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In this issue

Rob Siebers, Editor

For over 130 years the Ziehl-Neelsen stain has been the primary method for the laboratory detection of the genus *Mycobacterium*. The decolouriser, using acid-alcohol, is optimal for this purpose, delivering stained smears that are simple to examine and interpret, but is not reliable for the detection of nontuberculous mycobacteria. John Aitken and colleagues argue that where non-tuberculous mycobacteria are expected, it is not sufficient to use the acid-alcohol decolourising step on the Ziehl-Neelsen stain. They recommend 20-25% sulphuric acid as the decolouriser when looking for dormant forms of mycobacteria and non-tuberculous mycobacteria.

Javad Mozafari and colleagues from Iran determined the concentration of serum and urine levels of the S100B as a biomarker and compared them with mild brain trauma in children and adolescents, to provide an acceptable estimate of the presence or absence of positive and negative findings of brain CT scans. Their study concluded that use of serum and urinary levels of the S100B biomarker can reduce unnecessary brain CT scans in children and adolescents with mild traumatic brain injury.

Carbapenemase-producing *Enterobacterales* confer resistance to carbapenem antibiotics via the ability to hydrolyse the beta-lactam ring in carbapenem structures. Bronwyn Davison compared the NG-Test CARBA 5 and RESIST-4 O.K.N.V. immunochromatographic lateral flow assays for the detection of carbapenemase enzymes in *Enterobacterales*. She found these kits detected the main carbapenemase types in *Enterobacterales*, as currently found in New Zealand. These kits were user-friendly that provided results within 20 minutes, indicating the presence or absence of singular or multiple carbapenemase type(s) detected.

Mobile phones are increasingly being used in the hospital setting by health care workers and come in contact with various surfaces around the hospital. Thus, they are likely to get contaminated by a variety of organisms. Bhumi Tailor and colleagues from Fiji investigated the colonisation of microorganisms on students' mobile phones. All of the students' mobile phones showed evidence of bacterial colonisation with the most common bacteria isolated being *Bacillus* species. Contaminated mobile phones may be a vector in spreading nosocomial or community-acquired infections in a hospital setting.

Dennis Mok and Sharfuddan Chowdhury provide an update on selected international standards, guidance documents and relevant literature at the application level that are associated with the implementation of the strategic management stage of ISO 15189:2012 which contributes to the medical laboratory's development of implementations of ISO 15189:2012 in areas of operations as targeted interventions using reasonably practical references to achieve an acceptable level of conformance.

The A2756G polymorphism in the methionine synthase gene has been implicated in recurrent miscarriage risk. Zahra Asadnejad and colleagues present a case control study to determine whether there is an association between the methionine synthase A2756G polymorphism and recurrent miscarriage in Iran. Their results suggests that methionine synthase A2756G polymorphisms are associated with the risk of recurrent miscarriage in the northern Iranian population.

Uwen Akpan and Iva Bassey assessed biomarkers and frequency of metabolic syndrome in adult male smokers in Nigeria. They found that smokers had significantly higher diastolic blood pressure, total cholesterol, LDL-C and significantly lower HDL-C, compared to the controls. The frequency of smokers with metabolic syndrome was significantly higher than non-smokers.

Vanita Patil and Samarina Musaad present a case study of diabetic ketoacidosis to highlight laboratory results and raise high risk alert and inform to physician or refer them to appropriate centres for immediate management of the condition.

Beverley Pullon presents a case of haemoglobin Reading which was associated with normal haematological parameters. This haemoglobin variant was picked up as an interfering component on HbA1c testing using cation-exchange high performance liquid chromatography. Variable results returned from an HbA1c quality control survey highlights the difficulty of detecting haemoglobin Reading, demonstrating how this haemoglobin variant may be under reported.

Lauren Eddington interviewed Brandy Gunsolus, the first graduate of the Doctorate of Clinical Laboratory Science program in the USA while Michael Legge comments on this interview.

Jim Mann, previous principal technologist at Palmerston North Hospital recently passed away. Colvin Campbell reflects on Jim's working life in his obituary.

Bachelor of Medical Laboratory Science students at Otago University have to undertake research projects in their 4th year medical laboratory placements. In this issue are presented the abstracts of their projects from the first semester in 2019.

VIEWPOINT

A revaluation of the use of conventional Ziehl-Neelsen stain for detection of non-tuberculous mycobacteria

John M Aitken, Thomas J Borody and Gaurav Agrawal

ABSTRACT

Detection of non-tuberculous mycobacteria is difficult and not always consistent. These organisms are emerging as opportunistic pathogens in a number of clinical conditions, in part due to their remarkable stress intolerance. The Ziehl-Neelsen stain is optimal for the detection *of Mycobacterium tuberculosis* complex but is less reliable for the detection of non-tuberculous mycobacteria when using acid-alcohol as a decolouriser. Acid alcohol decolourisation should not be relied on where non-tuberculous mycobacteria are suspected, or the possibility of dormant forms of mycobacteria exists. In those instances other decolourisation methods should be considered, such 20-25% sulphuric acid or Gabbett's methylene blue.

Keywords: mycobacteria, Ziehl-Neelsen stain, non-tuberculous mycobacteria, mycobacteria staining methods, basic fuchsin.

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PERSPECTIVE

The Ziehl-Neelsen stain has been used since 1883 for the detection of *Mycobacterium tuberculosis* complex. The stain in the present form consists of carbol fuchsin as the primary stain, acid-alcohol as the decolourising agent, and methylene blue as the counter-stain. For non-tuberculous mycobacteria it has been recommended that the decolourising agent should be a strong acid in water without addition of alcohol (1). *Mycobacterium tuberculosis* complex are considered to be acid-alcohol fast while non-tuberculous mycobacteria are considered to be acid-fast. The acid alcohol decolouriser is now the common Ziehl-Neelsen decolouriser in routine use in medical and research laboratories.

A clinical distinction is also made between *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria. The former is considered to be invariably obligate pathogenic when detected in humans, while non-tuberculous mycobacteria may be present without causing disease, and can be described as saprophytes, commensals, and symbionts (2).

History of staining for *Mycobacterium* species

In 1882, Robert Koch announced the discovery of the causative organism of tuberculosis by application of a novel staining method (3). The procedure outlined in Koch's original publication was time consuming and difficult to reproduce. Within a year, Koch's original method had been supplanted by more rapid methods, the most notable example is the Ziehl-Neelsen stain.

As well as staining *Mycobacterium tuberculosis* complex, Koch found that his original method also stained *Mycobacterium leprae.* Koch used his stain on sectioned tissue, and it was left to Erlich to use his modification for the diagnosis of pulmonary TB through sputum examination. Erlich's modification, following soon after Koch's announcement, relied on basic fuchsin and aniline oil as the primary stain and the use of nitric acid as the decolourising agent (4). Erlich's modification was quickly adopted by Koch and other researchers (3).

In 1883 Ziehl described a modification that substituted Erlich's aniline oil and basic fuchsin with a primary stain containing basic fuchsin and phenol (carbolic acid) (4). Supplementing this, in 1883 Neelsen advocated the substitution of nitric acid as the decolouriser, with sulphuric acid. The concentration of phenol was increased, and the decolourising agent was improved with the addition of ethanol to the sulphuric acid (5). Thus the Ziehl-Neelson stain was born. Neelsen published the method for the Ziehl-Neelsen stain in a small book he compiled for anatomical pathologists (6) and records of the modifications exist in a paper written by Johne, (discoverer of Johne's disease, an inflammatory bowel disease in ruminants) recalling his discussions with Neelsen (7,8).

By 1892 the Ziehl-Neelsen stain was widely used and accepted as the "gold standard" for the diagnosis of tuberculosis. Since then there have been numerous modifications of the Ziehl-Neelsen stain, mainly involving the concentration of carbol-fuchsin and the composition of the decolouriser. Henry Gabbett proposed the use of Gabbett's methylene blue, which contained acid, ethanol and methylene blue, resulting in a two step stain which was used for many years with success and is still used in some parts of the world (9) In 1915 Kinyoun described the "cold stain" for the detection of *Mycobacterium tuberculosis* complex. (10). By 1972 it was common knowledge that if non-tuberculous mycobacteria were to be reliably excluded, an oxidising decolouriser was required (2).

DISCUSSION

For over 130 years, the Ziehl-Neelsen stain has been the primary method for the laboratory detection of the genus *Mycobacterium*. Over that time, the use of the Ziehl-Neelsen stain has been refined to suit cost-effectiveness, reliability and convenience in the medical laboratory. The Ziehl-Neelsen method used today is optimised for the detection of *Mycobacterium tuberculosis* complex, the primary human mycobacterial pathogen.

The decolouriser, using acid-alcohol is optimal for this purpose, delivering stained smears that are simple to examine and interpret, but is not reliable for the detection of non-tuberculous mycobacteria. Several other methods, using strongly acidic decolourising methods are recommended for that purpose. The recommended decolouriser when looking for dormant forms of mycobacteria and non-tuberculous mycobacteria is 20-25% sulphuric acid, which is a moderate oxidising agent. When mycobacteria are present in a dormant/

latent state, there is a reduced atmosphere. The reduced state will also occur in walled cavities containing *Mycobacterium tuberculosis* complex. Cell-wall-deficient mycobacteria in the dormant state will often also produce a positive Ziehl-Neelsen stain when the preparation is oxidised prior or during staining.

Medical laboratories are encountering increasing numbers of non-tuberculous mycobacteria in pathogenic roles including in opportunistic clinical infections, Latent, or dormant variants of the genus *Mycobacterium* are also thought to be responsible for recurrence of tuberculosis in treated patients and are increasingly linked to sarcoidosis, inflammatory bowel disease and similar 'autoimmune' diseases (11-13). Some researchers, using the acid-fast decolouriser, have reported identifying presumptive cell-wall-deficient mycobacteria present in blood cultures from patients presenting with these diseases(14).

We believe that, where non-tuberculous mycobacteria are expected, it is not sufficient to use the acid-alcohol decolourising step on the Ziehl-Neelsen stain. This may be particularly relevant where automated liquid culture-based detection methods of culture are used and cultures signaling positive growths are discarded based on the Ziehl-Neelsen stain alone.

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ORIGINAL ARTICLE

The diagnostic accuracy of serum and urinary S100B protein in children and adolescents with mild traumatic brain injury

Javad Mozafari, Mohammad Ali Fahimi, Kourosh Mohammadi, Hassan Barzegari, Mohammad Ghasem Hanafi and Amal Saki-Malehi

ABSTRACT

Objective: To determine the role of S100B as a serum and urinary biomarker in children with mild traumatic brain injury.

Methods: A cross-sectional study in children and adolescents (6 months to 18 years) with mild traumatic brain injury who were referred to the Emergency Department of Golestan Hospital, Ahvaz City, Iran. The patients were divided into two groups after a brain CT-scan: Group A with positive pathological findings and Group B with a normal brain CT scan. Serum and urinary levels of S100B biomarker were compared between the two groups.

Results: A total of 40 children and adolescents were evaluated in patient Groups A and B (20 in each group). The area under the ROC curve was 0.998 (P <0.0001) which indicated a high precision level of serum S100b in the differentiation between the two groups, the best cut-off point was 172.15 ng/l with 95% sensitivity and 100% specificity. In addition, the area under ROC curve of 0.985 (P <0.0001) indicated a high accuracy for S100B urinary concentrations to differentiate between the two groups, the best-obtained cut-off point was 67.75 with a sensitivity and specificity of 90% and 95 % respectively.

Conclusion: The results of this study demonstrated that use of serum and urinary levels of the S100B biomarker can reduce unnecessary brain CT scans in children and adolescents with mild traumatic brain injury. Considering the non-invasiveness of the urinary sample collection, this method can be used in an emergency instead of serum.

Key words: brain injury, biomarkers, S100B protein, diagnosis, emergency service.

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INTRODUCTION

Approximately 15% of patients with mild traumatic brain injury with Glasgow coma scale scores of 14 and 15 (classified as mild) from their brain computerised tomography scan (CT scan) have pathologies, such as subarachnoid haemorrhage, skull fracture, intracranial subdural and epidural haemorrhages, of which less than 1% need neurosurgery intervention. According to the available guidelines to determine the presence and extent of brain damage, (depending on clinical symptoms of the patient), a standard brain CT scan is administered in this group of patients. However, this imaging modality is expensive and time consuming and importantly, may increase the risk of cancer due to exposure to ionising radiation (3,4).

The S100 calcium-binding protein B, belongs to the family of Ca^{2+} binding proteins (5) and helps to regulate the amount of intracellular calcium (6). This protein has been identified in high concentrations in Schwann and Astrocytes cells (7) and has also been identified in other tissues, such as bone marrow cells, lymphocytes, adipocytes and melanocytes (8-10). S100B has the highest concentrations in the cerebrospinal fluid and serum, however, this protein can also be detected in other body fluids, including amniotic fluid, urine and cord blood (11). Based on previous research the initial level of S100B at 3 and 6 hours after a mild traumatic brain injury can be considered as a biomarker in predicting some outcome-related events as there is a correlation between CT scan pathological findings and a high level of S100B (12).

The aim of this study was to determine the concentration of serum and urine levels of the S100B as a biomarker and compare them with mild brain trauma in children and adolescents, to provide an acceptable estimate of the presence or absence of positive and negative findings of brain CT scans.

METHODS

Study design

This cross-sectional study was approved by the Jundishapur University of Medical Sciences Ethic Committee and parents/ guardians were approached for informed consent for children and adolescent with head injuries referred to the Emergency Department of the Ahvaz Golestan Hospital, Iran during April to September 2017. This study has been conducted according to the Standards for Reporting Diagnostic Accuracy (**STARD**) (14)**.**

Participants

All children and adolescent with diagnosis of mild traumatic brain injury who met the inclusion criteria were entered into this study. The inclusion criteria were the presence for an indication of a brain CT scan, aged 6 months to 18 years and a Glasgow coma score of 14 or 15. Injuries included those from traffic and home or sport events, and referrals less than 6 hours of the incident. Inclusion criteria were no previous history of alcohol or drug abuse, the absence of a history of previous neurological disease such as seizure or epilepsy, the absence of severe traffic injury and multiple trauma from motor vehicles, and absence of melanoma. Patients were excluded from the study if they had any of the following conditions: injuries except the brain mild trauma damage such as organ damage, previous illnesses such as diabetes, heart disease, asthma, pregnancy or recent febrile illness.

Test methods

Following initial examination and stabilisation, venous blood and urine samples were obtained by the attending nurse and the patients were then referred to a fixed imaging unit to obtain a skull CT scan. Primary information was recorded about the nature of injury and the presence of ligation, scratching,

ontogeny, and size and location of lesion in the scalp and face, Glasgow coma score, headache, nausea, vomiting, dizziness, neurological deficits, amnesia and decreased consciousness and its duration. The blood and urine samples were immediately transferred to the central laboratory of Golestan Hospital. Blood samples were centrifuged at room temperature for 10 minutes at 2200 g and the separated serum was stored at -70 ° C until analysis. Urine samples were centrifuged at room temperature for 10 minutes at 900 g and the supernatant was stored at -70° C until analysis. The S100B in both serum and urine samples were determined using S100B ELISA kits (Shanghai Crystal Day Biotech Co., Ltd). The concentration of S100B in serum and urine of each sample was recorded independently without knowledge of the brain CT scan results. The primary brain CT scans of all patients was interpreted using a 64 slice CTscan device and independently interpreted by a consultant neurologist who was not aware of the results of the corresponding S100B results. Patients were assigned to either Group A or Group B according to their CT scan results. **Data analysis**

Results from both the urine and serum concentrations of S100B and the results of the interpretation of brain CT scans were analysed by an independent t-test and the Mann-Whitney U test (SPSS 22). The area under the curve (AUC), sensitivities and specificities of the data were determined. The level of statistical significance was set at $p<0.05$.

RESULTS

Participants

In total 40 participants were evaluated, 20 who had positive pathologic findings associated with isolated head trauma (Group A) and those who lacked these findings in brain CT scans (Group B). The frequency of patients and their age distribution and the characteristics of both groups in brain CT scans are presented in Table 1.

Table 1. Patient's characteristics.

Variables		Group A (N= 20)	Group B (N= 20)
Median age in years (range)		9 (2-18)	6.6 (0.5 - 18)
Female N (%)		4 (20)	8 (40)
GCS (%)	14 score GCS (%)	4 (20)	13 (65)
	15 score GCS (%)	16 (80)	7 (35)
Average admission time after trauma in hours		2.79	2.66

Serum and urine S100B

In Group A the mean (±1SD) serum level of S100B was 561±283 ng/L, whereas in Group B it was 79.8±22.8 ng/L ($p<0.001$) (Table 2). A serum level of 172.15 ng/L had a sensitivity, specificity, PPA, and NPA of 95%, 100%, 100%, and 91% respectively for the diagnosis of intra cerebral lesions according to positive findings of the brain CT scan ($p<0.0001$). In group A, the mean urinary level of S100B was 134±63.5 ng/L, whereas in group B it was 25±19 ng/L ($p<0.001$) (Table 2). Urinary S100B levels of 67.75 and 56.4 ng/L with a sensitivity and specificity of 90% and 95% and 95% and 90% respectively were used to estimate CT scan results. There was a significant difference between the serum and urinary levels of S100B between the two groups and therefore had the potential to differentiate between these two groups of patients.

The area under the ROC curve of 0.998 ($P<0.0001$) indicated a high predictive value of serum S100B in the differentiation between positive and negative patients. The cut-off point of 172.15 ng/L with a sensitivity of 95% and a specificity of 100% was the best cutting point in the area under the ROC curve.

