Venturi counterpart. Additionally, the ESwab did have other subjective advantages. Being flocked, it was an easier swab system to use for collection as it allowed quicker uptake/absorption of surface mucous. Volunteers did not find this discomforting in comparison to the Dacron spun Venturi swabs. In fact, some volunteers found the ESwab more pleasant than the Venturi counterpart.

In the laboratory, the ESwab was much easier to manipulate than the Venturi swab due to the liquid media which allows the ability for multiple tests to be performed by using aliquots of the liquid media. Another advantage of the ESwab is its small size, flat base of the collection container, and the screw top cap which made the handling of the ESwab specimens much easier. The Venturi swab systems are long, have no screw-cap, and have a rounded base which makes them impossible to stand alone therefore one is forced to use both hands or a rack. The liquid phase is also an ideal platform for automated handling and specimen pooling. Two previous studies evaluated pooled against separate specimens for the detection of *S. aureus*. They concluded that pooling culture results for swabs from nare and the throat may be an appropriate method to optimise the yield of *S. aureus*-positive while saving the expectations of additional cultures (27,56). While the ESwab (NZ$1.39) is more expensive than the traditional Venturi swab (NZ$0.61), the additional cost may be offset by ability to pool samples.
Site specific MRSA colonisation

To our knowledge, this is the first study to have evaluated the importance of the throat in MRSA carriage in New Zealand. Previous studies have found a variable ratio of S. aureus throat colonisation fluctuating from 4% up to 64% (25-29). In our study, MRSA was found in the throats of 61.5% (PCR) to 85.6% (culture) of all the MRSA positive volunteers. In 38.5% (PCR) to 42.8% (culture) of these volunteers, the throat was the only site from where MRSA could be isolated suggesting that individuals can be colonised exclusively in the throat and would be missed on screening limited to the anterior nares. Similar findings have been reported previously in studies from the USA and Australia (26,57).

The addition of the throat swab increased the overall MRSA yield from 3% (anterior nares only swab sampling) to 4.8% (anterior nares and throat swab sampling). We believe that unidentified throat carriers may spread MRSA. Mertz et al. (27) suggests that these unidentified MRSA throat carriers may explain, in part, why many decolonisation schemes are prone to failure. During our study, we found one hospital patient who had MRSA in the throat only who would have been missed with routine nasal screening. Certainly, throat carriage has been documented to cause MRSA outbreaks. Kluytmans et al. (58) documented a large outbreak of MRSA infection, which was traced back to a health care worker who was exclusively colonised in the throat. Routine nasal screening failed to identify this carrier.

Throat swab specimens have been obtained routinely in the Netherlands for decades as part of the successful search-and-destroy policy (27). Like Mertz et al. (27), we also think that an additional throat screening is very important during the investigation of MRSA carriers. Marshall and Spelman (26) suggest either throat or nose swabs are essential for MRSA detection, but both are preferable. We disagree with this conclusion as both sites are important. Screening of the anterior nares only should be substituted with screening of the nares and the throat and the costs of cultures of the nose and throat can be minimised by pooling the samples in the laboratory, which is applicable for both conventional cultures and PCR techniques (56).

Strain variation

As expected, all of the MRSA isolated during the study were penicillin resistant. This is because mecA encodes PBP2a which is not inhibited by β-lactams. In the present study, the WSPP strain was the most frequently isolated strain, followed equally by the AK3 and WK/AK1 strains. While isolated in low numbers, our results are consistent with a previous report (59). However, it seems that the dominance of the WSPP clone in New Zealand is diminishing. Richardson and colleagues have documented that the prevalence of AK3 MRSA has increased each year since 2005, and in 2010 this strain accounted for the highest proportion (29.0%) of MRSA isolations (37). All the MRSA strains isolated in this study had type IV SCCmec elements. In New Zealand, it is now apparent that type IV SCCmec strains dominate MRSA isolations, and that this has been the case since 1995 (59). Since then, these strains have comprised on average almost three-quarters of all isolates per year, with a mean of 74% (37,59).

Although WPP, AK3, and WR/AK1 MRSA strains are considered community-associated, in our study they were all isolated from laboratory health workers and a hospital patient. We did not categorise volunteers according to where they acquired MRSA. Therefore, some participants who may have acquired MRSA in the community were categorised as hospital patients or staff. This is supported by the fact that the strains isolate in this study were not multi-resistant, a feature typical of community-associated MRSA (37). Additionally, as MRSA strains become progressively common in a variety of settings, the use of restrictive terms like HA-MRSA and CA-MRSA will become less meaningful as spread between all populations occur.

The potential to recover an increasing array of SCCmec types, especially from community-occurring MRSA strains, clearly exists (60).

Our study has some limitations. Firstly, by using clinical samples, we did not know the initial inoculum density therefore, the recovery percentage of the ESwab/conventional swab systems was not determined. Secondly, we did not include swabs utilised as zero-time controls. This was not possible for this real practice study as swabs would not be processed immediately after being taken. Thirdly, previous studies have focused on the CLSI M40-A protocol using a high initial inoculum for testing the swab systems. In our study, we tested the swab systems with clinical samples and therefore probably also with low numbers of microorganisms. Because of this, the results of the conventional sample with MRSA and the ESwab system was impossible to evaluate. Lastly, since we did not use non-selective agar, we were unable to determine if the PCR positive/culture negative results were due to methicillin-susceptible S. aureus isolates with a residual SCCmec right-extremity fragment following the deletion of a chromosomal segment containing mecA (32).

Conclusions

Our results with the Roche LightCycler MRSA Advanced Test show that it is an accurate and rapid method to detect MRSA colonisation, especially when compared to chromogenic culture. While the PCR method was more costly, it had superior sensitivity and specificity and was comparable to the culture method in regards to the amount of personnel hands on time per sample. The PCR assay can be performed by any microbiology personnel with minimal additional training and allows same day results. We therefore conclude that it wise to introduce PCR for MRSA screening.

Much effort has been expended to detect nasal MRSA carriers; however, throat carriers may contribute to spread MRSA infections. Our results support what has been previously reported; sampling from the anterior nares is insufficient for efficient detection of MRSA carriers. This study showed that the throat is an important habitat of MRSA hence any screening program for MRSA should include swab specimens from both, the anterior nares and the throat.

Both Copan swab types performed almost equally in maintaining the viability of MRSA during the study. While the ESwab was approximately double the price of the Venturi counterpart, it had a few advantages over the Venturi swabs system. The liquid phase allowed the pooling of samples and multiple testing to be performed from one swab specimen. Further studies are needed to determine if the higher cost of the ESwab will be offset by these advantages.

Acknowledgements

Based on a research project submitted in partial fulfilment of the requirements for the degree of Master of Science with Honours in Medical Laboratory Science, Institute of Veterinary, Animal, and Biomedical Sciences (IVABS), Massey University, Palmerston North, New Zealand. Financial assistance was provided by the Palmerston North Medical Research Foundation and the Massey University IVABS Postgraduate Fund. Roche Diagnostics New Zealand Ltd provided all the PCR equipment, PCR consumables, and on-site instrument training. Fort Richard provided the media and collection swabs at a discounted rate. We are thankful to: all the participants and those involved in volunteer enrolment; Southern Cross Hospitals for their support during the study period; Helen Heffernan and the team at ESR for typing the MRSA isolates; and Liz Burrows (Massey University) for confirming the culture negative, PCR positive MRSA isolates. The results of this project were presented at the Roche Molecular User Group Meeting September 2012. The authors declare no conflict of interest.
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References
NZIMLS Biochemistry SIG coming soon...........

Wellington, 29 June 2013

Further details to come........

Contact for presentations: Kim Allan, Aotea Pathology

email: kallan@apath.co.nz
Culture, identification and susceptibility testing of *Clostridium difficile* from EIA toxin positive faecal samples

Mary Stevens

Abstract

**Aim:** The aim of this study was to culture and identify *Clostridium difficile* from EIA toxins A/B positive and equivocal faecal samples. Breakpoint susceptibilities were performed on isolates to determine a baseline for future monitoring of emerging antimicrobial resistance.