The area under the ROC curve with a value of 0. 985 ($P<0.0001$) indicated a high accuracy of the urine S100B level in differentiating between positive and negative patients. Considering the area under curve, the cutting point was 67.75 with a sensitivity of 90% and specificity of 95%, and at the cutting point of 56.4 a sensitivity of 95% and specificity of 90%.

Table 2. S100B levels and clinical signs.

Variables	Group A (n= 20)	Group B (n= 20)	P
Urinary S100B ng/L Mean ± SD	134.59 ± 63.53	25.08 ± 19.32	<0.001
Serum S100B ng/L Mean ± SD	561.53 ± 283.37	79.83 ± 22.85	<0.001
Headache n (%)	9 (45)	12 (60)	0.9
Nausea & vomiting n (%)	13 (65)	10 (50)	
Confusion n (%)	1 (5)	2 (10)	
Functional neuro-logical disorder n (%)	1 (5)	0	
Vertigo n (%)	3 (15)	0	

DISCUSSION

Traumatic brain injury is one of the most common clinical complaints that lead to referral to the emergency department. Since most of the complications of head injury occur over passage of time, early detection of those who are likely to show these lesions plays a very important role in determining the clinical outcome for these patients. According to existing methods, brain CT scans detect brain damage in people with symptoms such as vomiting, however, this imaging method is expensive and not readily available in some centres and most importantly, most people who undergo a brain CT scan do not show any initial positive findings.

Considering our findings, when a child with mild traumatic brain injury is referred during the first 6 hours after the incident, serum or urinary levels of the S100B biomarker have an acceptable sensitivity and specificity for selecting patients requiring a definite brain CT scan. This approach could prevent unnecessary CT scans with negative findings, clinical risk of exposure to ionising radiation, reduce costs and stay in the Emergency Department. On the other hand, due to the non-invasive nature of the urine sampling and no significant difference between serum and urinary S100B levels in determining the presence and extent of brain CT scan, urinary samples may be considered as a non-invasive and more convenient method than serum samples.

Findings of our study showed that serum and urinary levels of S100B could significantly contribute to the positive pathological findings in brain CT scans, whereas no significant difference was observed between serum and urinary S100B levels. Previous studies have demonstrated the relationship between high serum levels of S100B and the presence of positive pathological findings in brain CT scans. Although these studies have been conducted in adults (14-17), studies on children and adolescent are few and studies that show the association between the urinary levels of this biomarker and positive findings of brain CT scans are scarce (14,18,19). Hallen *et al.*

did not show that urinary values of this biomarker increases during the first six hours of mild traumatic brain injury (20). Varying serum levels S100B, ranging from 100-240 ng/L, have been reported in studies with different sample sizes that should be interpreted according to the positive or negative predictive values and the sensitivity and specificity of the obtained cutoff values (21). In our study, the subjects who had positive findings in brain CT scans were all healthy before the recent incident and they did not have any effect on the levels of the biomarker, including multiple trauma, or symptoms such as rapid breathing or fever, and several cases such as head previous trauma and congenital toxoplasmosis.

In our study, a S100B serum level of 172.15 ng/L had a sensitivity, specificity, PPA, and NPA of 95%, 100% , 100%, and 91% respectively in the diagnosis of intra cerebral lesions and with the positive findings of brain CT scans ($p < 0.0001$). We have shown that a S100B urine level of 67.75 ng/L had a sensitivity and specificity of 90% and 95% respectively and a level of 56.4 ng/L had a sensitivity and specificity of 95% 90% respectively, in agreement with the positive findings of the CT scan.

Hallen *et al.* studied six children and adolescents with reported CT scan positive findings. The mean serum S100B level in the group that immediately underwent a brain CT scan was 111 ng/L after admission, range: 86-153ng/L (20). They showed that a serum S100B cut-off value of 195 ng/L with a sensitivity and specificity of 100% and 88% respectively, was consistent with CT scan findings. They also showed that urinary levels of S100B did not correlate with brain CT scan findings, which are acquired during the first 6 hours after trauma incident. Their study, however, showed a correlation between serum levels S100B levels and brain CT scan findings and these values were significantly helpful with prediction of CT scan findings (20).

Our choice of a 172.15 ng/L cut-off value for serum S100B is higher than the cut-off value of the Castellani *et al.* study (22). The differences in the values can be attributed to the severity and diversity of pathologic findings (22) as well as the differences in the manufacturing companies of the analytical kits.

Contrary to our study, few studies on the possible correlation between urinary S100B levels and positive brain CT scans have been previously conducted in children and adolescents. However, since findings of these studies showed no correlation between them, it is essential to conduct studies on urine specimens with a larger number of patients.

The average time for taking samples (urine and serum) was 2.79 hours in our study, while in the Hallen *et al.* study it was 3.5 hours. Due to the short half-life of this biomarker, this may explain the correlation with the positive brain CT scan in our study compared to the Hallen *et al.* study.

However, this biomarker may also be increased in other cases, such as brain infections (23). In our study, false positives and false negative were considered and were not included in the study from the very beginning.

In summary, previous studies have established a correlation between urinary S100B levels and positive findings of brain CT scans, however, few studies have been conducted in children and adolescents (21,24). Most of these studies reported no significant correlations. Our findings have demonstrated a significant relationship between serum and urinary S100B levels with positive findings of brain CT scans and further studies are required with a larger sample size of urinary samples. Our study had some limitations, namely patient follow-up was not possible and the sample size was small.

In conclusion, results of our study showed that the use of serum and urinary levels of the S100B biomarker could reduce unnecessary brain CT-scans in children and adolescents with mild traumatic brain injury. Considering the non-invasiveness of the urinary sample collection, this method can be used in an emergency instead of serum samples.

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ORIGINAL ARTICLE

Comparison of the NG Biotech NG-Test CARBA 5 and CORIS BioConcept RESIST-4 O.K.N.V. immunochromatographic lateral flow assays for the detection of carbapenemase enzymes in *Enterobacterales*

Bronwyn D Davison

ABSTRACT

Objective: To evaluate and compare the NG-Test CARBA 5 and RESIST-4 O.K.N.V. immunochromatographic lateral flow assays for the detection of carbapenemase enzymes in *Enterobacterales*. Both assays have the ability to detect four of the most widespread carbapenemases; OXA-48-like, NDM, KPC, and VIM. In addition, the NG-Test CARBA 5 is able to detect IMP types. The importance of rapid diagnostic tests in microbiology laboratories is becoming increasingly crucial with the emergence of Carbapenemase-producing *Enterobacterales* (CPE) in New Zealand as these isolates have limited treatment options.

Methods: NG-Test CARBA 5 and RESIST-4 O.K.N.V. were performed with 58 *Enterobacterales* isolates with reduced susceptibility to meropenem, including 45 CPE and 13 non-CPE.

Results: The respective sensitivity results for the NG-Test CARBA 5 and RESIST-4 O.K.N.V. were as follows: OXA-48-like – 94.7 (18/19) and 89.5% (17/19), NDM – 95.2% (20/21) and 90.5% (19/21), KPC – 100% (4/4) and 75.0% (3/4), VIM – 100% (2/2) and 100% (2/2). Additionally, the NG-Test CARBA 5 detected 80.0% (4/5) of IMP-types. Both assays produced 100% specificity .

Conclusions: NG-Test CARBA 5 and RESIST-4 O.K.N.V. detect the main carbapenemase types in *Enterobacterales*, as currently found in New Zealand. They are both highly specific assays and produced overall sensitivity values of 94.1% and 89.1% respectively. These products are robust, user-friendly kits that provide results within 20 minutes (including setup and incubation) indicating the presence or absence of singular or multiple carbapenemase type(s) detected.

Keywords: carbapenemase, *Enterobacterales*, immunochromatographic lateral flow assay, carbapenem.

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INTRODUCTION

Carbapenemase-producing *Enterobacterales* (CPE) confer resistance to carbapenem antibiotics via the ability to hydrolyse the beta-lactam ring in carbapenem structures. These enzymes are categorised into three major groups based on their molecular structures; Ambler class A carbapenemases including *Klebsiella pneumoniae* carbapenemases (KPCs), class B metallo-beta-lactamases (MBLs) including NDM, IMP, VIM carbapenemases and class D OXA carbapenemases (oxacillinases). The detection of carbapenemases is highly significant as their presence may result in a lack of effective antibiotics to treat infections caused by carbapenemase-producing bacteria (1). CPE may confer resistance to virtually all beta-lactam antibiotics as they have decreased susceptibility to carbapenems and are mostly, but not always, resistant to extended-spectrum cephalosporins. The majority of these enzymes are encoded by genes on transposable elements within plasmids, hence are an issue for infection control (2) as they have the ability to spread from one organism to another.

Other mechanisms of resistance to carbapenems in *Enterobacterales* include efflux pumps and ESBL or AmpC production coupled with porin loss (3). Differentiation as to whether it is carbapenemase-production resistance or another mechanism is optimal for laboratory analysis, results interpretation, clinical decision making, patient management and surveillance monitoring. Carbapenem resistance in *Pseudomonas* and *Acinetobacter* is also an emerging issue but are not discussed in this evaluation.

The EUCAST guidelines (July 2017) provide breakpoints for clinical treatment as well as breakpoints to indicate when screening for CPE should be carried out (2). Carbapenem MICs are set lower and disk diffusion zone breakpoints are set higher than the clinical breakpoints for *Enterobacterales*. Although some carbapenemase-producing organisms can have clinically susceptible breakpoints, it is still important to identify whether they harbour a carbapenemase in order to provide the best treatment options and to prevent the spread of resistance (4).

There are a variety of phenotypic methods currently available for the detection of CPE based on different principles. Combination disk testing methods, such as the D73C MASTDISCS® *Combi Carba plus* sets or ROSCO Neo-Sensitabs™, contain disks with meropenem +/- various carbapenemase inhibitors to detect the presence of CPE (2). Zones of inhibition are compared between disks containing meropenem alone and meropenem + inhibitors to determine if isolates produce carbapenemase activity. Colorimetric tests, such as the CarbaNP, utilise the principle of carbapenem hydrolysis indicated by a pH change, resulting in a colour change of the phenol red indicator (2). The Carbapenem inactivation method (CIM) and modified CIM also work by the principle of carbapenem hydrolysis. Following incubation of a meropenem disk in a broth inoculated with the test organism, the disk is placed on Mueller-Hinton agar inoculated with a carbapenem-susceptible indicator organism, incubated overnight and the zone of inhibition interpreted to determine whether meropenem has been hydrolysed (4).

Immunochromatographic lateral flow assays are a recent development, based on an antigen-antibody principle to identify the most widespread carbapenemases. In this study we evaluated two lateral flow assays, the NG-Test CARBA 5 and the CORIS BioConcept RESIST-4 O.K.N.V.

MATERIALS AND METHODS

A total of 58 *Enterobacterales* isolates were tested, including 45 previously characterised CPE and 13 non-CPE isolates harbouring other resistance mechanisms responsible for reduced susceptibility or resistance to carbapenems. Isolates used in this study were provided by the Institute of Environmental Science and Research Limited (ESR), Canterbury Health Laboratories and the RCPA. All non-CPE isolates tested negative for carbapenemase production by at least two phenotypic methods, including CarbaNP, together with one other method, such as the Carbapenem Inactivation Method (CIM), the modified CIM (mCIM), ROSCO MBL/KPC tabs or Cepheid Xpert® Carba-R PCR. Isolates were identified using the Vitek® MS matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry system (bioMérieux). The carbapenemase number and type of enzymes tested reflect those which are most commonly found in New Zealand to date (5), with a higher prevalence of OXA-48-like and NDM-type enzymes.

The RESIST-4 O.K.N.V. and NG-Test CARBA 5 assays utilise a nitrocellulose membrane cassette which is sensitised with antibodies directed against the different types of carbapenemases. A control capture reagent is incorporated to validate the test result. Tests were performed according to instructions provided by the manufacturers. Briefly, a bacterial test colony is homogenised in the provided buffer and a specified volume dispensed into the sample well of the cassettes and left to incubate at room temperature for 15 minutes. As the sample migrates through the well it produces a red line with the specific anti-carbapenemase antibodies if the isolate carries the corresponding carbapenemase.

The RESIST-4 O.K.N.V. kit has the ability to detect OXA-48-like, KPC, NDM, and VIM type carbapenemases with the respective detection limits of 0.125ng/ml, 0.625ng/ml, 0.25ng/ml and 0.23ng/ml (6). The NG-Test CARBA 5 is also able to detect OXA-48-like, KPC, NDM, VIM, and in addition IMP type carbapenemases with the respective detection limits of 300pg/ml, 600pg/ml, 150pg/ml, 300pg/ml and 200pg/ml (7) .

RESULTS

The results are displayed in Table 1. Both the NG-Test CARBA 5 and the RESIST-4 O.K.N.V. produced 100% specificity results as there were no false positive results. The overall sensitivity for each kit was calculated by considering each enzyme detectable to be an individual test. The NG-Test CARBA 5 therefore had a total of 290 tests (58 isolates tested against five enzymes), producing an overall sensitivity of 94.1%. The RESIST-4 O.K.N.V. had a total of 232 tests (58 isolates tested against 4 enzymes), producing an overall sensitivity of 89.1%.

The sensitivity for detection of OXA-48-like by the NG-Test CARBA 5 was 94.7% (18/19), due to one *Escherichia coli* OXA-48 producer being undetected. The same *E. coli* OXA-48 producer was also undetected by the RESIST-4 O.K.N.V. which also failed to detect OXA-48 in a *Klebsiella pneumoniae* co-producing NDM-1. These false negative results generated a sensitivity of 89.5% (17/19) for the detection of OXA-48-like enzymes. The sensitivity of NDM detection by the NG-Test CARBA 5 test was 95.2% (20/21), with one NDM-1-producing *Proteus mirabilis* undetected.

This NDM-1-producing *Proteus mirabilis* was also undetected by the RESIST-4 O.K.N.V., which also failed to detect a NDM-producing *Providencia stuartii* isolate, giving a sensitivity of 90.5% (19/21). All four KPC producers included in the study were detected by the NG-Test CARBA 5 kit resulting in a sensitivity of 100% (4/4). One KPC-2 enzyme produced by a *Klebsiella pneumoniae* was undetected by the RESIST-4 O.K.N.V. kit resulting in a sensitivity of 75.0% (3/4). Both kits detected the two VIM producers included in the study giving sensitivity results of 100% (2/2). The NG-Test CARBA 5 kit produced a sensitivity of 80.0% (4/5) for detection of IMP type carbapenemases, with one IMP-14-producing *Klebsiella pneumoniae* undetected. Although the RESIST-4 O.K.N.V. kit does not include detection for IMP types, the isolates were still tested in this study as all isolates were tested blindly.

Six of the isolates tested were co-producers of OXA-48-like and NDM-type carbapenemases, therefore these results have been included in the analysis of OXA-48 and NDM individually. Due to the frequency of CPE that co-produce carbapenemases it is important to recognise that both kits are able to detect multiple carbapenemases produced by the same isolate. Of the dual-producing isolates in this study, the only carbapenemase undetected by the RESIST-4 O.K.N.V. was a OXA-48 carried by a *Klebsiella pneumoniae,* which also produced an NDM.

Also of note is that there were two isolates which, although detected by both kits, produced a significantly weaker result with the RESIST-4 O.K.N.V. than the NG-Test CARBA 5. These isolates were a VIM-producing *Klebsiella pneumoniae* and a NDM-producing *Providencia stuartii*.

DISCUSSION

Global studies clearly suggest the emergence of CPE infections will continue to increase, impacting further on testing algorithms in diagnostic testing laboratories and requirements from health professionals for additional information on such isolates (8). Rapid confirmation reporting of CPE allows for more suitable patient management, including optimised treatment and implementation of contact precautions in order to minimise the risk of further spread between patients. The New Zealand National Antimicrobial Susceptibility Testing Committee (NZ NAC) has recently published guidelines for the “Minimum laboratory requirements for the detection of CPE from clinical samples and screening specimens” (9). This document provides laboratories in New Zealand clear cut instructions on how to identify *Enterobacterales* isolates that are suspicious for carbapenemase production , when further confirmatory testing should be performed and which isolates are required to be sent to ESR for confirmation and typing.

When performing phenotypic carbapenemase detection tests and a negative result is obtained it is crucial to consider patient risk factors and clinical information, with a low threshold for referring isolates to ESR for further testing, as indicated in the NZ NAC guidelines (9). As the NG-Test CARBA 5 and RESIST-4 O.K.N.V. lateral flow assays are specific to the kit profile carbapenemases, an option could be to perform a second phenotypic detection method when a negative result occurs and there is still high suspicion of carbapenemase. This may be useful if the isolate possesses a rare type, such as IMI or GES, or for detection of types with lowered sensitivity results from the lateral flow assays. Examples of such tests include the mCIM or the CarbaNP.

Meropenem MICs were performed alongside the NG-Test CARBA 5 and RESIST-4 O.K.N.V. cassettes. Those isolates with false negative carbapenemase results had meropenem MICs above the EUCAST screening cut-off for CPE (>0.125 mg/L). The decreased meropenem susceptibility indicates the carbapenemases were still active at time of testing therefore ruling out deterioration of the carbapenemase-bearing-plasmid as the reason for the false negatives. A possible explanation for these CPE not being detected may be due to potential low expression levels of the carbapenemases below the detection limits specified in the kit inserts.