**Methods:** After alcohol-shock treatment, samples were cultured onto *C. difficile* agar and incubated anaerobically at 36°C (±1°C) for 48 hours (±4 hours). Colonies visually resembling *C. difficile* were identified using Bruker MALDI-TOF and/or 16S rRNA sequencing. Agar dilution breakpoint plates with ciprofloxacin (2 and 8 µg/mL), vancomycin (8 and 32 µg/mL), metronidazole (8 and 32 µg/mL), amoxycillin/clavulanic acid (4/2 and 16/8 µg/mL) and meropenem (4 and 16 µg/mL) were inoculated to determine the susceptibility of isolates.

**Results:** Eighty five isolates were identified as *C. difficile* using MALDI-TOF. Isolates not identified by MALDI-TOF were subsequently identified using 16S rRNA sequencing. Antimicrobial susceptibility was determined for 91 *C. difficile* isolates. All were inhibited by the lowest concentration of vancomycin, metronidazole, amoxycillin/clavulanic acid and meropenem. Eighty nine isolates were resistant to ciprofloxacin.

**Conclusions:** Alcohol-shock treatment, combined with a selective agar, was an effective method of culture for *C. difficile*. MALDI-TOF was found to be a reliable, rapid and inexpensive tool in the identification of this organism. All the isolates showed susceptibility to vancomycin, metronidazole, amoxycillin/clavulanic acid and meropenem, while most were resistant to ciprofloxacin.

**Key words:** *Clostridium difficile*, antimicrobial susceptibility, 16S rRNA sequencing, MALDI-TOF, mass spectrometry, PCR ribotype 027.


Introduction

*Clostridium difficile* is a spore-forming, obligate anaerobic, Gram positive bacillus. It is ubiquitous in the environment and causes disease in humans and animals. *C. difficile* infection is primarily a nosocomial infection associated with prolonged antibiotic use, particularly cephalosporins and/or fluoroquinolones. Genetically encoded toxins (commonly toxins A and/or B) are responsible for the *in vivo* effects of this organism. If undiagnosed or untreated, pseudomembranous colitis, toxic megacolon and death may result. *C. difficile* infection is treated with metronidazole and/or vancomycin (1).

Since the early 2000s, hypervirulent strains (particularly PCR ribotype 027, also known as NAP-1 or BI) have been responsible for major outbreaks and subsequent increased mortality in the northern hemisphere, especially North America and Europe (2-4). Overseas studies have suggested a link between high-level fluoroquinolone resistance and PCR ribotype (5,6). Hypervirulent strains have recently been detected in New Zealand (7,8).

At Canterbury Health Laboratories, detection of toxins A/B from faecal samples is performed by enzyme immunoassay (EIA) (Premier™ Toxins A&B, Meridian Bioscience). Daily batch testing ensures results are available within 24 hours. Culture, in comparison, can take several days. In addition, accurate identification by traditional methods is difficult as other *Clostridium* species can be hard to differentiate from *C. difficile*. Identification by molecular sequencing, while accurate, takes a minimum two days and is limited to specialist laboratories.

The aim of this study was to culture and identify *C. difficile* from EIA toxins A/B positive and equivocal faecal samples. Breakpoint susceptibilities were performed on isolates to determine a baseline for future monitoring of emerging antimicrobial resistance.

**Methods**

**Faecal samples**

All faecal samples which tested positive or equivocal for *C. difficile* toxins A/B during the period September 2008 to March 2010 were included in the study. A total of 154 faecal specimens (146 toxin positive and eight specimens with equivocal results) were stored at -20°C (±3°C).

**C. difficile culture**

Stored faecal samples were subsequently thawed, subjected to alcohol-shock treatment (9), cultured onto Cycloserine, Cefoxitin, Fructose (CCF) Agar (Fort Richard Laboratories, Auckland), and incubated anaerobically at 36°C (±1°C) for 48 hours (±4 hours). After incubation, plates were examined for typical, ragged edged colonies (Figure 1) then observed under ultra violet light. Colonies with characteristic chartreuse fluorescence were stored at -80°C (±3°C) in brain heart infusion broth supplemented with 15% glycerol.

![Figure 1. *C. difficile* on CCF agar.](image-url)
**C. difficile** identification

Stored isolates were subsequently thawed and sub-cultured onto Columbia sheep blood agar (Fort Richard Laboratories, Auckland). Plates were incubated anaerobically at 36°C (±1°C) for 48 hours (±4 hours). Cultured isolates were identified using MALDI-TOF mass spectrometry (Bruker Daltonics) using MALDI Biotype version 3.0 software. A small number of isolates were also identified by 16S rRNA sequencing to confirm that MALDI-TOF identification was correct.

### Susceptibility testing

Breakpoint susceptibilities were tested by CLSI agar dilution method (10). Ciprofloxacin (2 and 8 µg/mL), vancomycin (8 and 32 µg/mL), metronidazole (8 and 32 µg/mL), amoxicillin/clavulanic acid (4/2 and 16/8 µg/mL) and meropenem (4 and 16 µg/mL), were incorporated into agar plates, prepared in-house using Oxoid Brucella agar base (Oxoid NZ Ltd) supplemented with 5µg hemin and 1µg vitamin K, per mL and 5% laked sheep blood. A sterilised replicator device with 32 x 3 mm pins was used to inoculate prepared plates with an inoculum density of 1 x 10⁶ CFU per 2.0 µL spot. Plates of lower antimicrobial dilution were inoculated first. An inoculation control plate (Columbia sheep blood agar, Fort Richard Laboratories, Auckland) was inoculated between each antibiotic series and all plates had been inoculated. C. difficile NCTC 11382 was used as the control strain. Results were interpreted by CLSI criteria, where available (10).

### Results

Of the 154 samples cultured, 120 yielded bacterial growth with colonial morphology resembling *C. difficile*; four of these were from eight samples with EIA equivocal results. No growth was obtained from 28 samples. Six samples grew bacteria not resembling *C. difficile*. After the exclusion of duplicates, the remaining 112 isolates were stored at -80°C (±3°C). However, 11 of these were non-viable on sub-culture, leaving 101 isolates for further analysis.

MALDI-TOF identified 85/101 (84.2%) isolates as *C. difficile*, two as *C. butyricum*, one each as *C. clostridioforme* and *C. symbiosum* and three as non-clostridia. Nine isolates were unable to be identified by MALDI-TOF, but were subsequently identified by 16S rRNA sequencing; six as *C. difficile*, one *C. neonatale*, and two *Clostridium* spp., unable to be specified. 16S rRNA sequencing confirmed the MALDI-TOF identification of a representative eight *C. difficile* isolates and the two *C. butyricum*. Neither the *C. clostridioforme* nor the *C. symbiosum* could be confirmed by sequencing.

A total of 91 confirmed *C. difficile* isolates were available for breakpoint susceptibility testing. Results are displayed in Table 1. One isolate grew at 2 µg/mL ciprofloxacin, but was inhibited at 8 µg/mL. All isolates grew on all inoculation control plates.

### Discussion

Although culture and identification of *C. difficile* is not routinely performed, it is necessary for antimicrobial susceptibility monitoring and for ribotyping; both of which are important epidemiologically. Therefore, rapid and inexpensive, methods of culture and accurate identification are desirable.

Alcohol-shock eliminates non-spor forming gastro-enteric organisms and promotes spore production. When cultured onto selective, enrichment medium, such as CCF agar, spores germinate and a pure growth of *C. difficile* (if present) should result. 16S RNA sequencing, the gold standard for organism identification, is specialised, time-consuming and costly. Colonial morphology and fluorescence are useful but other Clostridia mimic *C. difficile*, as shown in this study. For MALDI-TOF identification, organisms are taken from the anaerobic environment for only the short time taken to transfer them to the MALDI target. Therefore they remain viable, eliminating the need for repeated sub-culture if susceptibility testing and/or typing are required.

Culture and identification of *C. difficile* was confirmed for only 91 (59%) of 154 samples (62% of toxin A/B positive and 50% of equivocal samples). This is similar to the 57% recovery rate of Limbago et al (11), but lower than that achieved by Roberts et al (7). The failure to isolate *C. difficile* from toxin A/B positive samples could be attributed to a number of factors: suboptimal faecal storage conditions prior to culture, samples in which the organisms were no longer viable but toxin remained detectable, or cross reactivity of *C. sordellii* toxins with *C. difficile* toxins A/ B, which is a known limitation of the Premier™ Toxins A&B EIA procedure (12). Because *C. sordellii* does not fluoresce under ultra violet light, any isolates of this organism were not stored for further identification. Although no correlation between strength of EIA result and recovery of organisms was attempted, the lower isolation rate in EIA equivocal specimens suggests that organism recovery is more difficult in specimens with low numbers of toxigenic *C. difficile*. Furthermore, the failure of 11 isolates to survive -80°C (±3°C) storage h ad an impact on the confirmed culture rate. In the future, MALDI-TOF identification directly following isolation should eliminate the need for storage prior to analysis.