The sensitivity of NDM detection was lower for both kits as they showed false negative results for an NDM-1 *Proteus mirabilis* isolate. Similarly, this has been shown in other studies where a *Proteus mirabilis* NDM-1 producer was undetected using the RESIST-4 O.K.N.V. (10) as well as the RESIST-3 O.K.N. kit (11). This was analysed further to find an explanation for the false negative result, concluding that to increase the

performance of the CORIS RESIST kits for NDM detection the isolate could be cultured onto a blood-containing media, using an increased inoculum, or taking the test isolate directly adjacent to a carbapenem disk from Mueller Hinton agar (MHA) as there is a higher expression of the carbapenemase in that area (10,11). Studies show that the sensitivity of detecting NDMs can be increased by supplementing MHA with zinc ions, as MBLs bind zinc at their active site resulting in increased enzymatic activity (10). Using MHA with the addition of zinc therefore may prove beneficial by producing a darker line on the kits as they will have a stronger reaction for those isolates in the study which produced weaker lines using the RESIST-4 O.K.N.V. test (*Klebsiella pneumoniae* VIM and a *Providencia stuartii* NDM producer).

The detection of OXA-48-like carbapenemases can be challenging as they commonly have weak resistance to carbapenems, therefore providing a test which is highly specific to OXA-48-like carbapenemases which is important for CPE detection in areas where OXA-48 are one of the most prevalent types (12). High sensitivity values for OXA-48 detection have been portrayed in global studies (13,14). In a study by Kolenda et al including 11 OXA-48-like CPE, the sensitivity was 100% using the RESIST-4 O.K.N.V. (13). Likewise, the validation of the NG-Test CARBA 5 detected all OXA-48-like CPE (n = 37) (14).

The co-expression of OXA-48-like and NDM in six of the CPE tested were correctly detected by the NG-Test CARBA 5 assay. The RESIST-4 O.K.N.V. kit failed to detect one OXA-48 enzyme in co-expression with a NDM, however, correctly detected the co-expression of the other five isolates. Recent studies by Greissl et al and Boutal et al show both assays producing 100% sensitivity for the detection of isolates co-expressing OXA-48-like and NDM types (10,14).

Although only a small number of KPC and VIM producing isolates were available for this study, there have been larger studies performed with comparable high sensitivity results for detection of KPC and VIM using both kits. A validation study of the NG-Test CARBA 5 kit produced 100% sensitivity for KPC (n = 22) and VIM (n = 17) (14). Likewise in a small study, the RESIST-4 O.K.N.V. kit has shown to have a 100% sensitivity for KPC (n = 10) and VIM (n = 34) (13).

The sensitivity of IMP detection by the NG-Test CARBA 5 kit was decreased due to a false negative result of IMP-14 *Klebsiella pneumoniae*. Similarly, IMP-14 carbapenemases (n = 2) were undetected in an evaluation study by Hopkins et al contributing to the decrease in sensitivity for detection of IMP variants (15).

Overall both kits achieved acceptable sensitivity in our study. However, the sensitivity of the NG-Test CARBA 5 assay was higher than the RESIST-4 O.K.N.V. for the detection of OXA-48-like, NDM and KPC-types. In addition, the NG-Test CARBA 5 is able to detect IMP types. Both kits achieved a sensitivity of 100% for the detection of VIM-types. This evaluation of the NG-Test CARBA 5 and RESIST-4 O.K.N.V. produced 100% specificity as there were no false positive results.

Limitations of the study include that only *Enterobacterales* were investigated, therefore further testing would be required to evaluate the use of these kits for other Gram-negative organisms that can produce carbapenemases, including *Pseudomonas aeruginosa* and *Acinetobacter* species.

Table 1. Results

Resistance Mechanism	Organism	n*	Number carbapenemase detected	
Carbapenemase			**Carba5**	**RESIST-4 O.K.N.V**
OXA-48-like	*Escherichia coli*	10	9	9
	Klebsiella pneumoniae	3	3	3
OXA-48-like + NDM	*Escherichia coli*	3	3	3
	Klebsiella pneumoniae	3	3	2.5†
NDM	*Citrobacter freundii*	1	1	1
	Escherichia coli	6	6	6
	Klebsiella pneumoniae	4	4	4
	Klebsiella oxytoca	1	1	1
	Providencia stuartii	2	2	1
	Proteus mirabilis	1	0	0
KPC	*Klebsiella pneumoniae*	4	4	3
VIM	*Klebsiella pneumoniae*	2	2	2
IMP	*Escherichia coli*	2	2	0
	Klebsiella oxytoca	1	1	0
	Klebsiella pneumoniae	2	1	0
		45	**42**	**35.5**
Non-carbapenemase				
ESBL + AmpC (CIT Group)	*Escherichia coli*	2	0	0
pAmpC (ACC)	*Proteus mirabilis*	1	0	0
pAmpC (DHA)	*Klebsiella pneumoniae*	1	0	0
pAmpC (CIT Group)	*Citrobacter koseri*	1	0	0
	Escherichia coli	2	0	0
pAmpC	*Proteus mirabilis*	1	0	0
Hyper AmpC	*Enterobacter cloacae complex*	2	0	0
Hyper AmpC + porin	*Enterobacter aerogenes*	1	0	0
ESBL	*Escherichia coli*	1	0	0
None	*Escherichia coli*	1	0	0
		13	**0**	**0**

*n = number tested, † one co-producing isolate only NDM detected and OXA-48 not detected.

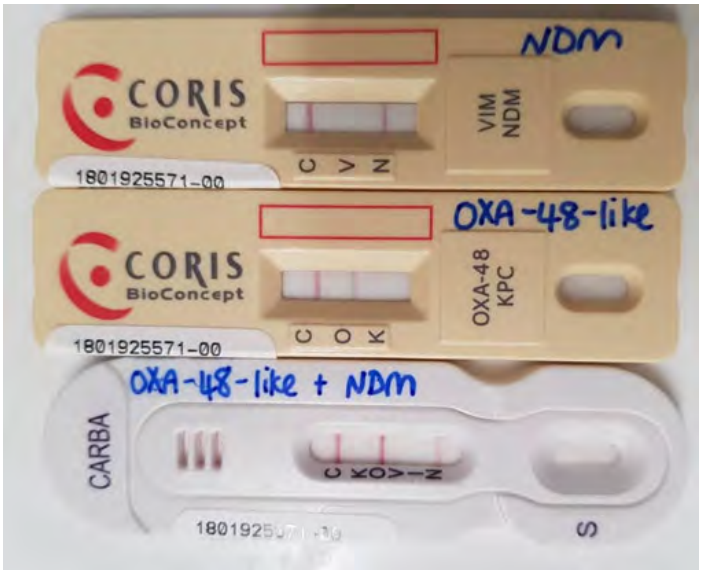

Figure 1. Biotech NG-Test CARBA 5 and CORIS BioConcept RESIST-4 O.K.N.V. detection of OXA-48-like and NDM co-expression.

The number of KPC, VIM, and IMP-type carbapenemases tested were limited, however, this reflects the current prevalence of carbapenemase-types in New Zealand. The fact that IMP-types are not detected by the RESIST-4 O.K.N.V. kit is a limitation. Although IMP-types are not commonly found in New Zealand, they are the highest reported type of carbapenemase detected in Australia (16). As carbapenemases have the ability to spread easily and with Australia being close geographically, New Zealand laboratories need to be vigilant and have methods suitable to detect IMP types.

Both kits tested were found to be robust as they can be stored between 4 - 30°C with a shelf life of up to 2 years, therefore suitable when testing volumes are low. They are user friendly, produced high specificity results and the sensitivity values produced for the assays were 94.1% for the NG-Test CARBA 5 and 89.1% for the RESIST-4 O.K.N.V. Results were rapidly produced within 20 minutes (15 minutes incubation plus set up of kit) indicating the presence or absence of singular or multiple carbapenemase type(s). These kits proved to be very straight forward to use, following a short number of steps required to prepare the isolates and no intensive training or specialised skills required to run the test.

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Aldosterone

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Prolactin
Progesterone
Testosterone
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hCG/ß-hCG
Androstenedione
SHBG

ANAEMIA
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HSV-1 IgG
HSV-2 IgG
HSV-1/2 IgM
Parvovirus B19 IgG
Parvovirus B19 IgM

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B. burgdorferi IgG
B. burgdorferi IgM

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ORIGINAL ARTICLE

What bacteria are present on the mobile phones of students?

Bhumi Tailor, Neha Nikita, Ashley Naicker, Taina Naivalu and Reginald Arvind Jnr. Kumar

ABSTRACT

Background: Mobile phones are an essential component of modern life and used by almost everyone. They are increasingly being used in the hospital setting by health care workers and come in contact with various surfaces around the hospital. Thus, they are likely to get contaminated by a variety of organisms.

Aim: To investigate the colonisation of microorganisms on students' mobile phones at the College of Medicine, Nursing and Health Science, Fiji National University.

Methods: A sample of 50 swabs were collected from randomly selected individuals' mobiles and cultured on blood agar, MacConkey agar and Sabarauds dextrose agar. Isolation of the organisms was processed according to laboratory standard protocol and each organism was identified.

Results: All 50 of the students' mobile phones showed evidence of bacterial colonisation. The most common bacteria isolated was *Bacillus* species (82%), followed by *Acinetobacter lowffii* (26%), coagulase-negative *staphylococcus* (16%), inactive *Escherichia coli* (12%), *Enterobacter agglomerans* (10%), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (8% each), *Staphylococcus aureus* (6%), *Klebsiella pneumonia*, *Klebsiella oxytoca* (2%) and *Micrococcus* species (2%).

Conclusions: This study revealed that mobile phones are contaminated by microorganisms and may be a vector in spreading nosocomial or community-acquired infections in a hospital setting. In order to combat this issue, proper handwashing, decontamination and infection control procedures should be practised adequately.

Key words: mobile phones, health care workers, bacterial colonization, nocosomial, community-acquired infections.

N Z J Med Lab Sci 2019; 73: 106-110

INTRODUCTION

Mobile phones have been around for more than thirty years since its introduction in the early eighties (1). In recent years there have been tremendous advancements and mobile phones nowadays are not only used for calling or messaging, but has evolved so that it can be used for basic tasks that were previously done on a computer. Although there are many advantages to the use of mobile phones, they are not usually cleaned or disinfected. Therefore, they serve as a reservoir for bacteria and may cause nosocomial infections. As mobile phones have evolved they have become an integral part of individuals daily lives and their potential to be hazardous to health is often overlooked (2,3). In hospitals, the use of mobile phones is a common sight, not just with patients or visitors but also with health care workers (1).

Studies have shown that mobile phones carried by dental and laboratory science students are at high risk of contamination as most times they are left in clinical coat pockets or laboratory benches that may be contaminated (1). Health care workers also touch their phones during or after patient examination, nursing care, sample processing and other such activities, which potentially leads to contamination or spread of pathogenic infections (4). Despite being used continuously, mobile phones are seldom cleaned or disinfected. Moreover, batteries keep mobile phones warm, thus making it ideal for bacterial growth and reproduction (5). Bacteria can easily be transmitted from mobile phones to an individual's face, eyes, mouth and even food. In addition to this, the sharing of mobile phones between health professionals and the community can transmit potential pathogens to the community (6). Smartphones have the potential of carrying more than 25,000 bacteria per square inch, of which most can be pathogenic to individuals, especially those who are immunocompromised (1).

Individuals are so accustomed to using mobile phones wherever they go and keeping their phones clean depends on personal hygiene and how often they disinfect their phones (1,7). Although mobile phones have been identified as a potential reservoir for microorganisms, very little research and awareness has been done to address this issue and provide evidence as to how contaminated cell phones can be (8). The aim of this study was to investigate the colonisation of microorganisms and the variety of bacteria present on students' mobile phones at the College of Medicine, Nursing and Health Science of the Fiji National University.

METHODS

A cross-sectional study was carried out by obtaining swabs from mobile phones of 50 randomly selected students of the College of Medicine, Nursing and Health Sciences. Informed consent was obtained from each individual. A sterile cotton swab was dipped into a nutrient broth then the mobile phones front, back and sides were swabbed. This was then reinserted in the nutrient broth test tube and incubated aerobically at 37^{o}C for 24 hours.

The next day the swab was cultured onto blood agar, MacConkey's agar and Sabouraud dextrose agar. Blood agars were incubated anaerobically at 37^{o}C for 24 hours while MacConkey's agars and Sabouraud dextrose agars were incubated aerobically. After 24 hours, each of the plates was observed for growth and a Gram stain smear was made from the different colonies from each plate. The growth pattern on the culture plate, i.e. the type of colonies, haemolysis shown, and heavy, moderate or light growth was noted. A standard laboratory protocol was followed for the identification of all organisms. Figure 1 below shows the scheme followed for the identification of Gram positive and Gram negative organisms.

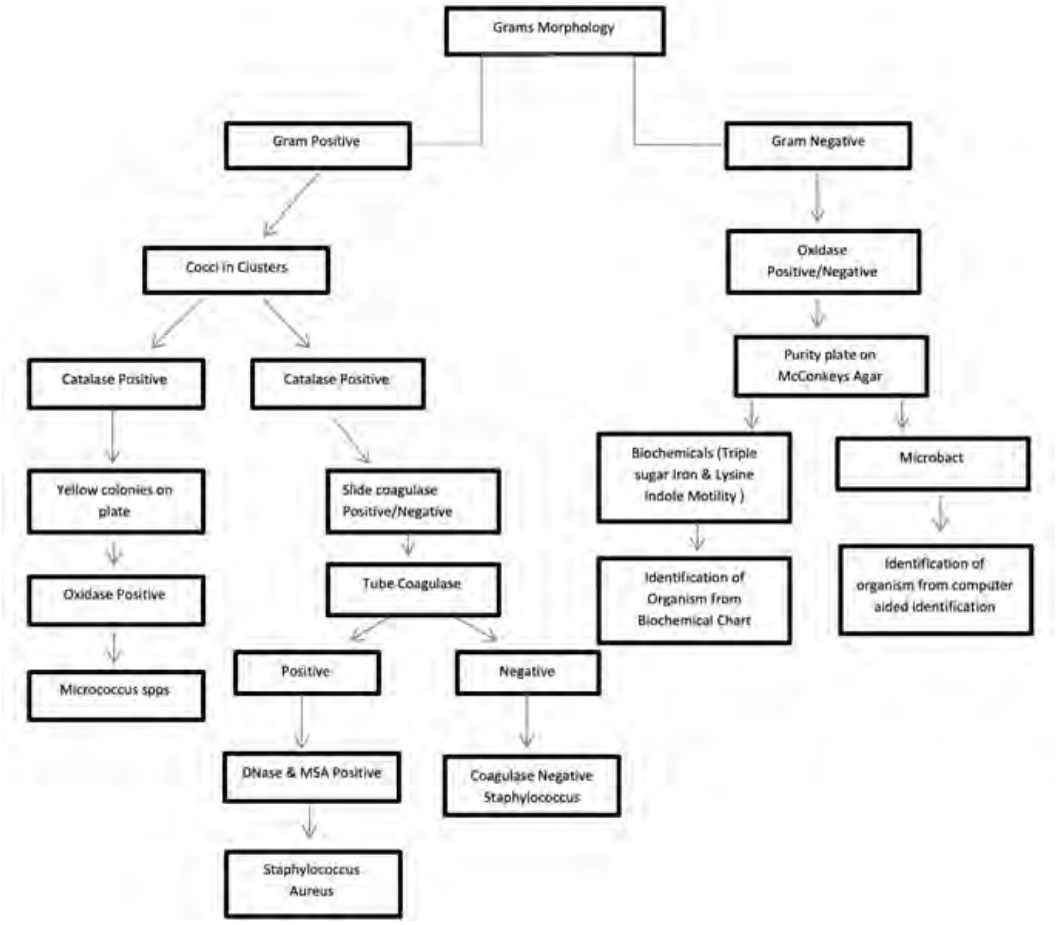

Figure 1. Schematic of identification of Gram-positive and Gram-negative bacteria.

RESULTS

All the 50 mobile phones that were swabbed and cultured showed growth and evidence of bacterial contamination. With regards to the bacterial contamination, 20 (40%) showed pure growth, 19 (38%) showed mixed growth of two types of bacteria and 11 (22%) showed diverse growth of three types of bacteria. The most common class of bacteria isolated morphology were Gram-positive bacilli (47%), followed by Gram-negative bacilli (39%) and the least common were Gram-positive cocci (14%).

Table 1 shows the different types of microorganisms cultured from mobile phones and the percentage of mobile phones on which they were present. The most common bacteria isolated was *Bacillus* species (82%), followed by *Acinetobacter lowffii* (26%), coagulase-negative *staphylococcus* (16%), inactive *Escherichia coli* (12%), *Enterobacter agglomerans* (10%), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (8% each), *Staphylococcus aureus* (6%), *Klebsiella pneumonia*, *Klebsiella oxytoca* (2%) and *Micrococcus* species (2%).

Table 1. Types of bacteria isolated .

Bacteria	Number of bacteria isolated	% of mobile phones
Gram-positive bacilli (47%)		
Bacillus species	41	82%
Gram-positive cocci (14%)		
Coagulase-negative *staphylococcus*	8	16%
Staphylococcus aureus	3	6%
Micrococcus species	1	2%
Gram-negative bacilli (39%)		
Inactive *Escherichia coli*	6	12%
Enterobacter agglomerans	5	10%
Pseudomonas aeruginosa	4	8%
Acinetobacter baumannii	4	8%
Klebsiella pneumoniae	1	2%
Klebsiella oxytoca	1	2%
Acinetobacter lowffii	13	26%

Figure 2 shows the number of mobile phones colonised by isolated bacteria. Out of the 11 types of bacteria that were isolated, seven (64%) were identified as harmful, while the remaining four (36%) were ubiquitous and not as harmful as the rest. The organisms that were classified as less harmful include *Bacillus* species, coagulase-negative *Staphylococcus*, *Micrococcus* species and *Acinetobacter lowffii.*

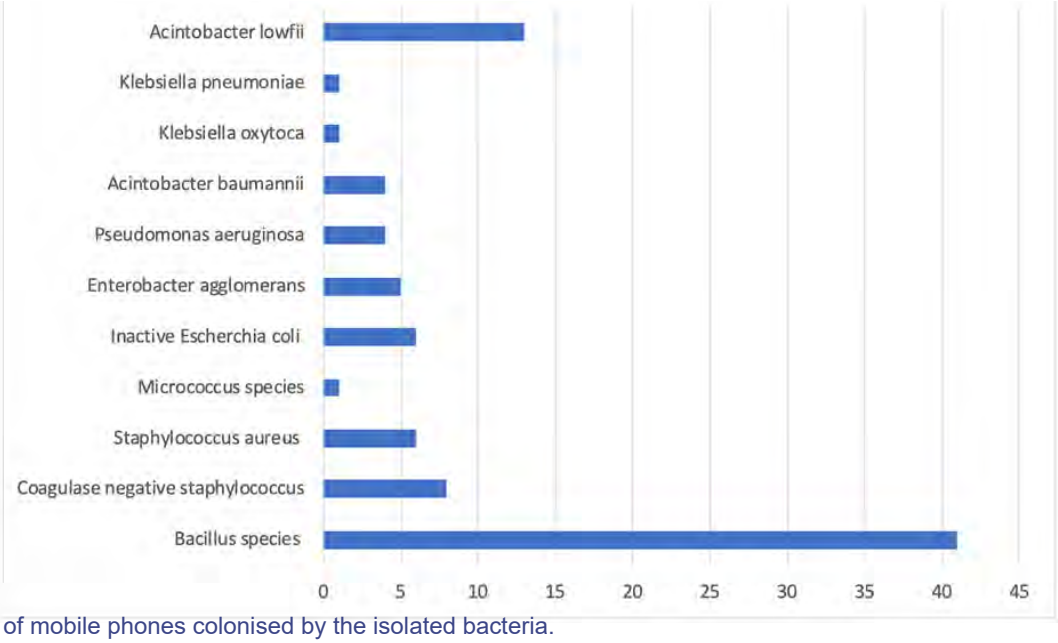

Figure 2. Number of mobile phones colonised by the isolated bacteria.

DISCUSSION

Mobile phones are the primary means of communication today and it is often overlooked that they may be a vector for causing infection. In recent times this has been a significant concern as microorganisms can be transmitted from one person to another or from non- human subject to people. Non-human items, such as stethoscopes and computers, or in this instance mobile phones, can be associated with hospital-acquired infections.

In our study we have shown that all 50 [100%] of the mobiles phones had bacterial colonisation. This finding is similar to that of Tagoe et al. (9) but comparatively higher than other studies that did not show 100% but still showed a high rate, which were between 90.98%, to 95% (4,10,11). This indicates that the presence of bacteria on mobile phones could be a possible mode of transmission of pathogens, potentially causing infections.

The most common bacteria isolated from students' mobile phones were *Bacillus* species [82%], similar to studies by Tagoe et al. (9) Kumar et al. (12) and Auhim (13). The high isolation of *Bacillus* species confirms the ubiquitous nature of this species giving it greater colonisation ability as well as the ability of its spores to resist environmental changes, withstand dry heat and certain chemical disinfectants for reasonable periods (13). The second most common bacteria isolated was *Acinetobacter lowffii (*26%) which is also due to its ubiquitous status in nature, and it is seen as a member of the normal flora that inhabits the oropharynx, human skin and the perineum in approximately 20 to 25% of healthy individuals (14).

In contrast, coagulase-negative *staphylococcus* was the most common bacteria isolated in studies from India (78%) ,Ethiopia (47.5%) and Nigeria workers (1,5,6). however, in the current study CNS isolation was 16% which is in agreement with a study by Datta et al. (13.19%) (15). The combination of constant handling and heat generated by cell phones creates a prime breeding ground for microorganisms that are typically found on our skin (1). Sepehri et al. reported that CNS such as *Staphylococcus epidermis* has emerged as a significant causative agent of nosocomial infections, which constitute the main component of the normal skin and are particularly responsible for catheter and other medical device-related infections (16).

Isolation of *Staphylococcus aureus* (6%) was low compared to the studies done by Tambekar *et al.* and Datta *et al.* who found *Staphylococcus aureus* as dominant bacteria present on mobile phones (15,17). *Staphylococcus aureus* is found on the skin and in the noses of up to 25% of healthy people and animals and can cause illnesses from pimples and boils to pneumonia, meningitis, and is a close relative of MRSA (7). Trivedi et al. (11) and Ulger et al. (18) documented that 50% of *Staphylococcus aureus* they isolated from mobile phones were MRSA. However, those studies were carried out on a limited scale and no further antimicrobial testing was done to identify methicillin-resistant strains thus it cannot be said that the 6% of *Staphylococcus aureus* isolated were MRSA negative (11,18).

Other bacterial isolations were inactive *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella species*, *Enterobacter agglomerans* and *Acinetobacter baumannii*. The role of these agents in spreading nosocomial infections is well established (19). In medical schools, students go for clinics and attachments to the hospital, which can play a critical role in the transmission of organisms associated with nosocomial infections. Micro-organisms can be transferred from person to person or from inanimate objects (such as stethoscopes, bronchoscopes, pagers, ballpoint pens, patient hospital charts, computer keyboards, mobile phones and fixed telephones) to hands and vice versa (16). Hence the present findings imply that mobile phones may serve as a vehicle of transmission of bacteria causing diseases, such as diarrhoea, pneumonia, boils and abscesses (20). *Pseudomonas aeruginosa* had been reported in the United States by the Centre for Diseases Control and prevention to be the most isolated nosocomial pathogen accounting for 10.1% of all hospital-acquired infections and has been implicated in gastrointestinal infection, primarily in immunocompromised individuals (19).

Acinetobacter baumannii was present on 8% of the mobile phones of the students in our study. Arora et al. isolated *Acinetobacter* species from the cell phones of two clinicians working in ICU of their hospital (21). A similar study done at a tertiary care hospital in Israel identified multidrug-resistant *Acinetobacter baumannii* in the hands and cells phones of health care workers and patients in ICU (22). The ability of *Acinetobacter* to contaminate cell phone is expected as this is an multidrug-resistant water and soil organism and is responsible for infection in predisposed patients in the hospital (21).

Escherichia coli is present in about 0.1% of gut flora and faecal-oral transmission is the major route through which pathogenic strains of this bacteria cause disease (1). The presence of *Escherichia coli* suggests faecal contamination of these phones and suggests poor hygiene and lack of proper handwashing practices by students. In our study there were 12% of the strain inactive *E. coli* which is similar to Auhim study, which was 12.5% (13). These findings are different compared to Famurewa et al. who had *E. coli* as one of the most frequently isolated organisms (23).

In addition, two of the student's mobile phones isolated different species of *Klebsiella*, one *K. pneumonia* and the other *K. oxytoca*, both have been considered as opportunistic pathogenic *Klebsiella* species (24). The low occurrence of *Klebsiella* species was similar to a study by Kawo and Musa, they documented that *Klebsiella* species might be associated with the fact that this bacterium is present in the respiratory tract and faeces of about 5% of healthy individuals, this could be a reason for isolating *Klebsiella* from student's mobile phones in our study (25). The role of this organism in both nosocomial and community-acquired infection has been well documented, *Klebsiella* species has been variously responsible for septicaemia, pneumonia, UTI and soft tissue infection with Podschun and Ullmann reporting that hospitalised, immunocompromised patients with underlying diseases is the main target of these bacteria (24).

The presence *of Enterobacter agglomerans* in our study was found to be 10%. Cataño et al. also isolated *Enterobacter agglomerans* from mobile phones of health care workers in Columbia (26). *Enterobacter agglomerans* is known to be a plant organism and was considered unimportant clinically until the mid-1960s, when it was identified in hospital-acquired infections in debilitated patients (especially those receiving broad-spectrum antibiotics) as causing postoperative wound infections and urinary tract infections after instrumentation, Al-Damluji et al. stating *Enterobacter agglomerans* as a new cause of primary pneumonia (27).

The overall implication is that mobile phones, which make communication easy and accessible, also form a carrier of pathogenic agents for disease transmission. If care is not taken, they could even be used as vehicles for the transmission of biological weapons. As most students attend clinics and are attached in the hospital, the use of personal mobile phones could cause harm to hospitalised patients. Heyba et al. documented that the use of mobile phones by health care workers in the intensive care unit, burns ward and operative rooms may have more severe hygiene consequences because, unlike fixed phones, mobile phones are often used close to the patient (28). ICU patients and burn patients are most vulnerable to infectious diseases, so the risk of transmission of the organism associated with nosocomial infection is increased (7).

There were some limitations to our study. Our study was small in numbers as only 50 cell phones were studied. Larger sample size would have increased workload, but the results would have been more valid, and more information could have been collected. Due to resource availability gloves could not be changed after taking each sample, thus gloves were changed after every five samples. To ensure that gloves did not contaminate other phones, they were wiped with SVM after every sample and replaced after five samples. Antibiotic sensitivity was not done as our budget was not sufficient to support this. Thus, the resistant mechanism of different bacteria was not able to be determined.

In conclusion, our study has shown that mobile phones are contaminated by microorganisms and may be a vector in spreading nosocomial or community-acquired infections in a hospital setting. Since mobile phones are used in close proximity to sensitive parts of the human body, such as the face, ears, lips and hands, this can lead to transmission of infections. Mobile phones were made for communications purposes, however, due to ignorance, lack of personnel hygiene and sanitation measures, such as hand washing and phone decontamination, mobile phones are slowly taking the path to becoming pathogenic agents of bacterial transfer.

We recommend the training of medical students and healthcare personnel on strict infection control procedures, hand hygiene and environmental disinfection. Since the restrictions of the use of mobile phones by health care workers may prove impractical in a public setting, strategies for preventing disease transmission are needed. Restrictions of mobile phone use can be used as a preventative measure. There are other recommendations, such as cleaning mobile phones and maintaining good personnel hygiene and sanitation measures for decreasing rates of bacterial contamination on mobile phones. It is essential to create awareness on the use of mobile phones being a possible vector for infection in a hospital setting and strict infection control procedures should be practised. Hand washing is considered the single most important intervention to prevent transmission of bacteria and viruses from the hands of a health care worker. Hand washing is also essential after using the bathroom as one can contaminate one's cell phone, due to the popularity of the use of mobile phones in the bathroom and there could also be the faecal-oral transmission of bacteria. Health facility could also provide alcohol hand wash or hand sanitisers in all wards and departments of the hospital.

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Now is a good time to start thinking about updating your CPD for 2019. Participants in the NZIMLS CPD Programme, need to have their 2019 points uploaded, plus their competency signed off prior to 1 January 2020. After that date there is an administration fee of $50 for us to add your points for you.

Scientists need to accumulate 300 points over a set three-year period, and require a minimum of 85 points per year during that period.

Technicians must accumulate 16 points per year.

ORIGINAL ARTICLE

The strategic management stage of ISO 15189:2012 management system standard: an implementation update

Dennis Mok and Sharfuddin Chowdhury

ABSTRACT

Objectives: The primary aim of this paper is to provide an update on selected international standards, guidance documents and relevant literature at the application level that are associated with the implementation of the 'strategic management' stage of ISO 15189:2012.

Methods: Additional relevant international standards and guidance documents were identified from the Union of International Associations segmented organisations (Types A, B, C, D, E and F) and supplemented with additional literature.

Results: Selected international organisations (*n* = 14), inclusive of additional organisations (*n* = 6), were found to provide relevant international standards and guidance documents (*n* = 79), inclusive of additional documents (*n* = 47), in support of implementation of the strategic management stage of ISO 15189:2012. An updated list of literature (*n* = 49) was also provided for further reference.

Conclusions: The present study contributes to the medical laboratory's development of implementations of ISO 15189:2012 in areas of operations as targeted interventions using reasonably practical references to achieve an acceptable level of conformance.

Key words: clinical competence, conformity, ISO 15189:2012, quality control, quality improvement, total quality management.

N Z J Med Lab Sci 2019; 73: 111-115

INTRODUCTION

The implementation of a relevant management system standard to support the delivery of pathology services is a common strategy for medical laboratories to maintain their technical competencies. The International Organization for Standardization (ISO) (1,pp.152-153, 2,pp.1874-1875) has been supporting such a strategy by collaborating with key international organisations, including the International Electrotechnical Commission (1,pp.138-139, 2,pp.1715-1716) and the International Telecommunication Union (2,pp.2001-2002), to produce relevant management system standards for implementation by the pathology services industry. The third edition of the ISO 15189 management system standard, ISO 15189:2012 entitled 'Medical laboratories - Requirements for Quality and Competence' (3), produced by the ISO remains the preferred management system standard in the pathology services industry for the provision of requirements against which the medical laboratory can claim conformance and achieve accreditation by an accreditation body (4).

ISO 15189:2012 can be represented using the ISO 15189:2012 process-based quality management system model (5) and one of the major processes is the 'strategic management' stage that comprises subclauses from both Clauses 4 (Management requirements) and 5 (Technical requirements) of ISO 15189:2012 (3,pp.6-39), and it has been determined that the strategic management stage of ISO 15189:2012 contains 19/119 (16%) administrative requirements (6) and 399/1515 (26%) conformance requirements (CRs) (5). The strategic management stage plays a significant role for the medical laboratory, especially in supporting the laboratory management to craft and execute strategic decisions (7), therefore management considerations need to be included to ensure long-term objectives and plans can add value to the medical laboratory operational processes.

The main challenge of the implementation of the subclause relating to the strategic management stage requires the medical laboratory to turn strategic activities into actual practices (8) while gaining and sustaining organisational competitive advantages (9,10). The updating process requires continual consideration in order to keep current regulatory and statutory requirements fulfilled in alignment with the organisation's strategic maturity (11). Nevertheless, the implementation effort is a continuous search for moving targets in continuous space (12,pp.121-153).

This paper provides an update on international standards and guidance documents associated with the strategic management stage of ISO 15189:2012 at the application level in the areas of interest and highlights areas of concern that may require reasonably practicable effort for the fulfilment of CRs. This update should be used in conjunction with the previously published 'ISO 15189:2012 implementation: an update of related international standards and guidance documents for medical laboratory quality management in the *New Zealand Journal of Medical Laboratory Science* (13). Overall, this update provides relevant information for medical laboratories on the implementation of subclauses relating to the strategic management stage of ISO 15189:2012.

MATERIALS AND METHODS

Selection criteria of international organisations

International organisations that were accepted by the Union of International Associations as either Type A, B, C, D, E or F (2,pp.xiv-xx) and published in 'Yearbook of international organizations 2018 - 2019: guide to global civil society networks' (2) were selected for inclusion.

Selection of recommended guidance documents associated with the strategic management stage of ISO 15189:2012

This update focused on the strategic management stage of ISO 15189:2012 published by the ISO. The subclauses of interest in the strategic management stage were Subclauses 4.1 (Organization and management responsibility) (3,pp.6-9), 4.2

(Quality management system) (3,pp.9-10), 4.3 (Document control) (3,pp.10-11), 4.4 (Service agreements) (3,pp.11-12), 4.13 (Control of records) (3,pp.15-16) and 4.15 (Management review) (3,pp.18-19) of ISO 15189:2012. Selected international organisations that have guidance documents that could provide reasonable support to the subclauses of interest were selected for inclusion.

Selection of relevant literature associated with the strategic management stage of ISO 15189:2012

This update provides additional resources associated with the strategic management stage of ISO 15189:2012. Relevant literature was screened and selected for inclusion. Literature listed in the previous update (13) is not included in this update.

RESULTS

Selected international organisations providing relevant guidance documents

This update includes additional international organisations (*n* = 6) that provide relevant guidance documents for the implementation of the strategic management stage of ISO 15189:2012 (Table 1).The full list of international organisations (*n* = 14), including the additional ones (*n* = 6), is listed in the supplementary section (Table S1).

Recommended guidance documents associated with the strategic management stage of ISO 15189:2012

This update has additional guidance documents (*n* = 47) to provide relevant information for the implementation of the strategic management stage of ISO 15189:2012 (Table 2). Recommended guidance documents were identified and classified in relation to relevant ISO 15189:2012 subclauses (Table S2).

Table 1. Additional international organisations providing relevant guidance documents in support of the implementation of strategic management stage of ISO 15189:2012.

Organisations	Classification (Type A to Type E)	References
CEN	D	2,p.902
IARC	E	2,p.1503
ICNIRP	D	2,p.1641
ISSA	B	2,p.1921
UN	A	2,pp.2658-2661
WHO/Europe	E	2,p.2739

CEN: European Committee for Standardization; IARC: International Agency for Research on Cancer; ICNIRP: International Commission on Non-Ionizing Radiation Protection; ISSA: International Social Security Association; UN: United Nations; WHO/Europe: WHO Regional Office for Europe.

Descriptions (2,pp.xiv-xx)**:**

Type A: federation of international organisations: comprises ≥ three international organisations; management and policy-making organisations reflect a well-balanced geographical distribution.