All isolates were susceptible to the antibiotics tested with the exception of ciprofloxacin. This is consistent with the findings of Roberts et al from other parts of New Zealand (7). The *in vitro* susceptibility of all isolates to metronidazole and vancomycin indicates that treatment with these agents is currently still valid in New Zealand. These isolates were not typed, but future surveillance to include PCR ribotyping, as well as susceptibility testing, is recommended.

In conclusion: alcohol-shock, combined with a selective, enrichment agar is an effective method of culture for *C. difficile*. MALDI-TOF is an inexpensive, simple and reliable tool for the identification of *C. difficile*. All isolates were susceptible to vancomycin, metronidazole, amoxicillin/clavulanic acid and meropenem, while most of the isolates appeared resistant to ciprofloxacin. However, close monitoring of antimicrobial susceptibility trends is important. PCR ribotyping of future isolates is recommended to detect possible emergence of hypervirulent strains.

### Acknowledgements

I am grateful to the following colleagues at Canterbury Health Laboratories for their assistance and encouragement with this project: Trevor Anderson, Dr Mona Schousboe, Julie Creighton and David Beckingham.

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**Table 1. Antimicrobial susceptibility of *C. difficile* isolates**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC breakpoints (µg/mL)</th>
<th>Susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤2</td>
<td>≥8</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤8</td>
<td>≥32</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>≤8</td>
<td>≥32</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>≤4/2</td>
<td>≥16/8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤4</td>
<td>≥16</td>
</tr>
</tbody>
</table>

Breakpoints obtained from Bourgault et al (5). CLSI breakpoints (10).
References
Cystic fibrosis in adulthood - an evolving spectrum disorder

Sandy Woods

Abstract
Cystic fibrosis has historically been described as a severe disease mainly confined to children. Over the last 2-3 decades the survival rate has improved dramatically and more patients with cystic fibrosis live into adulthood. Since 2005 Canterbury Health Laboratories has noticed a significant increase in adult sweat test requests. Those who present as adults usually have had mild symptoms or lack the common symptoms of cystic fibrosis that may have made the disease apparent in youth. This article looks at a period of 15 years of sweat testing in the laboratory, showing the increase in adult sweat test requests and will compare sweat chloride results and cystic fibrosis mutational analysis results and describe some atypical findings.

Key words: cystic fibrosis, sweat test, chloride, cystic fibrosis transmembrane conductance regulator gene, genotype


Introduction
Classically, cystic fibrosis (CF) is a childhood disease characterized by chronic lung disease, sinusitis, nasal polyps, pancreatic insufficiency, meconium ileus and elevated sweat sodium and chloride concentrations (1). It is an autosomal recessive disorder caused by impaired chloride transport across the apical membrane of cells as a result of mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (2). Amongst people of northern European ancestry, there is a carrier frequency of approximately 1 in 25 (1).

Although CF is traditionally regarded as a severe disease and presenting mainly in infancy or childhood, in recent years more adults have been diagnosed with the condition, reflecting an increased awareness of what has become termed "atypical cystic fibrosis" (1,3,4). The diagnosis may not be as "black and white" as originally thought, with increasing recognition that these atypical cases exist.

History
In the 1940s, the term "cystic fibrosis" was coined by Dr. Dorothy Andersen of the Babies' Hospital New York. She was the first doctor to give the disease its earliest definitive description. She also theorized that the condition was caused by deficiency in Vitamin A, although she was also instrumental in disproving this original hypothesis (5). Abnormal secretion of mucus was subsequently connected to the disease and the use of antibiotics, particularly penicillin for the treatment of the disease, had begun. The 1950s saw the introduction of the sweat test, which is still considered to be the gold standard test for CF (2).

The 1960s was a period when organisations specialising in CF research were founded. Rudimentary work on neonatal screening for CF began in the 1970s (6). The CF transmembrane conductance regulator (CFTR) gene was discovered in 1989 (2). The 1990s saw the introduction of the drug Pulmozyme to improve clearance of bronchial secretions (7). In 2005, the median life expectancy had reached 37 years compared with just 5 years in the 1950s (8). Over the last 2-3 decades, the survival rate has improved dramatically and more patients diagnosed with CF live well into adulthood.

In addition, it is increasingly recognised that many adults with respiratory symptoms such as persistent cough, bronchiectasis and sinusitis, who would otherwise have been assigned an alternative diagnosis, in fact had CF (1,3,4). Physicians in our environment have become increasingly aware of this atypical form of CF and have a lower threshold for diagnostic testing, including sweat testing and genotyping. We report our experience in this area over the last fifteen years.

Adult sweat tests at Canterbury Health Laboratories
The number of sweat tests carried out at Canterbury Health Laboratories over the past 15 years is shown in Figure 1. In the first 9 years from 1996 to 2004, the total number of sweat test requests remained relatively constant, at 50-70 requests per year, with around 4 adults tested per year over this period. In 2005, a new adult respiratory specialist was appointed in Christchurch, with a particular interest in CF. Although he is not the sole driver of the overall increase in sweat testing, it is apparent from this Figure that more adults have been tested since 2005.

Figure 1. Sweat tests at Canterbury Health Laboratories 1996-2010

Figure 2 shows the increase in adult sweat tests as a proportion of total requests. In the period 1996-2004, the average was 6% and in no year during this period was it ever above 10%. From 2005 onwards, however percentages ranged between 15 and 27%.

Figure 2. Adult sweat tests as a proportion of total requests
Table 1. Summary of the data 1996 – 2010

<table>
<thead>
<tr>
<th></th>
<th>Total no of adult sweat tests</th>
<th>Genetic testing</th>
<th>Number of genetic mutations</th>
<th>Heterozygous F508del</th>
<th>CF / atypical</th>
<th>Total mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996 – 2004</td>
<td>39</td>
<td>56%. n = 22</td>
<td>36%. n = 14</td>
<td>10%. n = 4</td>
<td>10% . n = 4</td>
<td>20%. n= 8</td>
</tr>
<tr>
<td>9 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005 – 2010</td>
<td>99</td>
<td>65%. n = 64</td>
<td>41%. n = 41</td>
<td>12%. n = 12</td>
<td>12% . n = 11</td>
<td>23%. n= 23</td>
</tr>
<tr>
<td>5 years</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

If there is so much more testing of adults, what was being found? In the 9 year period 1996 – 2004, there were 39 adult sweat tests performed and in the 5 year period from 2005 – 2010 there were 99 adult tests performed. The proportion of adults undergoing sweat test, together with genotyping, increased by 9% from the period 1996 – 2004 (56%) to the period 2005-2010 (65%). Of those adults who had an additional genetic screen, although a smaller percentage were found to have at least one mutation, absolute numbers detected between the two cohorts increased from 8 (20%) in the first 9 years to 23 (23%) in the subsequent 5 years. These figures are shown in summary form in Table 1.

Sweat chloride
Although measurement of sweat chloride is one of the most important tools on the road to making a CF diagnosis, interpretation of a sweat chloride result on its own is not straightforward. Traditionally, a sweat chloride >60mmol/L is regarded as supportive of CF, 40-60 mmol/L as intermediate and <40 mmol/L makes CF unlikely. Examination of the sweat results of all the patients who were found to be carriers of the F508del (p.Phe508del) mutation in the years 2005-2010 (Table 2) highlights this complexity.

Table 2. Sweat chloride results from patients found to be heterozygous for F508del from period 2005-2010

<table>
<thead>
<tr>
<th>Group A sweat Cl &lt;40mmol/L</th>
<th>Group B sweat Cl 40-60 mmol/L</th>
<th>Group C sweat Cl &gt;60 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>Cl</td>
<td>Na⁺</td>
</tr>
<tr>
<td>23</td>
<td>16</td>
<td>51</td>
</tr>
<tr>
<td>22</td>
<td>16</td>
<td>61</td>
</tr>
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<td>36</td>
<td>19</td>
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<td>44</td>
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<td></td>
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<tr>
<td>50</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Adult reference range for chloride <60 mmol/L (9)

The chloride results in group A are well within the reference range for adults and if only the sweat results were to be considered these patients would have been considered normal, whereas they were actually carriers. In Group B patients, with intermediate chloride values, genetic testing may well be justified depending on the clinical situation. In Group C, there are three abnormal results. The chloride result of 86 mmol/L was performed in late 2009 and the result of 104 mmol/L in March 2010. Both of these samples justified further investigations of the CFTR gene but at the time of writing this had not been requested by clinicians.