Type B: universal membership organisation: comprises either ≥ 60 countries or ≥ 30 countries in ≥ two continents with a well-balanced geographical distribution; management and policy-making organisations reflect a well-balanced geographical distribution.

Type D: regionally defined membership organisation: comprises ≥ three countries within one continental or sub-continental region; management and policy-making organisations reflect a well-balanced geographical distribution.

Type E: organisation emanating from places, persons or other bodies: no criteria for membership; reference to, and to some degree limited by, another international organisation, or a person, or a place.

Table 2. Additional relevant guidance documents associated with the strategic management stage of ISO 15189:2012.

Subclauses	Organisations	References
4.1.1.3	ISO	**ISO 37001:2016** Anti-bribery management systems — Requirements with guidance for use
4.1.1.4 e)	CEN	**CWA 15793:2011** Laboratory biorisk management **CWA 16393:2012** Laboratory biorisk management – Guidelines for the implementation of CWA 15793:2008 **EN 166:2001** Personal eye protection — Specifications **EN 407:2004** Protective gloves against thermal risks (heat and/or fire) **EN 420:2003+A1:2009** Protective gloves — General requirements and test methods **EN 421:2010** Protective gloves against ionizing radiation and radioactive contamination **EN 511:2006** Protective gloves against cold **EN 840-6:2012** Mobile waste and recycling containers — Part 6: safety and health requirements

4.1.1.4 e)	IARC	Arsenic, metals, fibres, and dusts Chemical agents and related occupations Non-ionizing radiation, Part 1: static and extremely low-frequency (ELF) electric and magnetic fields Non-ionizing radiation, Part 2: radiofrequency electromagnetic fields Radiation
	ICNIRP	Guidelines for limiting exposure to electric fields induced by movement of the human body in a static magnetic field and by time-varying magnetic fields below 1 Hz *Health Phys* Guidelines for limiting exposure to time-varying electric and magnetic fields (1 Hz to 100 kHz) *Health Phys* Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz) *Health Phys* Guidelines on limits of exposure to broad-band incoherent optical radiation (0.38 to 3 μm) *Health Phys* Guidelines on limits of exposure to static magnetic fields *Health Phys* Guidelines on limits of exposure to ultraviolet radiation of wavelengths between 180 nm and 400 nm (incoherent optical radiation) *Health Phys* ICNIRP guidelines on limits of exposure to incoherent visible and infrared radiation *Health Phys* ICNIRP guidelines on limits of exposure to laser radiation of wavelengths between 180 nm and 1,000 mm *Health Phys* Revision of guidelines on limits of exposure to laser radiation of wavelengths between 400 nm and 1.4 μm *Health Phys*
	IEC	**IEC 60825-1:2014** Safety of laser products – Part 1: equipment classification and requirements **IEC 61010-1:2010** Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: general requirements **IEC 61010-1:2010/COR1:2011** Corrigendum 1 – Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: general requirements **IEC 61010-1:2010/COR2:2013** Corrigendum 2 – Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: general requirements **IEC 61010-1:2010/AMD1:2016** Amendment 1 – Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: general requirements
	ISO	**ISO 11014:2009** Safety data sheet for chemical products — Content and order of sections **ISO 15190:2019** Medical laboratories — Requirements for safety **ISO 30061:2007** Emergency lighting **ISO 45001:2018** Occupational health and safety management systems — Requirements with guidance for use
	ISSA	ISSA guidelines on prevention of occupational risks
	UN	Globally harmonized system of classification and labelling of chemicals (GHS)
	WHO	Electromagnetic fields (400 Hz to 300 GHz) Extremely low frequency fields Guidance on regulations for the transport of infectious substances 2017—2018 International minimum requirements for health protection in the workplace Lasers and optical radiation Magnetic fields Noise Radiofrequency and microwaves Static fields Ultraviolet radiation
	WHO/Europe	WHO guidelines for indoor air quality: dampness and mould WHO guidelines for indoor air quality: selected pollutants

4.2	ISO	**ISO 9004:2018** Quality management — Quality of an organization — Guidance to achieve sustained success
4.13	ISO	**ISO 30301:2019** Information and documentation — Management systems for records — Requirements

CEN: European Committee for Standardization; IARC: International Agency for Research on Cancer; ICNIRP: International Commission on Non-Ionizing Radiation Protection; IEC: International Electrotechnical Commission; ISO: International Organization for Standardization; IFCC: International Federation of Clinical Chemistry and Laboratory Medicine; ISSA: International Social Security Association; UN: United Nations; WHO: World Health Organization; WHO/Europe: WHO Regional Office for Europe.

Relevant literature associated with the strategic management stage of ISO 15189:2012

Additional references (*n* = 49) that were found to provide further relevant information for the implementation of the strategic management stage of ISO 15189:2012 were identified (Table S3).

DISCUSSION

Strategic management stage of ISO 15189:2012

The strategic management stage of ISO 15189:2012 comprises Subclauses 4.1, 4.2, 4.3, 4.4, 4.13 and 4.15 of ISO 15189:2012, containing 399/1 515 (26 %) CRs (5). Additional international organisations (*n* = 6) (Table 1) were identified to provide further guidance documents (*n* = 47) (Table 2) to support the implementation. The completed list of selected international organisations (Table S1), recommended guidance documents (Table S2) and additional resources (Table S3) that could support the implementation are presented. It is highly recommended that this update should be used by medical laboratory implementers and internal auditors in conjunction with the previously published update in the *New Zealand Journal of Medical Laboratory Science* (13).

Subclause 4.1 (Organization and management responsibility) of ISO 15189:2012

Subclause 4.1 of ISO 15189:2012 specifies the strategic management implementation aspects of the medical laboratory. Additional guidance documents were identified and the medical laboratory should extract the relevant 'good practice and applicable requirements' for implementation as specified in Subclause 4.1.1.4 e of ISO 15189:2012 (3,p.7).

Implications for implementers: additional resources should be allocated for the control of relevant factors that may affect the safety of medical laboratory personnel, such as electric, magnetic and electromagnetic fields, as appropriate to the areas of operations as specified in Subclause 4.1.1.4 e) of ISO 15189:2012 (3,p.7). More environmental conditions are specified in Subclause 5.2.6 (Facility maintenance and environmental conditions) of ISO 15189:2012 (3,p.23).

Implications for internal auditors: the internal audit process should ensure the medical laboratory is in alignment with all principal good practices and regulatory requirements for ethical conduct arrangements as specified in Subclause 4.1.1.3 d) of ISO 15189:2012 (3,p.6) as well as safety of personnel aspects as specified in Subclause 4.1.1.4 e) of ISO 15189:2012.

Subclause 4.2 (Quality management system) of ISO 15189:2012

Subclause 4.2 of ISO 15189:2012 specifies that the medical laboratory must establish, implement and maintain an effective quality management system to support the medical laboratory service operations and processes.

Implications for implementers: the medical laboratory should establish processes by incorporating requirements from ISO 9004:2018 entitled 'Quality management — Quality of an organization — Guidance to achieve sustained success' (14) to optimise operational effectiveness.

Implications for internal auditors: no additional notes.

Subclause 4.3 (Document control) of ISO 15189:2012

Subclause 4.3 of ISO 15189:2012 specifies that all relevant documented information relating to the medical laboratory quality management system is maintained to provide effective operations.

Implications for implementers: no additional notes.

Implications for internal auditors: no additional notes.

Subclause 4.4 (Service agreements) of ISO 15189:2012

Subclause 4.4 of ISO 15189:2012 specifies that the medical laboratory must manage suitable service agreements relating to the provision of medical laboratory services.

Implications for implementers: the medical laboratory must treat each valid service request as a service agreement as specified in Subclause 4.4.1 (Establishment of service agreements) of ISO 15189:2012 (3,p.11) and determine the timeframe for retaining the agreement required at the completion of each request.

Implications for internal auditors: the internal audit process must check each valid service request is kept in an acceptable format and is retrievable within a reasonable timeframe when requested.

Subclause 4.13 (Control of records) of ISO 15189:2012

Subclause 4.13 of ISO 15189:2012 specifies that the medical laboratory must manage all relevant quality and technical records relating to access, amendment, collection, disposal, identification, indexing, maintenance and storage.

Implications for implementers: the medical laboratory must determine what types of records need to be retained and for how long. Additionally, the retention timeframes must be in alignment with all principal legal and regulatory requirements. It is important to note that the retention requirements vary greatly between locations, such as Australia (15) and the United Arab Emirates (16). The medical laboratory should consider requirements from ISO 30301:2019 entitled 'Information and documentation — Management systems for records — Requirements' (17) while establishing the documented procedure where it is operationally feasible.

Implications for internal auditors: the internal audit process must check carefully that the organisational, national, regional and international requirements pertaining to the particular activity and the nature of the records are competently fulfilled.

Subclause 4.15 (Management review) of ISO 15189:2012

Subclause 4.15 of ISO 15189:2012 specifies that laboratory management must review the medical laboratory quality management system at planned intervals to ensure continual adequacy, effectiveness, suitability and support of patient care.

Implications for implementers: the medical laboratory must ensure the review input information as specified in Subclauses 4.15.2 a) to 4.15.2 o) of ISO 15189:2012 (3,pp.18-19) are provided to the management review process.

Implications for internal auditors: the internal audit process must seek evidence that information in Subclauses 4.15.2 a) to 4.15.2 o) of ISO 15189:2012, including information in Subclauses 4.15.2 a) to 4.15.2 l) of ISO 15189:2012 (3,p.18) referred subclauses, is provided to the management review.

CONCLUSIONS

This update has provided further references to support the implementation of the medical laboratory quality management system, particularly in Subclauses 4.1, 4.2, 4.3, 4.4, 4.13 and 4.15 of ISO 15189:2012. These findings are relevant to both implementers and internal auditors. Considerably more work must be done by the medical laboratory to determine the pertinent content of relevant guidance documents and additional literature with an appropriate level of scientific certainty. In sum, ensuring reasonably practicable implementation of the strategic management stage of ISO 15189:2012 should be a priority for the medical laboratory if the sustainment of organisational competitive advantages becomes relevant for competition in the contemporary marketplace.

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ORIGINAL ARTICLE

Association of the methionine synthase gene polymorphism with recurrent miscarriage in Mazandaran province, Iran

Zahra Asadnejad, Majid Alipour and Seyedeh Elham Norollahi

ABSTRACT

Purpose: Methionine synthase is one of the main regulatory enzymes for homocysteine metabolism. The A2756G polymorphism in the methionine synthase gene has been implicated in recurrent miscarriage risk. The present case-control study was conducted to determine whether there is an association between the methionine synthase A2756G polymorphism and recurrent miscarriage in Mazandaran province, north of Iran.

Methods: In this case-control study, 60 women with a previous history of at least three recurrent miscarriages and 100 healthy women without a history of miscarriage were analysed for methionine synthase A2756G polymorphism using PCR-RFLP method. The results obtained by estimating the genotype of each polymorphism were analysed using Excel software SPSS *v*16. Chi-square test was used to analyse the data.

Results: Twenty-four (40%) and thirty-four women (34%) in the case and control groups respectively were heterozygous for methionine synthase A2756G polymorphism. Additionally, GG (homozygous mutant) genotypes in the case and control groups were five (8%) and two (2%) respectively. Thus, frequencies of mutant GG and AG genotypes for methionine synthase polymorphisms was significantly different between the controls and cases. G allele frequency (28%) in the patient group was higher than the control group (19%). The case group, however, had significantly higher prevalence of the AG and GG genotypes (p=0.002*)* than the control group.

Conclusion: Our study suggests that methionine synthase A2756G polymorphisms are associated with the risk of recurrent miscarriage in the northern Iranian population.

Keywords: methionine synthase, hyperhomocysteinemia, miscarriage, polymorphism, methionine synthase.

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INTRODUCTION

Recurrent miscarriage is classically defined as the loss of three or more consecutive pregnancies before 20 weeks gestation (1). Several key enzymes, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase and methionine synthase reductase, are important in homocysteine metabolism and methylation reactions. Methylenetetrahydrofolate reductase reduces 5,10 methylene-tetrahydrofolate to 5-methyltetrahydrofolate. Methionine synthase requires vitamin B12 (cobalamin) as a coenzyme that uses the methyl group from 5-methyltetrahydrofolate for re-methylation of total homocysteine to methionine (2). Over time, the cobalamin (I) cofactor of methionine synthase is oxidized to form cobalamin (II), leading to inactivation of methionine synthase. Methionine synthase reductase is required for reversion of oxidized cobalamin (II) to CH-3-cobalamin (III) to maintain the activity of methionine synthase (3).

Since the source of vitamin B12 is meat and dairy products, vegetarians are prone to a deficiency of this vitamin (4). The human methionine synthase gene (gene ID: 5448) has been mapped to chromosome 1q43and encoding a product of 1,265 amino acids. Methionine synthase A2756G polymorphism (exon 21) results in substitution of aspartic acid for glycine decreases methionine synthase activity and increases the cellular homocysteine concentration (5). Polymorphism is defined as a heritable DNA alteration occurring in at least 1% of alleles, types with frequencies higher than 10% are considered common polymorphisms.

Single-nucleotide polymorphisms (SNPs) constitute the most frequent type of polymorphisms in human population (6). Previous reports have indicated that methionine synthase gene polymorphism had an effect in increasing total homocysteine concentration (7,8). Another study investigating cardiovascular disease observed that the methionine synthase gene polymorphism was not associated with plasma homocysteine concentration, however the GG phenotype had a four-fold association with the disease cohort (9). Thrombophilia, vascular endothelial cell injury, and toxic effects on the embryo could result from a high level of plasma homocysteine and could result in pregnancy loss (10). To date, no studies have been conducted on the prevalence of polymorphism methionine synthase gene on recurrent miscarriage in pregnant women in the northern Iranian population. In this study, we investigate the relationship between polymorphism A2756G methionine synthase gene and recurrent miscarriage in northern Iran.

MATERIALS AND METHODS

Sampling and DNA extraction

In this study, 60 women referred to the obstetrician with at least three recurrent miscarriages were selected as cases and 100 healthy women without a history of miscarriage as controls. Venous blood samples (5ml) were taken and immediately transferred to a sterile tube containing 50µl of 0.5M EDTA. Patient and volunteer blood samples were collected after informed consent. Following DNA extraction, the tubes containing the blood samples were stored at -20°C. DNA extraction kit from Sinacolon Company (Iran) was used to extract DNA from whole blood. The extracted genomic DNA was stored at -70°C.

PCR-RFLP for detection methionine synthase A 2756G polymorphism

The PCR–RFLP method was used to detect the methionine synthase A 2756G genotype. The transition creates a HaeIII recognition site, which was used for mutation analysis.

The primers used in this study include, F: 5'-GAACTAGAAGACAGAAATTCTCTA-3' and R: 5' -CATGGAAGAATATCAAGATATTAGA-3' as previously described (3). Thermo Cyclic device from Eppendorf (Germany) was used to perform PCR. The PCR was performed with a 25μL reaction mixture con-taining 1μL of the solution containing DNA, 2.5μL of 10x PCR buffer, 0.5μL of 10mM mixture of deoxynucleoside triphosphates, 1.5μL of 50 mM magnesium chloride. 0.2μL of 5U of Taq DNA polymerase, 1μL of each primer (20pmol), and 17.8μL of distilled water. The reactions were performed as follows: initial denaturation at 95°C for 3min, fol-lowed by 35 cycles of denaturation at 94°C for 1min, annealing at 53°C for 1min, extension at 72°C for 1min, and a final extension at 72°C for 5min. The PCR prod-ucts were electrophoresed on a 3% agarose gel and visual-ized with UV transilluminator after ethididium bromide staining. PCR products were digested with HaeIII according to manufactures instruction (Thermo Fisher Scientific, Waltham, MA, USA) and fragments were separated by 3% agarose gel electrophoresis. The substitution of A to G in the methionine synthase 2756 creates a HaeIII recognition site. After digestion of PCR product was created fragments of 159 and 30 bp. The band 30 base pair is not visible in the gel, therefore, a single 189 bp band on the gel indicated that there was no recognition site for HaeIII (AA homozygous), two fragments of 189 and 159 bp was indicator of AG genotype (heterozygous) and a single band 159 bp was indicator of GG genotype (homozygous) (Figure 1).

Data analysis

Data were analysed by Excel software and SPSS version 16. Chi-square test was used to analyse the data. Statistical significance was set at the P 0.05 level.

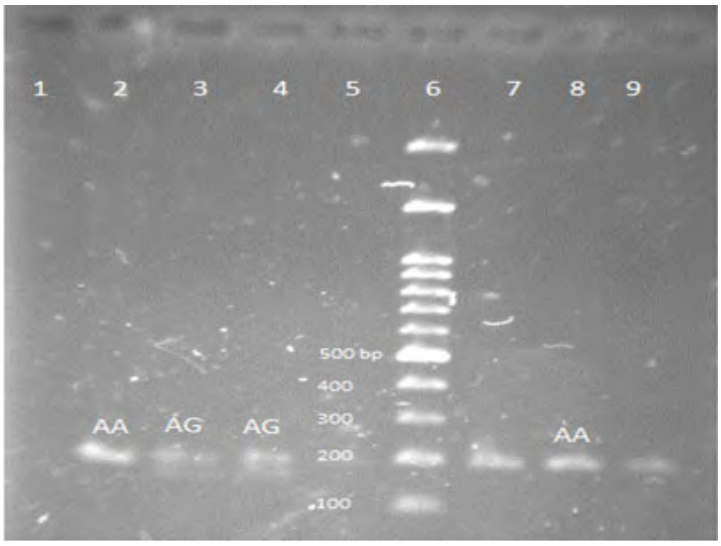

Figure 1. Agarose gel Electrophoresis of digested PCR products. Lane 6: DNA ladder. Lane 1: negative control. Lanes 2,7,8 and 9: AA genotype. Lanes 2 and 3: AG genotype.

Results

Genotype and allele frequencies of the A2756G methionine synthase gene polymorphism are shown in Table 1.

Table 1. Prevalence of genotypic and allelic polymorphism of A2756G methionine synthase gene in case and control individuals.

Genotypes and alleles	Cases number (%)	Controls number (%)
AA	31 (52)	64 (64)
AG	24 (40)	34 (34)
GG	5 (8)	2 (2)
A	72	81
G	28	19

The results of our study showed that the prevalence of GG and AG genotypes among recurrent miscarriage patients is much higher than the controls with a significant difference between the AG and GG genotypes (p=0.002) compared to the control group.

DISCUSSION

Mutation in genes of encoding homocysteine metabolism enzymes, such as methionine synthase, could increase plasma homocysteine levels (11). A polymorphism in the methionine synthase gene A2756G (rs1805087) results in decreased enzyme activity (12). Hyper-homocysteinemia results in endothelial damage and has a direct influence on atherogenesis, activation of coagulation factor V and VII, increased thrombin and platelet aggregation (4,13). Methionine synthase A 2756 G polymorphism is also a maternal risk factor associated with the birth of a child with Down syndrome, recurrent pregnancy loss, and is a risk factor for breast cancer (14-17). In addition, placental thrombosis in pregnancies might be responsible for reduced utero-placental blood flow that can cause spontaneous miscarriages (18). A number of studies have been conducted relating to the role of V A 2756 G polymorphism in spontaneous miscarriage. In the study by Shi *et al*. (19), it was determined that the genetic variant methionine synthase gene may serve as a biological marker of pregnancy loss. In another study it was suggested that the maternal and fetal methionine synthase A 2756 G allele is an important risk factor for the development of uteroplacental insufficiency (20). In our study, the frequency of G allele was (28%) in the recurrent miscarriage group and (19%) in the control group respectively, which is similar to the previous studies mentioned above. The p-value was 0.002, thus the null hypothesis was rejected. Therefore, our study has shown that there is a significant relationship between recurrent miscarriage and methionine synthase A2756 G polymorphism. In the study performed by Madjunkova *et al*. (21), methionine synthase A2756G mutations did not differ between the studied groups, which is different to our study. The variability in different results may indicate that the prevalence of methionine synthase A2756G polymorphism varies in different geographical regions. Based on our study, it can be concluded that methionine synthase A2756G polymorphism plays a role in recurrent miscarriage in Mazandaran province, north of Iran. As methionine synthase is involved in the metabolism of methyl groups the role of epigenesis, including DNA methylation, should be considered (22-31).

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ORIGINAL ARTICLE

Biomarkers of metabolic syndrome in male cigarette smokers in Calabar, Southern Nigeria

Uwem Okon Akpan and Iya Eze Bassey

ABSTRACT

Background: Metabolic syndrome has recently attracted much attention due to increasing knowledge of its relationship with cardiovascular mortality, morbidity and other conditions.

Objective: This study assessed the biomarkers and frequency of metabolic syndrome in adult male smokers.

Methods: 141 apparently healthy male cigarette smokers and 60 apparently healthy non-smokers aged 18 to 65 years were recruited for the study. The smokers were classified as light, moderate or heavy smokers. Anthropometric indices, blood pressure, fasting plasma glucose, lipid profile and serum insulin were measured. Insulin resistance was calculated using homeostasis model assessment of insulin resistance (HOMA-IR). Data was analysed using SPSS version 20.0; $p<0.05$ was considered statistically significant.

Results: The smokers had significantly higher diastolic blood pressure (p=0.0001), Total cholesterol (p=0.008) and LDL-C (p=0.0001) and significantly lower HDL-C (p = 0.0001), compared to the controls. The frequency of smokers with metabolic syndrome was significantly higher than non-smokers using the Adult Treatment Panel III (p=0.032) criteria with dyslipidemia being the most prevalent metabolic risk factor.

Conclusions: The unfavorable changes in the lipid profile and blood pressure observed in this study may predispose smokers to a higher risk of cardiovascular disease and there is a higher frequency of metabolic syndrome among smokers in Calabar compared to the non-smokers.

Keywords: Metabolic syndrome; cigarette smoking; dyslipidemia.

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INTRODUCTION

Tobacco smoking is one of the leading causes of preventable death in the world today and is associated with high morbidity and mortality. Smoking harms almost every organ or tissue in the body and greatly reduces both the quality of life and life expectancy (1).

Tobacco kills more than 7 million people each year. More than 6 million of those deaths occur as a result of direct tobacco use while around 890,000 deaths result from non-smokers exposure to second-hand smoke. This is expected to rise to more than 8 million annually by 2030 (2). Around 80% of the world's 1.1 billion smokers live in low and middle-income countries, including Nigeria (3). According to the 2008 Nigeria Demographic and Health Survey, less than 1% of women aged 15-49 and 11.5% of men aged 15-49 used tobacco products with those who smoked cigarettes constituting 9% (4). By the year 2018, it is expected that about 8.5% of Nigerian males aged 15 years and above will smoke cigarettes with 7.1% daily smokers (2).

Metabolic syndrome may be defined as a collection of inter-related metabolic risk factors that directly increase the risk of coronary heart disease, other forms of cardiovascular atherosclerotic diseases and type 2 diabetes mellitus (5). The components of metabolic syndrome are obesity, insulin resistance, high blood pressure and dyslipidemia but, with the upsurge of new findings, the list keeps increasing. The components now include hyperinsulinemia, insulin resistance, high blood pressure, central obesity and atherogenic dyslipidemia (increase in LDL-C, plasma triglycerides and decrease in HDL-C), endothelial dysfunction, genetic susceptibility, prothrombotic state, pro-inflammatory state and chronic stress (6), but, the diagnosis and recognition of metabolic syndrome depends on the particular criterion used. Many factors, including physical inactivity, obesity, excessive alcohol consumption and unhealthy diet have been identified as important modifiable metabolic syndrome risk factors and its consequences but the mechanisms underlying the onset are not fully elucidated (7).

Presently, the prevalence of metabolic syndrome globally is multiplying and reports from several countries have comparable prevalence rates ranging between 10-20%. This is evident in a study conducted in Hong Kong with a prevalence of 13.1% using the WHO criteria and Asian criterion of BMI (25kg/m^2 and above) and waist circumference (>90cm for men), and 9.6% using the NCEP criteria (8).

In African populations the prevalence of metabolic syndrome ranges from as low as 0% to as high as around 50%, or even greater, depending on the study population and design (9). In a study of adults in semi-urban and rural areas in Enugu state, South-East Nigeria, the metabolic syndrome prevalence was reported to be 18% (10). Another study in Benin, Nigeria using the three diagnostic tools - World Health Organization (WHO), Adult Treatment Panel (ATP III) and International Diabetes Federation (IDF) criteria, revealed a prevalence of 33.4%, 22.6% and 30.9% respectively (11).

The current pattern of increase in the metabolic syndrome prevalence is mostly, but not completely, attributed to embracing a western lifestyle, which is characterised by increased physical inactivity and replacement of the traditional African diet rich in fresh vegetables and fruits for the more calorie-laden foods (12). However, documented studies on metabolic syndrome in Nigerian cigarette smokers are scarce (13).

We therefore assessed the risk of metabolic syndrome and biomarkers of metabolic syndrome in male cigarette smokers and to determine any relationship between cigarette smoking and metabolic syndrome in male cigarette smokers in Calabar.

METHODS

Study area

The study was carried out within the Calabar South and Calabar Municipality Local Government Areas of Cross River State, Nigeria.

Study design/subject selection

A case control study design was used for the study. One hundred and forty-one (141) apparently healthy active male cigarette smokers and sixty (60) apparently healthy non-smokers aged 18 to 65 years were consecutively recruited for this study. They were all residents of Calabar. Each participant was duly informed on the objectives of the study and their informed consent was obtained. Participation was voluntary and confidentiality of participants' data was maintained. A structured questionnaire was administered to obtain information from the participants about their age, family and medical history, and dietary and physical activity and lifestyle. Ethical approval was obtained from the Health Research and Ethics Committee of the Cross River State Ministry of Health, Nigeria.

Inclusion and exclusion criteria

The test participants included males who smoked cigarettes at least once every day for one month or more and were asymptomatic of any disease (apparently healthy). The control participants had never smoked cigarettes in their life. Smokers who were diagnosed of any smoking-related disease (such as lung cancer, coronary heart disease), diabetes mellitus, hypertension, terminal disease or on drugs, were excluded from participation.

Sample collection

Venous blood samples were obtained from participants between 7a.m and 10a.m after an overnight fast. Six milliliters of blood was aseptically collected by venipuncture via the median cubital vein with a well tied tourniquet. Two milliliters were dispensed into a fluoride oxalate bottle for fasting plasma glucose determination while four milliliters were dispensed into a plain bottle and allowed to clot and retract at room temperature for about 45 minutes. After clotting, the blood was centrifuged at 4000 rpm for 5 minutes and the serum extracted was stored frozen at -20 ºC prior to lipid profile and insulin studies. All blood samples were free from haemolysis.

Calculation of sample size

A sample size of 125 was obtained using the formula described by Daniel with a prevalence rate of 9% (14) and 95% confidence limit (15).

Assessment of metabolic syndrome

Metabolic syndrome was assessed using the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) criteria. Metabolic syndrome for men according to the (NCEP-ATP III) criteria is the presence of three or more of the following; fasting plasma glucose level ≥5.6mmol/L, triglycerides level ≥1.7mmol/L, HDL-C level <1.03mmol/L, waist circumference ≥102cm and blood pressure ≥130/85 mmHg (16).

Measurement of blood pressure and anthropometric indices

The measurement of blood pressure was done using a digital blood pressure monitor (Omron Healthcare Ltd, UK). Weight and height of participants was measured in Kg and metres respectively using appropriate equipment. Body mass index (expressed in Kg/m^2) and waist/hip ratio were calculated using appropriate formulae (17,18).

Laboratory analyses

Serum insulin was measured using ELISA kits (Calbiotech Inc., California, USA). Plasma glucose and HDL-C levels were measured using enzymatic colorimetric methods (Giesse Diagnostics, Italy). Total cholesterol and triglycerides were measured using enzymatic colorimetric methods (ELITech Clinical Systems, SAS, France). Very low density lipoprotein-cholesterol (VLDL-C) and low density lipoprotein-cholesterol (LDL-C) were calculated using the Friedewald equation (19). Insulin resistance was determined using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) formula (20).

Smoking pack years

Smoking pack years was calculated as the product of the number of packs of cigarettes smoked in a day and the number of years an individual has smoked. For example, 1 pack year implies smoking 1 pack of cigarettes (20 sticks) per day for one year. Smokers were classified as light smokers (<8 pack years), moderate smokers (8-30 pack years) or heavy smokers (>30 pack years) (21, 22).

Statistical analysis

Data was analysed using Microsoft Excel (MS office 2010) for Windows and Statistical Package for the Social Sciences (SPSS), version 20.0. Student's t-test, Analysis of Variance (ANOVA), Statistics calculator, Pearson's correlation and least significant difference (LSD) post hoc analysis were all employed in data analysis. Statistical significance was set at the p 0.05 level.

RESULTS

Diastolic blood pressure was significantly higher in smokers compared to non-smokers. There was, however, no significant difference in age, body mass index, waist circumference, waist/hip ratio or systolic blood pressure in smokers compared to non-smokers (Table 1).

Total cholesterol and LDL-C levels were significantly higher in the smokers compared to non-smokers while HDL-C was significantly lower in smokers compared to non-smokers. However, fasting plasma glucose, HOMA-IR, triglycerides and VLDL-C levels showed no significant difference between the two groups (Table 2).

Table 3 shows no significant differences in blood pressure or anthropometric indices in smokers based on their smoking pack years. Table 4 shows that there was a significant difference in the levels of fasting plasma glucose, triglycerides, TC and LDL-C among the three categories of smokers. Other parameters showed no significant differences.

Using LSD post-hoc analysis, BMI was significantly lower in heavy smokers than in the light or moderate smokers. Fasting plasma glucose was significantly higher in the light smokers than in the moderate and heavy smokers. Serum triglyceride levels were significantly higher in heavy smokers than in moderate smokers while triglycerides levels were significantly higher in heavy smokers than in light or moderate smokers. Serum LDL-C levels were also significantly higher in heavy smokers compared to light or moderate smokers. However, VLDL-C levels were significantly lower in moderate smokers compared to heavy smokers (Table 5).

Table 6 shows the cardio-metabolic risk factors in smokers and non-smokers. It was observed that dyslipidemia (increased LDL-C levels and decreased HDL-C) was the most predominant risk factor of metabolic syndrome in smokers compared to non-smokers. Additionally, some other risk factors were higher in smokers compared to non-smokers, though not statistically significant.

Table 7 shows the percentage of smokers and non-smokers with metabolic syndrome using the NCEP-ATP III criteria. The percentage of smokers with metabolic syndrome was significantly higher than that of the non-smokers.

Table 1. Age, blood pressure and anthropometric indices in male smokers and non-smokers.

Parameter	Smokers N=141	Non-smokers N=60	P
Age (years)	32.99 ± 9.21	30.87 ± 9.93	0.160
BMI (Kg/m^2)	23.04 ± 4.30	23.79 ± 2.65	0.134
WC (cm)	78.40 ± 8.29	79.65 ± 7.43	0.296
W/H	0.86 ± 0.07	0.85 ± 0.07	0.406
Systolic BP (mmHg)	129.96 ± 15.99	128.65 ± 12.22	0.527
Diastolic BP (mmHg)	82.55 ± 11.97	75.92 ± 9.34	0.0001

Values are expressed as mean ± SD.

BMI=body mass index; WC=waist circumference; W/H=waist/hip ratio; BP=blood pressure.

Table 2. Fasting plasma glucose, insulin, HOMA-IR and lipid profile in smokers and non-smokers.

Parameter	Smokers N=141	Non-smokers N=60	P
Glucose (mmol/L)	4.77 ± 0.96	4.91 ± 1.00	0.349
Insulin (µIU/ml)	1.98 ± 2.18	2.49 ± 1.86	0.092
HOMA-IR	0.42 ± 0.51	0.52 ± 0.40	0.118
TG (mmol/L)	0.99 ± 0.50	0.90 ± 0.36	0.150
TC (mmol/L)	4.68 ± 1.40	4.38 ± 1.03	0.008
HDL-C (mmol/L)	0.53 ± 0.17	1.43 ± 0.38	0.0001
LDL-C (mmol/L)	3.88 ± 1.39	2.67 ± 0.95	0.0001
VLDL-C (mmol/L)	0.45 ± 0.23	0.41 ± 0.17	0.147

Values are expressed as mean ± SD.

HOMA-IR=homeostasis model assessment of insulin resistance; TG=triglycerides; TC-total cholesterol; HDL-C=high density lipoprotein; LDL-C=low density lipoprotein,; VLDL-C=very low density lipoprotein.

Table 3. Blood pressure and anthropometric indices of male smokers based on smoking intensity.

Parameter	Light smokers (<8 pack yrs) N=94	Moderate smokers (8-30 pack yrs) N=27	Heavy smokers (>30 pack yrs) N=20	P
BMI (Kg/m^2)	23.27 ± 4.02	23.83 ± 5.83	21.07 ± 2.36	0.070
WC (cm)	78.84 ± 7.38	79.19 ± 10.51	75.30 ± 8.79	0.192
W/H	0.86 ± 0.07	0.87 ± 0.06	0.85 ± 0.09	0.723
Systolic BP (mmHg)	129.64 ± 15.11	134.33 ± 18.89	125.60 ± 15.21	0.170
Diastolic BP (mmHg)	81.22 ± 11.54	85.33 ± 11.97	85.55 ± 11.97	0.175

Values are expressed as mean ± SD.

BMI=body mass index; WC=waist circumference; W/H=waist-Hip ratio; BP=blood pressure.

Table 4. Fasting plasma glucose, insulin, HOMA-IR and lipid profile in smokers based on smoking pack years.

Parameter	Light smokers (<8 pack yrs) N=94	Moderate smokers (8-30 pack yrs) N=27	Heavy smokers (>30 pack yrs) N=20	P
Glucose (mmol/L)	5.0 ± 0.83	4.4 ± 1.21	4.2 ± 0.82	0.0001
Insulin(µIU/ml)	1.8 ± 2.27	2.1 ± 2.25	2.5 ± 1.57	0.475
HOMA-IR	0.39 ± 0.50	0.45 ± 0.64	0.48 ± 0.37	0.717
TG (mmol/L)	0.99 ± 0.55	0.85 ± 0.36	1.20 ± 0.34	0.054
TC (mmol/L)	4.82 ± 1.49	4.41 ± 0.97	5.65 ± 1.18	0.009
HDL-C (mmol/L)	0.52 ± 0.18	0.56 ± 0.15	0.55 ± 0.17	0.624
LDL-C (mmol/L)	3.86 ± 1.49	3.45 ± 0.97	4.56 ± 1.14	0.023

Values are expressed as mean ± SD.