With such a wide range of sweat chloride results seen in Table 2 and in particular those within the normal range, a genetic screen is not routinely performed on all the adults, given the underlying costs. At Canterbury Health Laboratoties the cost of a sweat test is $160. The next step in the testing path is the Oligonucleotide Ligation Assay, which detects 31 common mutations of the CFTR gene at a price of $333 (at the time of the last tested group in this review in 2010). If there is still a high index of suspicion of cystic fibrosis the clinician can request full CFTR gene sequencing of all exons for $972 (in 2010).

The full extent of investigation within the current technology available at CHL is the MLPA (Multiplex Ligation-dependent Probe Amplification) which has the capacity to detect deletions and insertions of whole exons. This is an additional cost of $200 (also in 2010). And yet, the combined use of all these techniques still cannot guarantee detection of all mutations. Approximately 2% of mutations remain undetected in CF patients with the classical form and even more in patients with atypical presentations. And what exactly is this term atypical? To understand atypical CF, there needs to be an understanding of what is meant by classical CF.

Classical and atypical CF
Classical CF tends to have a number of different criteria such as:

- Two disease-causing mutations in the gene for CFTR on chromosome 7 and changes to the electrolyte concentrations on cell surfaces. In sweat glands, CFTR gene defects result in reduced transport of sodium and chloride in the reabsorptive duct and therefore sweat with a higher salt content. In most instances, sweat chloride levels above 60mmol/L are considered diagnostic.
- There are abnormal secretions, inflammatory responses, obstructions and infections.
- There is organ disease, which can include some, or all, of these tubular structures, such as airways, vas deferens, gut, liver and pancreas and may vary in severity.

So if the criteria state that the identification of two disease causing mutations means CF then absence of detectable mutations would presumably exclude the diagnosis, although that is not necessarily the case. Interpretation of sweat test results is similarly not straightforward.

Formed out of this grey area of diagnostic uncertainty is a classification of patients whose type of CF is non-classical, often known as “atypical cystic fibrosis”2-4. There is no documented formal definition of atypical CF but typically within this group there are individuals who:

- Have two CFTR mutations and maybe a raised sweat chloride but do not develop the classical phenotype, or have no symptoms at all.
- Or there are the patients who have one or many clinical features associated with CF but have a normal or intermediate sweat test result and genetic tests that are equivocal or negative.

Patients with atypical CF tend to have milder disease, including less severe respiratory symptoms which can go undetected for a number of years until clinicians investigate retrospectively the cause of the chronic symptoms (1,3,4). Looking back through the Canterbury Health Laboratory’s “shades of grey”, it is clear the number of atypical or unusual cases is growing.

Case studies from Canterbury Health Laboratories
In 1998 there were 7 adult sweat tests performed and one male went on to have genetic analysis. His chloride of 47 mmol/L was in the intermediate range. At that time, we were only able to screen for 7 CF mutations.
The F508del mutation was identified. A repeat sweat test, after fertility issues were noted in 2003, gave similar results to those in 1998 (Table 3). In 2010, full CFTR gene sequencing was performed. The presence of F508del was confirmed along with two other probable pathogenic variants. The finding of these sequence variants supported a diagnosis of atypical CF for this patient. Both these variants have been found to be associated with CBAVD (congenital bilateral absence of the vas deferens) when found with another CF mutation such as F508del, thus explaining his infertility.

### Table 3.

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>Sweat test results (mmol/L)</th>
<th>Genetic analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Na+: 59</td>
<td>Heterozygous F508del</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl: 47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Na+: 56</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl: 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Full CFTR gene sequencing</td>
<td>F508del, R668C, A1067V</td>
<td></td>
</tr>
</tbody>
</table>

In 1999 there were 8 sweat tests performed on adults and all had sweat chloride results <60 mmol/L. One patient went on to have genetic testing which identified the F508del mutation. Another patient had a low sweat chloride of 24mmol/L and with such a low level, no genetic testing was requested. A repeat sweat test was conducted 6 years later and the results were significantly different (Table 4). Some variability in chloride can be expected but not usually to this extent.

### Table 4.

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>Sweat test results (mmol/L)</th>
<th>Genetic analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Total = 8</td>
<td>Normal = 8</td>
<td>N=2</td>
</tr>
<tr>
<td></td>
<td>Patient A 43 yr</td>
<td>Na+: 51</td>
<td>F508del</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl: 44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient B 29 yr</td>
<td>Na+: 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl: 24</td>
<td>Not performed</td>
</tr>
<tr>
<td></td>
<td>Patient B (6 years after)</td>
<td>Na+: 97</td>
<td>F508del, P67L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl: 90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient B (6 years after)</td>
<td>Na+: 62</td>
<td>3 benign polymorphisms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl: 60</td>
<td></td>
</tr>
</tbody>
</table>

Because the patient’s health had deteriorated in those six years since the initial sweat test, there was more than a strong suspicion of CF, and now that improved technology was available, a more thorough examination of the CFTR gene for other mutations was initiated. CFTR gene screening by DHPLC was performed with additional sequencing to find the P67L (p.Pro67Leu), in addition to F508del and also 3 benign polymorphisms. The finding of one inactivating mutation F508.del together with the P67L mutation supports a diagnosis of non-classical CF. The P67L mutation has been shown to be associated with minimal disease and patients exhibiting pancreatic sufficiency. This patient had severely impaired lung function and has since died of type 2 respiratory failure.

In 2000 there were 6 adult sweat tests and five had normal sweat chloride levels (Table 5). One patient had raised sweat chloride and was found to be heterozygous for the F508del and R117H mutations. If the testing for this patient had stopped at this point, the interpretation would be that the results are consistent not only with a diagnosis of cystic fibrosis but also with CBAVD in males. This is because there are two forms of the R117H allele which are dependent on the form of an additional variation in intron 8 within the allele.

One form with the 5T allele is usually found in CF, whereas the 7T allele is often seen in CBAVD and 9T is usually not disease causing. Further genetic testing showed this patient carries one CF allele with both the R117H mutation and 5T allele and one allele with both the F508del mutation and the 9T allele. This genotype is consistent with a diagnosis of chronic pancreatitis, but also of cystic fibrosis. If the partner were a CF carrier, this couple would have at least a 1 in 4 chance of having a child with CF or a CF-like illness, thus genetic counselling would be strongly indicated.

This patient’s result is a very good example of how, in some cases, there is a correlation between genotype and phenotype. Sadly this correlation is not so dependable that it can predict the type or course the disease may take in all cases. Although some mutations are recognised as conferring a more severe phenotype, additional gene-gene or gene-environment interactions may also have a strong influence on the phenotype.

### Table 5.

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>Sweat test results (mmol/L)</th>
<th>Genetic tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Adult sweat tests</td>
<td>Total = 6</td>
<td>abnormal = 1</td>
</tr>
<tr>
<td></td>
<td>Patient A 30 yr</td>
<td>Na+: 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl: 69</td>
<td>(R117H-5T/F508del-9T)</td>
<td></td>
</tr>
</tbody>
</table>

In 2002 there were two very interesting cases, both of similar age (Table 6). What is remarkable about these two cases is that one with sweat chloride levels below 60 mmol/L had CFTR mutations (patient A), while in the other with markedly raised sweat chloride (patient B), a mutation was not identified, illustrating the complexities of making a diagnosis.