HOMA-IR=homeostasis model assessment of insulin resistance; TG=triglycerides; TC=total cholesterol; HDL-C=high density lipoprotein; LDL-C=low density lipoprotein; VLDL-C=very low density lipoprotein.

Table 5. Glucose, TG, TC, LDL-C and VLDL-C in light, moderate and heavy smokers using LSD post hoc.

Parameter	Groups		Mean diff.	Std. error	P-value
	Light smokers (n=94)	**Moderate smokers (n=27)**			
BMI	23.23±4.02	23.83±5.83	-0.604	0.927	0.516
Glucose	5.0±0.83	4.4±1.21	0.590	0.199	0.004
TG	0.99±0.55	0.85±0.36	0.141	0.107	0.191
TC	4.82±1.49	4.41±0.97	0.416	0.297	0.164
LDL-C	3.86±1.49	3.45±0.97	0.402	0.296	0.178
VLDL-C	0.45±0.25	0.39±0.17	0.062	0.049	0.207
	Light smokers (n=94)	**Heavy smokers (n=20)**			
BMI	23.23±4.02	21.07±2.36	2.156	1.046	0.041
Glucose	5.0±0.83	4.2±0.82	0.741	0.225	0.001
TG	0.99±0.55	1.20±0.34	-0.213	0.121	0.082
TC	4.82±1.49	5.65±1.18	-0.822	0.335	0.016
LDL-C	3.86±1.49	4.56±1.14	-0.708	0.334	0.036
VLDL-C	0.45±0.25	0.55±0.15	-0.096	0.055	0.084
	Moderate smokers (n=27)	**Heavy smokers (n=20)**			
BMI	23.83±5.83	21.07±2.36	-2.760	1.253	0.029
Glucose	4.4±1.21	4.2±0.82	0.151	0.270	0.576
TG	0.85±0.36	1.20±0.34	-3.354	0.145	0.016
TC	4.41±0.97	5.65±1.18	-1.238	0.402	0.003
LDL-C	3.45±0.97	4.56±1.14	-1.109	0.401	0.006
VLDL-C	0.39±0.17	0.55±0.15	-0.158	0.066	0.018

Table 6. Metabolic abnormalities in male cigarette smokers and non-smokers.

Metabolic abnormalities	Smokers N=141 (%)	Non-smokers N=60 (%)	P
Hypertension[a]	16 (11.3)	3 (5)	0.163
Diabetes[b]	3 (2.1)	1 (1.7)	0.852
High LDL-C[c]	61 (43.3)	5 (8.3)	0.0001
Low HDL-C[d]	139 (98.6)	4 (6.7)	0.0001
Hypertriglyceridemia[e]	14 (9.9)	4 (6.7)	0.468
Central obesity[f]	11 (7.8)	5 (8.3)	0.904
High BMI	8 (5.7)	1 (1.7)	0.212
High Waist circumference	8 (5.7)	4 (6.7)	0.785
Dyslipidemia[g]	139 (98.6)	8 (13.3)	0.0001

a. Defined as blood pressure ≥140/90 mmHg.
b. Defined as fasting plasma glucose ≥7.0mmol/L.
c. Defined as LDL ≥4.0mmol/L.
d. Defined as HDL<0.9mmol/L.
e. Defined as triglycerides ≥1.70mmol/L.
f. Defined as waist circumference ≥94cm and/or BMI ≥30Kg/m^2.
g. Defined as triglycerides ≥1.70mmol/L and/or HDL<0.9mmol/L.

Table 7. Percentage of smokers and non-smokers with metabolic syndrome based on NCEP-ATP III criteria.

Metabolic Syndrome	N	NCEP-ATP III N (%)
Smokers	141	19 (13.5)
Non-smokers	60	2 (3.3)
t-test		2.163
P		0.032*

DISCUSSION

Cigarette smoking could predispose an individual to a variety of diseases, including metabolic syndrome (23). The findings from this study showed that the smokers and non-smokers have a comparable BMI with values within the normal range (18-25Kg/m^2) (24). This observation agrees with a study in Delta State, Nigeria which reported that smokers have a comparable BMI with non-smokers (13). However, while a study by Zbikowski *et al.* reported no significant association between smoking status and BMI (25), another study reported that BMI was significantly lower in smokers than in non-smokers (26). Our study showed a negative and significant relationship between smoking pack years and BMI. This implies that with increase in smoking pack years, there is loss in weight. This may be due to the adverse effects of smoking on food consumption such as loss of appetite, increased olfactory and gustatory receptor insensitivity, increase in energy expenditure (via cortisol enhanced lipolysis) and increase in metabolic rate (27). This finding is in line with a study by Pragti and Sunil (28).

Fasting plasma glucose, insulin and HOMA-IR were comparable in both smokers and non-smokers. The association between cigarette smoking and blood glucose levels remains controversial. Our observation of fasting blood glucose agrees with the findings of Nakanishi *et al.* (29) but, disagrees with the study of Oli *et al.* (30) who reported a transient increase in plasma glucose concentration in smokers. However, another study reported decreased fasting blood glucose levels in smokers compared to non-smokers (13). Nicotine has been linked to hyperglycemia. In small concentrations, it increases the activity of nicotinic acetylcholine receptors which provokes an increase in catecholamines (epinephrine and norepinephrine) as well as cortisol production. These hormones impair insulin action by stimulating hepatic glycogenolysis and gluconeogenesis leading an increase in plasma glucose levels (31). However, the decrease in plasma glucose levels with increased smoking intensity found in our study could be as a result of poor feeding habits of the smokers and fake satiety usually experienced by cigarette smokers in our community.

In our study, HOMA IR, a measure of insulin resistance, was not associated with smoking status. This is in agreement with a study by Berlin *et al.* (32). It has been observed that metabolic syndrome plays a key role between cigarette smoking and cardiovascular disease (33). This suggestion is mainly based on short-term human laboratory studies (34) and on the observations that smoking may result in reduced blood flow to skeletal muscles (increased peripheral resistance), vascular changes and central obesity, all potentially associated with decreased insulin-mediated glucose uptake and increased insulin insensitivity. However, another study showed that insulin levels and HOMA-IR were significantly higher in cigarette smokers than in non-smokers (29), which is in contrast with the findings of our study. Other studies have reported an increase in insulin resistance and a significant increase in HOMA-IR in cigarette smokers after an hour of smoking (35,36). Dietary lifestyle might contribute to the comparable results observed in both smokers and non-smokers. Future studies are, therefore, necessary to explore specifically the relationship between smoking and insulin resistance in this community.

Diastolic blood pressure was significantly increased in the smokers compared to the non-smokers. This finding is consistent with the observations of others (37). This may be due to the resultant effect of rapid mobilisation of catecholamines by nicotine during smoking, which is accompanied by high blood pressure and increased heart rate (38).

Smoking has been linked to increased synthesis and release of catecholamines, thereby resulting in an upsurge in circulating free fatty acids via lipolysis, which could be responsible for the high triglyceride and LDL-C concentrations previously observed (39). The dyslipidemia observed in our study is in line with findings of others who reported that tobacco smoking is associated with high levels of TG, LDL-C and reduced levels of HDL-C (40,41). However, other investigators reported no significant difference in lipid profile pattern among smokers and non-smokers (42).

The mechanisms through which smoking reduces HDL-C are not completely understood but has been linked to alteration in some important enzymes of lipid transport; by reducing lecithin-cholesterol acyl transferase activity, lowering cholesterol ester transfer protein and hepatic lipase activity (43). High density lipoprotein-cholesterol may also become vulnerable to oxidative changes by cigarette smoke thereby losing its atheroprotective function. Based on smoking pack years, our study demonstrated that triglyceride and LDL levels were associated with increased intensity and duration of smoking. These observations are similar to the findings of Omar *et al.* who reported that high levels of atherogenic lipoproteins, mainly LDL and LDL, in relation to increased smoking intensity most likely result in production of high concentration of oxidized LDL via increased oxidative alterations in the LDL molecule (43,44).

Our study showed a high prevalence of metabolic syndrome among smokers than non-smokers in Calabar using the NCEP-ATP III classification. However, the relationship between smoking status and metabolic syndrome is still controversial. Metabolic syndrome is estimated to affect around 20-25% of the adult population globally (45). The current trend of high metabolic syndrome prevalence is mostly and generally attributed to adoption of sedentary lifestyle characterised by reduced physical activity and replacing the traditional African diet rich in vegetables and fruits with high calorie containing foods (12). Findings from the NHANES III survey indicated that smoking was associated with a high risk of metabolic syndrome in adult males and females when adjusted for modifiable lifestyle factors (46).

In Sub-Saharan Africa and in Nigeria, there is paucity of data on epidemiological characteristics and prevalence of metabolic syndrome in cigarette smokers. In the general population, the first reported metabolic syndrome study in Sub-Saharan Africa was carried out in Cameroon in the mid-90s (47) which found a metabolic syndrome prevalence of 1.3% among men living in urban areas using the IDF classification (9), though, HDL-C concentration was not measured in that study. A similar study carried out in Seychelles found a high metabolic syndrome prevalence which was evident in 25-30% of the study participants (48). In Ethiopia the metabolic syndrome prevalence among working men, based on the NCEP-ATP III and IDF criteria, were 10% and 14% respectively (49). In a study involving adults in semi-urban and rural areas in Enugu state, Nigeria, the metabolic syndrome prevalence was found to be 18% (10). Using the NCEP-ATP III criteria, we found a significant prevalence estimate of metabolic syndrome to be 13.5% in smokers as against 3.3% observed in non-smokers. In the Sokoto state, Nigeria, a 27.36% metabolic syndrome prevalence rate in men (non-smokers) was reported based on the NCEP-ATP III criteria and also identified hypertension and low HDL-C to be the most predominant components of the metabolic syndrome (50). However, our study identified dyslipidemia (high LDL-C and low HDL-C) as the major predominant risk factors of metabolic syndrome. This may be because our study was conducted in an urban area where physical activity is reduced as well as nutrition transition to refined, low fiber and calorie dense meals.

Smokers in the Delta state of Nigeria are at high risk of metabolic syndrome based on some components of the syndrome (dyslipidemia and hypertension) (13). The high prevalence of metabolic syndrome and its components found in our study may be partly due to the epidemiological and

nutritional transition that have occurred in the world, including Sub-Saharan Africa, where lifestyle modifications and behavioral changes, both products of urbanisation and modernization, have occurred (51). The unfavorable changes in the lipid profile and blood pressure observed in our study may predispose smokers to a higher risk of cardiovascular diseases and there is a high frequency of metabolic syndrome among cigarette smokers in Calabar compared to the non-smokers.

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2019/2020 NZIMLS CALENDAR

Dates may be subject to change

DATE	COUNCIL	CONTACT
November	Council Meeting, Rangiora	fran@nzimls.org.nz
March 2020	Council Meeting, Rangiora	fran@nzimls.org.nz
DATE	**SEMINARS**	**CONTACT**
09 November	Mortuary SIG, Henley Room, LabPlus, Auckland City Hospital	jsucich@adhb.govt.nz
09 November	Immunology SIG, Commodore Hotel, Christchurch	rodger.linton@gmail.com

NZIMLS ANNUAL GENERAL MEETING

The Annual General Meeting of the NZIMLS for 2020 will be held in conjunction with the Annual Scientific Meeting at Waipuna Hotel and Conference Centre, Auckland on 13 August 2020

DATE	CONFERENCE	CONTACT
11—14 August 2020	Annual Scientific Meeting Waipuna Hotel and Conference Centre, Auckland	fran@nzimls.org.nz
DATE	**NZIMLS EXAMINATIONS**	**CONTACT**
02 November 2019	QMLT Examinations	fran@nzimls.org.nz

CASE STUDY

Diabetes ketoacidosis - a case study

Vanita Patil and Samarina MA Musaad

ABSTRACT

Uncontrolled hyperglycaemia can lead to diabetic ketoacidosis. It evolves within a short time and if not addressed urgently can lead to serious complications. The purpose of this case study was to interpret laboratory results and raise high risk alert and inform to physician or refer them to appropriate centres for immediate management of the condition.

Key words: diabetic ketoacidosis, Type 1 diabetes, Type 2 diabetes, glycogenesis, gluconeogenesis, glutamic acid decarboxylase antibodies, islet cell autoantibodies.

N Z J Med Lab Sci 2019; 73: 126-129

INTRODUCTION

Diabetic ketoacidosis is a serious acute metabolic condition that is a complication of diabetes (1). It is life threatening yet preventable and results from elevated concentrations of ketone bodies and glucose in blood; a combination decreases blood pH and increases serum osmolality, which has deleterious effects on multiple body organs including the kidneys. Diabetic ketoacidosis occurs more frequently in Type 1 diabetes particularly if triggered by infection, surgery, or trauma but it can also occur in Type 2 diabetes. Timely management of this condition reduces its morbidity and mortality. We present a case of diabetic ketoacidosis in a nine-year old with previously undiagnosed Type 1 diabetes.

Background

Glucose is the primary energy source for the human body and is mostly derived from the breakdown of carbohydrates from diet. Glucose can also be synthesized endogenously from amino acids and triglycerides by the hepatocytes, by gluconeogenesis (2). Excess glucose is converted to glycogen by a process known as glycogenesis and stored in the liver and muscles. The liver glycogen stores are the main source of energy to maintain the blood glucose primarily during periods of fasting. The concentration of glucose and glycogen metabolism are regulated by multiple complex metabolic pathways. Glycogenesis is stimulated by insulin which is a hormone produced by the β-cells of the islets of Langerhans in the pancreas whereby glycogen is converted back to glucose and this process is mainly regulated by glucagon, a hormone produced by the α-cells of the pancreas (2).

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized due to insulin deficiency resulting in hyperglycaemia (2). Insulin deficiency can be quantitative, as is the case in Type 1 diabetes, in which there is loss of pancreatic cells leading to low levels of insulin or no insulin due to autoimmune destruction of insulin secreting cells (2), This differs in Type 2 diabetes in which insulin concentrations may be normal or elevated but there is peripheral resistance to insulin action.

In Type 1 diabetes the islet cells are damaged by autoantibodies to insulin and glutamic acid decarboxylase (GAD). These autoantibodies are detectable years before symptoms of hyperglycaemia develop (2).

The first full description of diabetic ketoacidosis was in 1886 by Julius Dreschfeld, a German pathologist working in the United Kingdom, 35 years before insulin was discovered (3). Insulin deficiency results in a reactive increase in the activity of glucagon, cortisol, catecholamines and growth hormone in response to the inability of the body to utilise glucose (2). Figure 1 summarises pathways involved in the pathogenesis of diabetic ketoacidosis. Diabetic ketoacidosis can occur very quickly and may develop in less than 24 hours (4).

When carbohydrate metabolism is impaired, as is the case in diabetes mellitus or during prolonged starvation, stored fat becomes a source of energy (2). The “counter regulatory hormones” glucagon, cortisol, catecholamines and growth hormone trigger the breakdown of fat (lipolysis), and the release of free fatty acids from adipose tissue. Free fatty acids then are oxidised and produce large quantities of acetyl Co-A. The tricarboxylic acid cycle is a common pathway for oxidation of all food materials. In normal carbohydrate metabolism oxaloacetate produced from the tricarboxylic acid cycle gets condensed enzymatically with acetyl Co-A to produce citrate and enters the citric acid cycle, which is rate limited. In the case of diabetic ketoacidosis the excess acetyl-co-A is diverted into an alternative metabolic pathway and forms acetoacetic acid, β-hydroxybutyric acid and acetone (ketone bodies) with resultant ketonemia and metabolic acidosis. Accumulation of ketoacids increases the anion gap [anion gap= (Na^+) - $(Cl^- + HCO3^-)$], the reference range of which is +/- 12 mmol/L (1). The most important metabolic features of diabetic ketoacidosis are a high anion gap metabolic acidosis, hyperglycaemia and ketonemia (1). Peripheral high glucose leads to glycosuria, osmatic diuresis and loss of electrolytes.

Diabetic ketoacidosis is mostly observed in Type 1 diabetes patients and can be triggered by conditions of stress such as infection, myocardial infarction, stroke, anorexia or inadequate insulin administration and certain medications such as isoproterenol which is often used for treatment of bradyarrhythmia (slow irregular heart rhythm) (4,5). Diabetic ketoacidosis itself is a risk factor for stroke (6). Higher mortality rates, increased incidence of cerebral odema, sepsis, shock and renal failure have been identified in children with diabetic ketoacidosis from developing countries (7). A root cause for such complications and for increased mortality in diabetic ketoacidosis could be delayed diagnosis in children, which is common in these regions (7). Prior to the discovery of insulin, diabetic ketoacidosis was associated with a very high mortality rate. Due to awareness and eventual availability of synthetic insulin, the rate of diabetic ketoacidosis associated mortality is reduced and the incidence of diabetic ketoacidosis associated serious conditions, such as cerebral odema and renal failure, have decreased significantly (0.15%-0.31%) in developed countries (7).