### Table 6.

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>Sweat test results (mmol/L)</th>
<th>Genetic tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>Adult sweat tests</td>
<td>Total = 5</td>
<td>abnormal = 1</td>
</tr>
<tr>
<td></td>
<td>Patient A 36 yr</td>
<td>Na+: 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl: 23</td>
<td>(F508del-9T; SS49N-7T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient B 42 yr</td>
<td>Na+: 118</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl: 132</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In 2006 there was a marked increase in adult sweat tests, with 18 requests (Table 7). Fifteen out of the 18 adults tested had normal sweat chloride concentrations. Fourteen patients with normal sweat chlorides had genetic screening performed and two were found to carry the F508del mutation. Patients B and C, who had quite elevated electrolyte results, had extensive testing by DHPLC and full CFTR gene sequencing and despite these methods being extremely sensitive, no mutations were found. It remains possible, however that mutations may still exist in the non-coding regions of the CFTR gene or large insertions and deletions which account for less than 2% of known CFTR mutations. Patient F was found to be heterozygous for the G85E (P.Gly85Glu) mutation. Several sequence variants were also detected, one of which had been observed with increased frequency in individuals with atypical CF and may have clinical significance despite being listed on the CFTR mutation database as a benign sequence change. A second mutation, which would have confirmed a diagnosis of classical CF, was not found.

These results indicate that the patient is a CF carrier. By this time, a family pregnancy had been conceived and the foetus found to have an echogenic bowel on the 18 week scan. It was considered that there was an increased probability that the foetus may have had CF and this should not be excluded on the basis of one parent’s results. The partner was gene tested and no mutations were found.
This does not, however, completely exclude the possibility that the partner is a CF carrier with an undetected mutation.

In 2007 we performed sweat tests for 15 adult patients (Table 8). Of these, 12 had normal sweat chlorides, and seven underwent additional genetic testing. Of those seven, three were found to carry the F508del mutation. All patients with abnormal sweat test results had genetic tests. Patient A, with extremely high chloride levels, presented with a family history of a sibling who was heterozygous for the severe Q493X (p.Gln493X) mutation. Q493X was also identified in Patient A along with the R117H mutation. This patient also carries both the 5T allele and the 7T allele was present. Further testing confirmed that patient D had co-inherited the 7T allele with the R117H mutation, thus probably indicating a milder phenotype. These results are consistent with atypical CF. Patient D is also one of the oldest patients with CF at 79 years of age. Patient E presented to the respiratory department in Christchurch with likely CFTR dysfunction or CF. This patient had a brother who died of CF following an adult diagnosis. Patient E had CBAVD, bronchiectasis, sinusitis and one CF gene mutation, F508del, identified some years previous. Full CFTR gene sequencing was performed which revealed the R1066H (p.Arg1066His) mutation. The finding of two pathogenic variants supports a diagnosis of CF in this patient.

Patient C, with moderately raised sweat electrolytes, had full CFTR gene sequencing to identify this sequence variant. The

In 2009 there were a number of adults with very raised sweat electrolyte results (Table 9). Patient A presented to the respiratory department initially in 2007 with respiratory symptoms and azoospermia. At that time, genetic testing only was performed and the results showed that the patient was a carrier for the G85E mutation. The subsequent identification of another mutation is consistent with a diagnosis of azoospermia due to CBAVD, if the two mutations were inherited on different chromosomes. Because we were unable to identify the allele specific phases, the patient’s parents were genotyped and the mutations were indeed inherited on different chromosomes, thus consistent with atypical CF. Patient D was found to be heterozygous for one severe mutation (F508del) and one potentially damaging mutation (R117H), depending on whether the 5T allele was present. Further testing confirmed that patient D had co-inherited the 7T allele with the R117H mutation, thus probably indicating a milder phenotype. These results are consistent with atypical CF. Patient D is also one of the oldest patients with CF at 79 years of age. Patient E presented to the respiratory department in Christchurch with likely CFTR dysfunction or CF. This patient had a brother who died of CF following an adult diagnosis. Patient E had CBAVD, bronchiectasis, sinusitis and one CF gene mutation, F508del, identified some years previous. Full CFTR gene sequencing was performed which revealed the R1066H (p.Arg1066His) mutation. The finding of two pathogenic variants supports a diagnosis of CF in this patient.

In 2010 we had our first adult with classical CF. Patient C had been diagnosed with cystic fibrosis overseas at age 10. A formal confirmation of this patient’s medical condition was requested in New Zealand. Sweat chloride levels were very high and a blood test confirmed the patient to be homozygous for the F508del mutation. This patient put their longevity down to healthy lifestyle and a good positive outlook and had a resting heart rate of 45 bpm.

Patient D was heterozygous for the F508del mutation only, had pancreatitis, markedly elevated sweat chloride levels and a sibling with CF. Further gene sequencing was probably indicated, although the patient may have been assigned a diagnosis on clinical grounds. Two patients, E & F with normal sweat tests had quite rare mutations detected. Both these results indicate they are at least a CF carrier.
Conclusions

Adult cases investigated through Canterbury Health Laboratories over the last 15 years with clinical suspicion of CF have been reviewed. It is evident that there are many cases with atypical CF and that with the emergence of more sophisticated genotyping technologies, the boundaries of the phenotype are gradually being re-defined. What was previously considered to be a “black and white” diagnosis is clearly an evolving spectrum disorder and this is reflected in the various definitions of CF. The clinical index of suspicion is also clearly shifting and with the expectation that more cases of atypical CF will be recognised.

It is critical not to dismiss a CF diagnosis just because a patient’s sweat chloride levels is below 60 mmol/L. And furthermore, a diagnosis of CF in adults requires appropriate clinical awareness and interpretation of both sweat testing and full CFTR genesequencing data. Although the number of the latter genetic data is too small for useful statistical analysis, there does appear to be an increased frequency of uncommon mutations, and also a significant number of patients who are at least mutation carriers.

A historical footnote – did Chopin have cystic fibrosis? He was emaciated and had a serious “lung condition” from early childhood and died at the age of 39, presumed to be of tuberculosis. He fathered no children. His sister, Emily also died at age 14, possibly of CF. Scientists want to exhum his heart to do genotyping, although Polish authorities won’t allow it. In all likelihood, he probably did have cystic fibrosis.

Acknowledgements

I would like to acknowledge the NZIMLS for awarding me the Barrie Edwards/Rod Kennedy Scholarship and to travel to the South Pacific Congress in Australia in 2011 to present this paper. Thank you also to Chris Florkowski, Richard Mackay, Andrew Fellowes and Peter George for your invaluable knowledge and Canterbury Health Laboratories for your support.

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References


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- Scientific programme
- Workshop information
- Speaker profiles
- Call for papers & posters
- General information
- Online registration

Important Dates

- Early bird registration closes 1 July 2013
- Tuesday 20 August for workshops
- Wednesday 21 – Friday 23 August conference
- Thursday 22 August – NZIMLS Annual General Meeting

NZ J Med Lab Science 2013
Measured LDL – an unnecessary expense or a customer service?

Alan Simkins

This article briefly describes our experience arising from the New Zealand national cardiovascular disease (CVD) and diabetes screening programmes which evaluates a patient’s risk factors and probability of a CVD event over 5 years (1). For high risk individuals identified by the programmes, intensive lifestyle changes are recommended, supplemented by drug interventions for blood pressure lowering, diabetes control and lipid modification with regular follow up assessments. The programmes have increased early morning attendances for fasting blood tests leading to increased waiting times, patient dissatisfaction and stressed phlebotomists. Government targets are to have a five year risk prediction for 95% of the eligible population within the next two years so these pressures will continue. Screening for diabetes now utilises HbA1c for which fasting is not required.

But fasting is still a requirement for lipids under New Zealand guidelines, though a non-fasting sample for total cholesterol (TC) / high density lipoprotein cholesterol (HDL) ratio is acceptable for CVD risk assessment (2). Internationally, the Emerging Risk Factors Collaboration confirmed that non-fasting lipid measurements are acceptable for CVD risk screening (3) and, based on this evidence, some of our local Practitioners have already adopted non-fasting lipids for CVD screening. These developments have helped reduce the early morning pressures. However, our data show that 35% of all community-generated biochemistry requests have a lipid profile requested and >40% of these lipid requests still require fasting. This likely reflects the monitoring of lipid modification (Statin therapy) by estimation of low density lipoprotein cholesterol (LDL).

In New Zealand LDL is estimated by calculation using the Friedewald formula (4). This formula allows calculation of the contribution of very low density lipoprotein cholesterol (VLDL) to serum TC (LDL = TC - HDL - triglyceride / 2.22) with triglyceride / 2.22 as proxy for the estimation of VLDL. The current national guidelines state that a fasting sample is mandatory due to effect of food intake on the triglyceride which causes the equation to ‘overestimate’ VLDL and, therefore, artefactually ‘underestimate’ LDL. Internationally, measured LDL methods (LDLm) were introduced to overcome this shortcoming (5) and we decided to investigate whether this approach could further reduce the need for patient fasting.

We used the 2nd generation Roche LDL-C plus method running on a Cobas 6000 analyser. The method principle is a homogenous enzymatic cholesterol assay (cholesterol esterase, cholesterol oxidase / peroxidase coupling reaction) modified by the addition of a non-ionic detergent, a sugar compound and magnesium which enables the selective determination of LDL (6). The method is standardised against the reference beta quantification method and Roche data indicates little interference from high levels of VLDL, chylomicrons and triglyceride. We calculated LDL (LDLc) by the Friedewald equation using Roche TC, HDL and triglyceride reagents on the same analyser. Application parameters and calibrations were as per the manufacturer’s recommendations. Samples were freshly drawn lithium–heparin plasma and fasting status determined by direct questioning of the patients.

Data on 543 samples (243 fasting, 300 non-fasting) is summarised in Figure 1. The difference between the two LDL methods (LDLc minus LDLm) is plotted against the triglyceride in the same sample. It can be seen that at lower triglyceride levels the LDLm has a negative bias compared to LDLc but at triglyceride levels >2.5 mmol/L the LDLc has an increasing negative bias to LDLm. Plotting fasting and non-fasting data separately revealed exactly the same “tip over” point (2.5 mmol/L).
Comparing all samples with triglyceride <2.5mmol/L showed a strong correlation between the two methods (Figure 2). Our data supports the conclusions of van Deventer et al, who compared LDLc and multiple LDLm assays with reference methods (6). They also found lower LDLm levels compared to LDLc in fasting samples but found LDLc more accurate compared to reference methods. With raised triglyceride, LDLc became negative compared to LDLm which was also closer to the reference method target. Their “tip over” point was a triglyceride of 2.3mmol/L.

We conclude that it is the triglyceride level that differentiates, not fasting status, and we conclude it is necessary to estimate LDLm only on patients whose triglyceride levels are >2.5mmol/L (fasting or non-fasting). This was achieved by programming an appropriate rule in our computer middleware (IT3000, Roche Diagnostics). If triglyceride ≥2.5 mmol/L, LDLm is automatically added to the lipid profile, the LDLc result nullified and an appropriate interpretive comment added. This rule operates reflexively – the sample is re-sampled for a LDLm assay before the sample rack leaves the analyser. No operator input is required and auto-validation completes the reporting. By combining the two methods in this way we are able to give more consistency to our LDL results and target LDLm to where it is most effective. The reflex rule approach also minimises extra reagent costs which has been an impediment to the use of LDLm in NZ.

In Kaitaia our data indicate an LDLm added to 18% of lipid requests, though this may vary in other communities. Current reagent, calibration and QC costs approximates to an extra $1 per reportable sample. Obviously, for larger laboratories this could be a significant reagent cost increase, but this should be set against the appreciable benefit, for the laboratory and the wider local community. For doctors, it allows patients to be referred to the laboratory straight from the health centre thus improving result turn-around-time. For patients, it eliminates the need for overnight fasting which many find onerous, particularly if repeated for therapy monitoring. It gives flexibility of attendance and, there is no need for patients to return home from the GP surgery to fast overnight and return for a blood test – a problem for remote communities. For the laboratory, it removes uncertainty about sample validity and assures accurate LDL results regardless of patient fasting status or diagnosis. The net effect would be to reduce early morning phlebotomy queuing, reduce patient waiting times, increase customer satisfaction and encourage patient compliance with the screening programmes.

In Kaitaia, our patient attendance time profiles have changed in the last year with our peak now at 10.30 – 11.00am with more patients attending after 2pm. Complete elimination of the need for fasting would continue this process but given the demonstrated bias between LDLm and LDLc an amendment to the national guidelines regarding the exact role of LDLm may be required to achieve this aim.
The current practice of laboratories reporting inaccurate LDLc results in samples with raised triglyceride should also be reviewed. In summary, LDLm is an opportunity for New Zealand laboratories to manage their phlebotomy services better and also contribute cost effectively to the well being of their local population by encouraging compliance with the national CVD screening programme. Targeted LDLm should be considered for wider application in New Zealand.

Acknowledgements
I am grateful to Vivien Goldsmith, Laboratory Manager, Northland District Health Board for her professional support and encouragement as this local initiative developed.

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References

Correction
In the November 2012 issue the abstracts of the Annual Scientific Meeting of the NZIMLS were published. Unfortunately the name of a second author was omitted from one of the abstracts (page: 90). Below is the corrected abstract.

The emerging roles of medical scientists in humanitarian assistance operations
L Hristov1, D.Mok2,3
1Sullivan Nicolaides Pathology, Cairns, Queensland, Australia
2Headquarters 2nd Division, Randwick Barracks, Randwick, New South Wales and 3Medical Management Consulting, Eastwood, New South Wales, Australia

There is a growing demand for medical scientists (MSs) to provide pathology services on short notice to high risk operating environments. Recent major humanitarian aid (HA) operations including the Rwanda genocide, the Indian Ocean tsunami and the Haiti earthquake have identified that the delivery of pathology services remains to be challenging. High risk areas such as Afghanistan, Colombia, Somalia and the Darfur region of Sudan that pay little attention to humanitarian law and principles pose extra stress to HA providers. Additionally, the operational uncertainty makes planning and forecast difficult and it is likely that each affected population will have a different demographic profile for treatment. The MSs required to support task-specific land-based operations in complex terrain must learn new skills to meet these emerging challenges. The further development of standards, accountability and training requirements should enhance their overall delivery efficiency. The provision of specialised pathology support on HA operations is dependent upon many new factors. Firstly, they must possess communication skills enabling them to work more effectively together, both interculturally and interprofessionally, so that critical decisions can be made more quickly. Secondly, performance of mission-specific tasks must include operational flexibility and multitasking considerations in support of wider interests. Thirdly, survivability and mobility must be improved, so that the MSs can operate with better preparation to sustain prolonged operations. This paper will highlight several challenges that the MSs are likely to encounter. The information will enable future MSs to maintain a competitive advantage when selected for deployment.
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I would like to thank the NZIMLS for the opportunity to attend the American Association of Blood Banks (AABB) annual meeting in Boston, October 6-9, 2012. Receiving the Barrie Edwards and Rod Kennedy Scholarship made it possible for me to attend this conference.

The conference was held over 4 days in the Boston Convention Centre and was attended by over 6000 delegates. Several tracks made up the conference including Education and Quality, Scientific and Technical and Patient Blood Management. I attended presentations in the Education and Quality and Scientific and Technical tracks, and presented my paper in an Education and Quality forum.

Many of the presentations were from internationally renowned experts in their transfusion fields. The presentations were of very high quality, with an emphasis on latest information. This will allow me to update my teaching, particularly in the area of molecular typing. Molecular typing in Transfusion Medicine is now becoming routine (in addition to continuing to be a valuable research tool). For example, extended genotyping across ABO, Rh, Duffy, Kidd, Kell, MNS systems for multi-transfused patients, followed by genotypic matching to a donor, means that these patients receiving on-going challenges to their immune systems are less likely to form allo-antibodies post transfusion.

In USA, there is a large population of people living with Sickle Cell Disease, who are transfusion dependent. In the past, these people have made multiple allo-antibodies and it has become difficult or nigh impossible to provide them with compatible blood (not least because their blood types of African descent are very different to Caucasian blood donors). The advent of genotyping (using such technologies as micro-array) to extensively type patients and ethnically matched donors, is improving the level of match, and thus the transfusion outcomes for this group of patients. It is interesting to think that we may need to increase our donor pools in the future if we wish to provide this enhanced level of matching amongst different populations in New Zealand.

The dry match (genotype matching rather than the traditional cross match) was discussed by a panel in 1 session, with interesting and varying viewpoints as to whether or not this would be routine for all patients in the future. Multi-transfused patients also face increased transfusion transmitted infectious disease risk, due to their increased exposure to donors. Pathogen inactivation was also a current topic at the conference. At one of the presentations on pathogen inactivation, a patient with sickle cell disease was part of the presentation panel. In addition to hearing the latest scientific information, we heard the patient viewpoint, which I found a real privilege. We do not often have this opportunity as laboratory scientists, and to hear from a woman first-hand how regular exchange transfusion has changed her life for the better, was very special.

My own paper, entitled “Measuring serologic reaction grading skill with kodecytes at an accredited teaching institution” was well received. I spoke in an Education and Training session to an audience of scientists, nurses and doctors. I received some questions from the floor after the presentation.

Breakfast meetings were run each day and I attended three of these; on Rh blood group system, pathogen inactivation and an AABB meeting to welcome new members. The CTTXPO was also on site and consisted of 1 floor of trade displays. I spent time in these displays. In another area were the posters and I spent time studying the posters relevant to my teaching (namely blood group systems).

The conference was magnificently organised, with the inclusion of such features as on-line upload of presentations prior to arriving in Boston, on site ability to print posters, abstracts published in Transfusion, speed networking sessions and on site AABB bookstore with authors signing their newly launched Transfusion books. The prize money allowed me to purchase Sync to slide, which gives me access to all the presentations from the meeting, and will allow me to further update my teaching to BMLS students. Delegates were issued with an electronic card, which we swiped on entry to a session. This was used by USA delegates to register their professional development programme points. These cards could also be swiped in the trade display, for easy contact with the company later.

I also attended the annual meeting of the AABB which, as a new member, I found interesting and informative. One item on the agenda I found interesting was Necrology, which was the reading of a list of members who had passed away during the previous year, followed by a minute’s silence. The opportunities to network were most valuable, and I took full advantage of these. Boston is a beautiful city and I also managed 1 afternoon of sightseeing before the conference proper started.

Once again, I would like to thank the NZIMLS Council for this wonderful opportunity.

Holly Perry
Programme Leader BMLS
School of Applied Science, AUT, Auckland
Below are 10 questions based on articles from the April 2013 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 can only claim 5 CPD points. You cannot then claim additional CPD points for reading the articles from which the questions were derived.

The site will remain open until Friday 12th July 2013. You must get a minimum of 8 questions right to obtain 5 CPD points.

The Editor sets the questions but the CPD Co-ordinator Jillian Broadbent marks the journal questionnaire. Please direct any queries to her at cpd@nzimls.org.nz.

April 2013 journal questions

1. How has Neisseria gonorrhoeae detection traditionally been performed?
2. Muco-purulent discharge, commonly associated with Neisseria gonorrhoeae infection, can block the pipettor on the cobas 4800 analyser. How can this be overcome?
3. What are the advantages and disadvantage of using the BBL CHROMagar II MRSA media?
4. What does the LightCycler (LC) MRSA advanced test target?
5. Why is high sensitivity of any MRSA test necessary?
6. If undiagnosed or untreated, Clostridium difficile infection can lead to what? Which antibiotics are commonly used to treat Clostridium difficile infection?
7. The failure to isolate Clostridium difficile from toxin A/B positive samples could be attributed to a number of factors. Name these.
8. All Clostridium difficile isolates were susceptible or resistant to which antibiotics?
9. Traditionally, what sweat chloride levels are regarded as supportive of cystic fibrosis diagnosis, what levels are deemed intermediate, and what levels makes cystic fibrosis diagnosis unlikely?
10. What does a CFTR gene defect in sweat glands result in?

Questions and answers for the November 2012 journal questionnaire

1. What do the mean cell volume (MCV) and mean cell haemoglobin (MCH) represent? The average size and haemoglobin content of red blood cells produced in the bone marrow over the last 120 days.
2. What reflects the body's iron status and over what time span? The proportion of hypochromic and microcytic cells. Previous couple of months.
3. The area under the curve (AUC) for both % LScRBC and % Micro R were both close to 1.0. What did that indicate? Low false positive prediction for determining restricted erythropoiesis in both thalassemia and iron deficiency.
4. Peripheral primitive neuroectodermal tumours (PNET) affect mainly whom and what anatomical sites does it commonly involve? PNET affects mainly children and young adults. Thoracopulmonary region, pelvis, abdominal region, and extremities.
5. What are PNETs normally composed of? Small round cells normally lacking morphological evidence of neuroblastic differentiation in the form of neuropil or ganglion cell formation.
6. Why does the cytologic diagnosis pose a diagnostic challenge? Due to overlapping cytomorphologic features with those of other small cell tumours.
7. What rules out the diagnosis of lymphoma in the case of PNET? Absence of lymphoglandular bodies and presence of large clusters of adhesive cells and a negative stain for LCA.
8. What could possibly be the explanation for the observed differences in the detection rate of bacteria between the Gram stain/culture method and the PCR assay? Bacteria being below the limit of detection (bacterial load) of Gram stain and culture and/or the ability of the PCR method to detect dead organisms.
9. What may these reasons be attributable to and why? The effect of the antibiotic treatment which inhibits the growth of Neisseria meningitidis but does not interfere with PCR amplification of the organism's DNA.
10. Which organisation registers medical laboratory scientists/technicians and issues the Annual Practicing Certificate; and who enrolls for and administers the CPD Programme. The Medical Sciences Council of New Zealand (MSCNZ) registers medical laboratory scientists/technicians and issues the Annual Practicing Certificate. The NZIMLS enrolls for and administers the CPD Programme.
Greetings from the PPTC
The PPTC wishes to extend to you the very best wishes for this year 2013.

Courses held at the Centre
Blood transfusion course
A course in transfusion medicine was provided by the PPTC from the 5th November – 30th November 2012, at its centre in Wellington, and the following students attended:
Mele Vea Fonua from Tonga, Elizabeth Tekanene from Kiribati, Newton Banisi and Robert Qalolilio from the Solomon Islands and Lindburch Dribo from Ebeye, Marshall Islands.

The course included units of study covering the theoretical and practical aspects of the following topics; routine blood grouping, blood group antigens, cross match techniques, antibody detection, transfusion reactions, haemolytic disease of the newborn, screening blood for infectious agents, blood donor selection, organisation of a blood bank and the appropriate use of blood components in transfusion medicine. Practical sessions were also provided, focusing on correct technique and fundamental basic procedure. One week of the course was set aside for an overview of current techniques in the detection of transfusion transmissible infections including, HIV, syphilis, and Hepatitis B and C.

We are sincerely grateful to Susan Evans, and the Blood Bank staff of the New Zealand Blood Service, Wellington Hospital, for the excellent tuition and practical training provided throughout the duration of this course.

We would also like to sincerely thank Dr Dorothy Dinesh for presenting to our students their certificates of attendance.

The PPTC receives a donation from both the Norman Kirk Trust and NZ Red Cross each year to help sponsor a student from the Pacific Islands to attend the blood bank course offered each year in Wellington. The student sponsored for 2012 was Mele Vea Fonua from Tonga and the PPTC is very grateful to both organisations for helping to create this learning opportunity for Mele in terms of extending her knowledge and skill in blood transfusion technique. During their stay in New Zealand, the students were taken on tour of the Hawkes Bay and were given the opportunity to meet with NZ Red Cross representatives who graciously provided a luncheon as a welcome gesture.

Luncheon with NZ Red Cross representatives, Hawkes Bay, November 2012

Country visits
The STI programme
The goal of the STI teaching programme which is a WHO initiative, sets out to provide laboratory technicians working in microbiology sections of Pacific Island country laboratories with the information and skills needed to accurately perform the various laboratory tests related to the laboratory diagnosis of the common sexually transmitted infections [STIs] including HIV and also Hepatitis B & C.

The STI programme was developed by the PPTC as a hybrid course and consists of two main parts; a comprehensive theory component which was made available to Pacific Island students through distance learning covering all aspects of STIs and RTIs [reproductive tract infections], and a practical workshop that is currently being conducted in national laboratories of participating countries. This programme has been enormously successful to date and both Russell Cole, our Laboratory Quality Co-ordinator and Navin Karan our Teaching and Training Programme Co-ordinator have contributed greatly to the on-going success associated with this programme. Since October 2012, Russell has visited the Cooks, American Samoa, Samoa, and the Marshalls. In April he is scheduled to visit Yap and Vanuatu. Navin has visited Fiji and travels to the Solomons in mid March, moving onto Pohnpei in April and Kiribati and Tonga in May.
Laboratory quality management and external quality assessment
Both Navin and Russell continue to be enormously involved in the implementation of the PPTC Laboratory Quality Management Programme in Samoa, Vanuatu, Cooks, Kiribati, and Tonga. Russell is to visit Samoa in May and Tonga in June. Navin will visit Vanuatu early in June and Kiribati later in the month.

Phil Wakem, the PPTC Manager and Christine Story the PPTC Administrator plan to visit the Cook Islands in September to meet with the laboratory staff at Rarotonga hospital and carry out an assessment of the PPTC’s EQA programme currently operating in the laboratory.

Haematology training,
Phil is also scheduled to visit the Solomons for two weeks in May to conduct a Haematology and Blood cell morphology course for students and staff in Honiara.

Wellington based courses scheduled for 2013
The PPTC’s Wellington based courses for 2013 are as follows:

Haematology and blood cell morphology
11 March – 05 April 2013

External quality assessment
22 April – 10 May 2013

Laboratory quality management systems
20 May – 14 June 2013

Biochemistry
15 July – 09 August 2013

Microbiology
02 September – 27 September 2013

Phlebotomy
07 October – 25 October 2013

Blood transfusion science
04 November – 29 November 2013

Online Distance Learning Courses
The PPTC will be offering 6 POLHN modules leading to the Diploma in Medical Laboratory Science [PPTC] commencing with the module: laboratory technology. The Diploma course is delivered over a two year period to students who have registered and have been accepted for the programme. The cycle of the programme will begin in March 2013. The scheduled dates for the modules to be released in 2013 and 2014 are as follows:

2013 modules:
Laboratory Technology: 08 March 2013
Haematology: 09 June 2013
Biochemistry: 16 Sept 2013
Examination Part 1: November 2013

2014 modules:
Microbiology: 03 March 2014
Transfusion science: 02 June 2014
Laboratory quality management systems: 15 September 2014
Examination Part II: November 2014

Welcome to our new Board Member
The PPTC Board of Management would like to welcome Filipo Faiga as its newly appointed co-opted Board member. Filipo is currently the Section Head in the Dept of Biochemistry, Wellington Hospital. Filipo has a wealth of experience in biochemistry and has had involvement with the PPTC and the Pacific for many years. We are very fortunate to have him join us.
COME AND BE BLOWN AWAY AT ESR
34 Kenepuru Drive, Porirua, Saturday 25th May 2013

Registration and coffee 9.30am
Programme starts at 10.00am until approximately 5.00pm
Dinner at 7pm

Shuttles will leave Wellington Airport for ESR at 8.45am and return, leaving ESR at 5.15pm

Submit your presentation proposals to Rosemary Hawkes at Rosemary.Hawkes@esr.cri.nz

Online registration available now at www.nzimls.org.nz
## 2013 NZIMLS Calendar

**Dates may be subject to change**

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<tr>
<td>20 - 23 August</td>
<td>NZIMLS Annual Scientific Meeting, Bridging Science &amp; Education</td>
<td><a href="mailto:Mary-Ann.Janssen@waikatodhb.health.nz">Mary-Ann.Janssen@waikatodhb.health.nz</a></td>
</tr>
<tr>
<td></td>
<td>Claudia Event Centre, Hamilton</td>
<td></td>
</tr>
<tr>
<td>13 April</td>
<td>South Island Seminar, Regent Theatre, Hokitika</td>
<td><a href="mailto:eileen.chappell@westcoastdhb.health.nz">eileen.chappell@westcoastdhb.health.nz</a></td>
</tr>
<tr>
<td>4 May</td>
<td>North Island Seminar, Waipuna Hotel &amp; Conference Centre, Auckland</td>
<td><a href="mailto:rossh@adhb.govt.nz">rossh@adhb.govt.nz</a></td>
</tr>
<tr>
<td>17-19 May</td>
<td>NICE Weekend, Wairakei Resort, Taupo</td>
<td><a href="mailto:Raewyn.Cameron@lsr.net.nz">Raewyn.Cameron@lsr.net.nz</a></td>
</tr>
<tr>
<td>May</td>
<td>Microbiology SIG Seminar, ESR, Kenepuru</td>
<td><a href="mailto:Rosemary.Hawkes@esr.cri.nz">Rosemary.Hawkes@esr.cri.nz</a></td>
</tr>
<tr>
<td>22 June</td>
<td>Molecular Diagnostics SIG Seminar, Copthorne Hotel Commodore, Christchurch</td>
<td><a href="mailto:Jill.Taylor@cdhb.health.nz">Jill.Taylor@cdhb.health.nz</a></td>
</tr>
<tr>
<td>29 June</td>
<td>Biochemistry SIG Seminar, Wellington</td>
<td><a href="mailto:kallan@apath.co.nz">kallan@apath.co.nz</a></td>
</tr>
<tr>
<td>October</td>
<td>PreAnalytical SIG Seminar</td>
<td></td>
</tr>
<tr>
<td>19 October</td>
<td>Histology SIG Seminar, Nelson</td>
<td><a href="mailto:alannah_z_@hotmail.com">alannah_z_@hotmail.com</a></td>
</tr>
<tr>
<td>23 November</td>
<td>Immunology SIG Seminar, Copthorne Hotel Commodore, Christchurch</td>
<td><a href="mailto:angela.horridge@sclabs.co.nz">angela.horridge@sclabs.co.nz</a></td>
</tr>
<tr>
<td>November</td>
<td>Mortuary SIG Seminar</td>
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### NZIMLS Examination Information

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<tr>
<td>30 April</td>
<td>Applications close for Fellowship Examinations</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>2-3 May</td>
<td>Council Meeting, Auckland</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>20 May</td>
<td>Applications close for QMLT/QSST Examinations</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td></td>
<td><em>(No late applications accepted)</em></td>
<td></td>
</tr>
<tr>
<td>6 November</td>
<td>QMLT and QSST Examinations</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>12-13 November</td>
<td>Fellowship Examinations</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
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### Council Matters

<table>
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<th>Date</th>
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<tbody>
<tr>
<td>25 June</td>
<td>Nomination forms for election of Officers and Remits to be with the Membership (60 days prior to AGM)</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>Mid July</td>
<td>Material for the August issue of the Journal must be with the Editor</td>
<td><a href="mailto:rob.siebers@otago.ac.nz">rob.siebers@otago.ac.nz</a></td>
</tr>
<tr>
<td>16 July</td>
<td>Nominations close for election of officers (40 days prior to AGM)</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>3 August</td>
<td>Ballot papers to be with the membership (21 days prior to AGM)</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>11 August</td>
<td>Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM)</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>18 August</td>
<td>Ballot papers and proxies to be with the Executive Officer (7 days prior to AGM)</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>18 &amp; 19 August</td>
<td>Council Meeting, Hamilton</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>22 August</td>
<td>Annual General Meeting, Hamilton</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
</tr>
<tr>
<td>Mid September</td>
<td>Material for the November issue of the Journal must be with the Editor</td>
<td><a href="mailto:rob.siebers@otago.ac.nz">rob.siebers@otago.ac.nz</a></td>
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<td>November</td>
<td>Council Meeting</td>
<td><a href="mailto:fran@nzimls.org.nz">fran@nzimls.org.nz</a></td>
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NZIMLS
MOLECULAR DIAGNOSTICS SIG
SEMINAR 2013

Keep Saturday 22nd June 2013 free for this seminar to be held at the Copthorne Hotel Commodore, Memorial Avenue, Christchurch.

Presentations please — contact Jill Taylor at Jill.Taylor@cdhb.health.nz

Further information will be sent to Laboratories and details will be available at www.nzimls.org.nz soon

NZ J Med Lab Science 2013
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NZIMLS presents

NORTH ISLAND SEMINAR

“The Broad Spectrum of Pathology”
Saturday May 4th 2013
Waipuna Hotel & Conference Centre
Auckland

Registration & coffee
9.00 am to 10.00 am
Finish 5.00 pm approx

WANTED!!!

Presentations on Phlebotomy, Specimen Services, Molecular Diagnostics, Biochemistry, Haematology, Microbiology, Anatomical Pathology, Cytology, Virology, Immunology, Point of Care, Automation, Information Technology, Quality Management, Laboratory Management, Case Studies.
Prizes for Best Presentation,
Best First Time Presenter

Contact for Presentations
Ross Hewett
LabPLUS, Auckland City Hospital
PO Box 110031, Auckland
Email: rossh@adhb.govt.nz