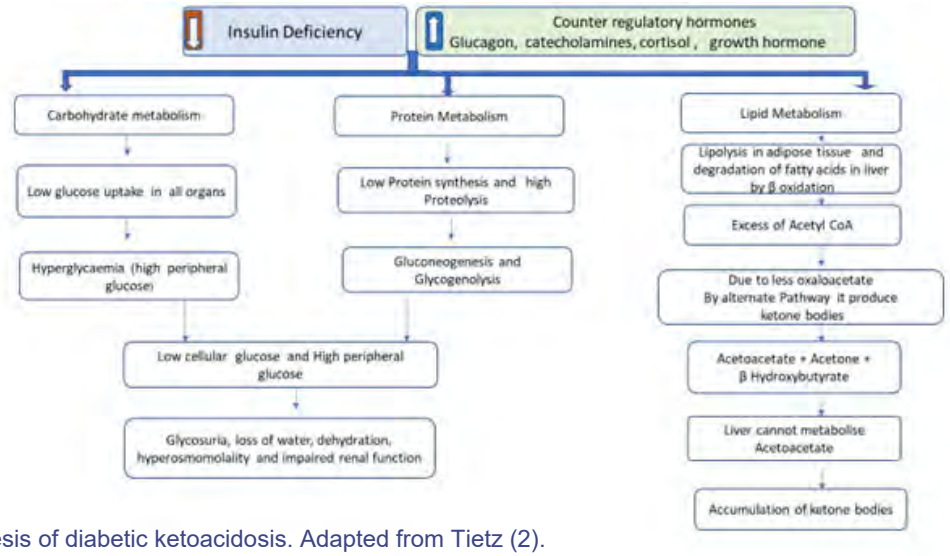

Figure 1. Pathogenesis of diabetic ketoacidosis. Adapted from Tietz (2).

CASE

A nine-year old boy presented to his general practitioner (GP) in the morning with a week-long history of extreme lethargy, polydipsia, and polyuria, particularly at night. The mother had noticed recent weight loss and faster breathing. He had a history of a leg injury one year prior but otherwise no recent illness. On examination he had dry mucous membranes, reduced skin turgor, and Kussmaul breathing (rapid deep breathing with a fruity smell, also called acidotic breathing). There was no known family history of Type 1 diabetes. The GP requested laboratory investigations which are summarised in Table 1.

In the laboratory blood glucose was measured on a Roche COBAS platform by the hexokinase enzyme method. Bicarbonate was a reflex test added because of the elevated glucose as per the laboratory's protocol. It was measured on a Siemen's Advia 2400 platform utilising phosphoenolpyruvate carboxylase and malate dehydrogenase catalysed reactions. Creatinine and electrolytes were also measured on the Siemens Advia 2400 platform. Creatinine was measures by the kinetic Jaffe methodology with rate blanking and intercept correction. (Package insert, Advia Chemistry XPT)

Table 1. Laboratory results.

Analyte	Reference range (Laboratory 1)	Day 1 Lab 1	Day 1 Lab 2	Day 2 Lab 3	Day 6 Lab 3
Sodium mmol/L	135-145	137	137		
Potassium mmol/L	3.5-5.2	4.2	4.5		
Creatinine umol/L	<65	99	115	58	
Bicarbonate umol/L	22-31	12			
Glucose mmol/L	3.5-7.7	32.2	41.9		
β-hydroxy butyrate mmol/L	0.0-0.27		10.3	3.6	
Urea mmol/L	3.2-7.7		6.8	5.2	
Albumin g/L	32-48		46	35	29
Protein g/L	66-84		90		
Globulin g/L	25-41		44		
Bilirubin umol/L	<25		3	4	
GGT U/L	0-60		12		
ALP U/L	80-450		370	261	
ALT U/L	<45		20	10	
Phosphate mmol/L	1.00-1.85			0.35	1.06
Calcium mmol/L	2.1-2.55				2.29
Blood pH	7.30-7.40		6.9		
Haemoglobin g/L	115-145		135		
Red cell count 12e9/L	4.20-5.60		4.96		
Platelets 10e9/L	150--425		439		
White cell count 10e9/L	4.30-12.00		21.40		
Neutrophils 10e9/L	1.5- 7.00		16.95		
HbA1c mmol/mol	<41		111		
Total cholesterol mmol/L	< 5.0		6.3		
Triglycerides mmol/L	<2.0		4.8		
High density lipoprotein mmol/L	>1.0		0.87		
Glutamic Acid Decarboxylase Autoantibodies(GAD) GAD IgG IU/mL	<10				447
Islet cell autoantibodies (IA-2 IgG) IU/mL	<10				>4000

Based on the results and clinical presentation diabetic ketoacidosis was suspected and the patient was referred to hospital. On admission he was alert, had Kussmaul breathing, blood pressure was stable, had dry mucous membranes and reduced skin turgor, chest was clear and abdominal examination showed no organomegaly (enlarged organs such as the liver). Weighing confirmed a 5 Kg weight loss (according to the mother). Further tests were carried out in the hospital which showed a rising glucose and creatinine, elevated β-hydroxybutyrate and low pH and high HbA1c (Table 1).

DISCUSSION

Classic clinical symptoms of diabetic ketoacidosis include polyuria and dehydration, weight loss contributed to by fluid loss, vomiting, abdominal pain, weakness and change in mental status (1). Typical physical signs include loss of skin turgor due to dehydration, Kussmaul breathing due to acidosis, tachycardia, and less commonly seizures (1). Our patient had most of these symptoms and signs.

Diabetic ketoacidosis can be classified as mild, moderate or severe based on the concentration of blood bicarbonate and on mental status (1). A bicarbonate between 15 and 18 mmol/L is classified as mild diabetic ketoacidosis; <15 mmol/L as moderate diabetic ketoacidosis; and <10 mmol/L as severe diabetic ketoacidosis. Our patient had a bicarbonate of less than 12 umol/L which suggested moderate to severe diabetic ketoacidosis (1). Studies show that serum osmolarity and mental alertness correlate (1). Calculated osmolarity in this case in hospital (laboratory 2) was >320 mOsm/kg (reference range: 280-300 mOsm/kg).

Creatinine was elevated in the first day but had decreased by the next day (Table 1). Dehydration was probably the main reason for this with the creatinine normalising after in-hospital rehydration and management. Ketone bodies and high glucose levels are known to falsely elevate creatinine measured by some Jaffe methods (2). However, the manufacturer's package insert makes no reference to interference by glucose or ketone bodies.

Insulin promotes the movement of potassium into cells, therefore in the absence or deficiency of insulin potassium concentrations in the plasma may be within the reference range or elevated in spite of tissue deficiency. However, administration of insulin would facilitate the transfer of potassium back into the cells and may promote hypokalaemia if not done with caution and regular monitoring. In our patient's case potassium was found to be normal when presented to the community laboratory and remained stable after admission. Insulin also promotes the intracellular movement of phosphate and it may have contributed to the low level of phosphate (0.35 mmol/L) on the third day. Our patient also had elevated triglycerides and low high density lipoprotein cholesterol. These findings are consistent with the derangement in lipid metabolism in diabetes mellitus.

Glycated haemoglobin (HbA1c) is formed by condensation of glucose with the N-terminal valine residue of each β-chain of haemoglobin to from a stable compound known as HbA1c. The amount of HbA1c depends on the life span of red blood cells that is an average 120 days. The rate of formation of HbA1c is directly proportional to glucose in the blood and thus HbA1c is a marker of blood glucose in last 120 days (2). In our patient HbA1c was significantly elevated, which indicated longstanding hyperglycaemia.

Our patient had elevated white blood cell, neutrophil and platelet counts (Table 1). These findings cannot exclude the existence of an infection that may have worsened his already deranged glucose metabolism and precipitated diabetic ketoacidosis. On the other hand, diabetic ketoacidosis can in itself increase white cell count (4).

Laboratory results confirmed the presence of glutamic acid decarboxylase antibodies and islet cell autoantibodies which indicates that the patient has Type 1 diabetes. These autoantibodies are associated with the development of Type 1 diabetes.

In New Zealand approximately 4,263 new cases of diabetes (Type 1 and Type 2 diabetes inclusive) were registered in 2017 (8). In the same year, data published by the Australian Institute of Health and Welfare revealed that 9% of diabetics were diagnosed with Type 1 diabetes in Australia (9). Data from three registries and audits from five countries (Austria, Germany, England, Wales, and the Unites States) was analysed for 49,859 individuals less than 18 years old with Type 1 diabetes (10). The rates of diabetic ketoacidosis ranged from 5% to 7.1% (10).

The incidence of diabetic ketoacidosis is higher in developing countries compared to developed countries (7). Admission for diabetic ketoacidosis was 10 times higher for young diabetics (0 - 24 years old) compared to other age groups in developing countries, due to factors like delayed diagnosis of diabetes mellitus and of diabetic ketoacidosis (7). Reasons for a delayed diagnosis of diabetic ketoacidosis include a lack of parental awareness about diabetic symptoms, lack of awareness among physicians, mis-interpretation of diabetic symptoms, lack of finger prick estimation of blood glucose in known diabetics, not recognizing laboratory abnormalities consistent with diabetic ketoacidosis, lack of immediate referral, delay in transport to an appropriate centre or emergency department and delayed referral for specialist care (7). Knowledge and awareness can reduce the incidence of diabetic ketoacidosis and its complications. An example of a successful awareness program exists in Italy. Simple awareness programs in Italian schools and physicians' offices over a five-year period, in the form of posters depicting signs of diabetes, have helped to significantly reduce the occurrence of diabetic ketoacidosis there (7).

Our patient is thriving and put on 7 Kg within a few weeks of management. He has learnt to self-administer insulin and his HbA1c dropped to 55 mmol/mol after 3 months. His growth is within the 95th to 98th percentile range.

Take home messages

- The diagnosis of diabetic ketoacidosis can easily be missed in children due to lack of awareness
- Diabetic ketoacidosis should be considered as a potential cause when a child presents with abdominal pain, a relatively common and potentially vague symptom
- It is prudent on laboratories to implement reflex testing of bicarbonate if blood glucose levels are above a pre-set threshold, particularly in children, to support timely detection of metabolic acidosis and diabetic ketoacidosis.
- It is worthwhile including as much clinical information on request forms to guide scientists and pathologists to interpret critically abnormal results.

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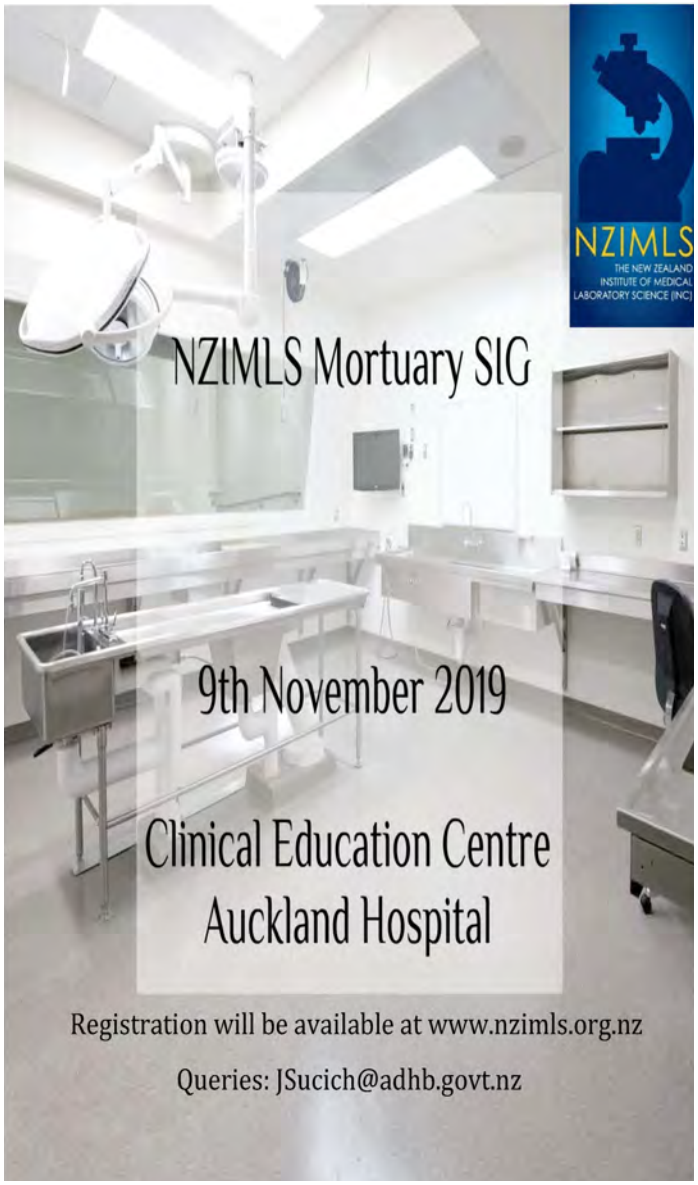

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CASE STUDY

Haemoglobin Reading [α48Leu>Pro; HBA2: c.146T>C]: a comparison of different HbA1c methods in its detection

Beverley Pullon

ABSTRACT

We report a second occurrence of haemoglobin (Hb) Reading [α48Leu>Pro; *HBA2*: c.146T>C], and like the initial case, this was associated with normal haematological parameters. Mass spectrometry showed the variant α-chain was expressed at 21% of the total α- chain material and this was consistent with normal isopropanol stability. The Hb variant was picked up as an interfering component on HbA1c testing using cation-exchange high performance liquid chromatography (HPLC). Variable results returned from an HbA1c quality control survey highlights the difficulty of detecting Hb Reading, demonstrating how this haemoglobin variant may be under reported.

Key words: Haemoglobin Reading, haemoglobin variant, HPLC, capillary electrophoresis, HbA1c.

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INTRODUCTION

There has only been one previous report of Hb Reading, where it was listed as a personal communication on the Hb variant server (1). It was associated with normal blood count parameters but of unknown pathogenicity in a British subject. In this paper we report a second case of Hb Reading.

Glycated haemoglobin A1c (HbA1c) is widely used to monitor long-term glycaemic control in diabetic patients. HbA1c measurements can be performed using different laboratory analysers. There are several methods of HbA1c measurement which can be divided into two major categories; assays based on molecular charge (HPLC, electrophoresis) or assays based on molecular structure or mass (boronate affinity chromatography, immunoassay) (2). The presence of Hb variants are known to cause interference in HbA1c measurement and can result in aberrant HbA1c values. In general, detection and interference depends upon HbA1c method of assay, and the type of Hb variant (3).

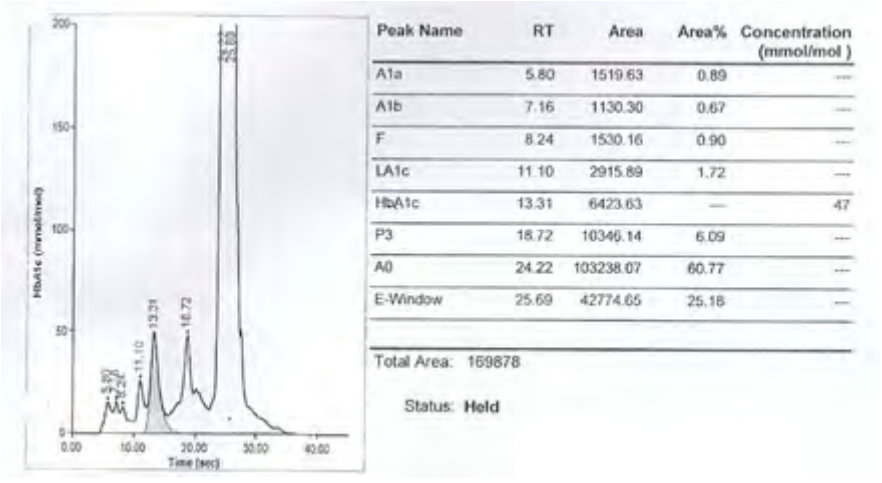
This Hb Reading specimen was sent to laboratories across New Zealand for HbA1c analysis as part of an HbA1c quality control (QC) survey. The aim was twofold: firstly, to see if the Hb variant affected the HbA1c assay with aberrant values, and secondly to compare which HbA1c method/s detected Hb Reading.

CASE STUDY

The subject of this investigation was a 63 year old NZ Maori male. He was at risk of diabetes thus had recurrent HbA1c's with values over previous years ranging between 40 and 47 mmol/mol (reference range <40). Prior to this he had serial fasting glucose measurements which varied between 4.7 and 5.9 mmol/L (normal fasting reference range 3.5-5.4mmol/L). His full blood count was unremarkable: haemoglobin 152 g/L, RBC 5.31 x 10^{12}/L, MCV 89 fl, MCH 29 pg, RDW 12.7%, WBC 8.8 x 10^{9}/L and platelets 262 x 10^{9}/L.

Cation-exchange HPLC for HbA1c testing was undertaken using a Bio-Rad D100 system (Hercules, California, USA) where the Hb variant was picked up as an interfering component. Even though this subject had previous HbA1c tests, this was the first occasion in which an interfering component was detected in his HbA1c testing. Initially, Hb Reading appeared as an abnormal peak of 25.18% eluting in the Hb 'E' window (25.56-27.36) at retention time (RT) 25.69 seconds on the Bio-Rad D100 chromatogram (Figure 1).

A new sample was requested and a week later an abnormal peak of 24.78% eluted in the 'unknown' window (25.33-25.55) at RT 25.49 seconds on the same analyser. The HbA1c values from the two samples were similar at 47 and 46 mmol/mol respectively. The HbA1c (IFCC) result confirmed by affinity method was similar at 45 mmol/mol.



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.80	1519.63	0.89	---
A1b	7.16	1130.30	0.67	---
F	8.24	1530.16	0.90	---
LA1c	11.10	2915.89	1.72	---
HbA1c	13.31	6423.63	---	47
P3	16.72	10346.14	6.09	---
A0	24.22	103238.07	60.77	---
E-Window	25.69	42774.65	25.18	---

Total Area: 169878

Status: **Held**

Figure 1. HbA1c chromatogram with High Performance Liquid Chromatography on a Bio-Rad D100 showing the haemoglobin variant eluting in the 'E'-window.

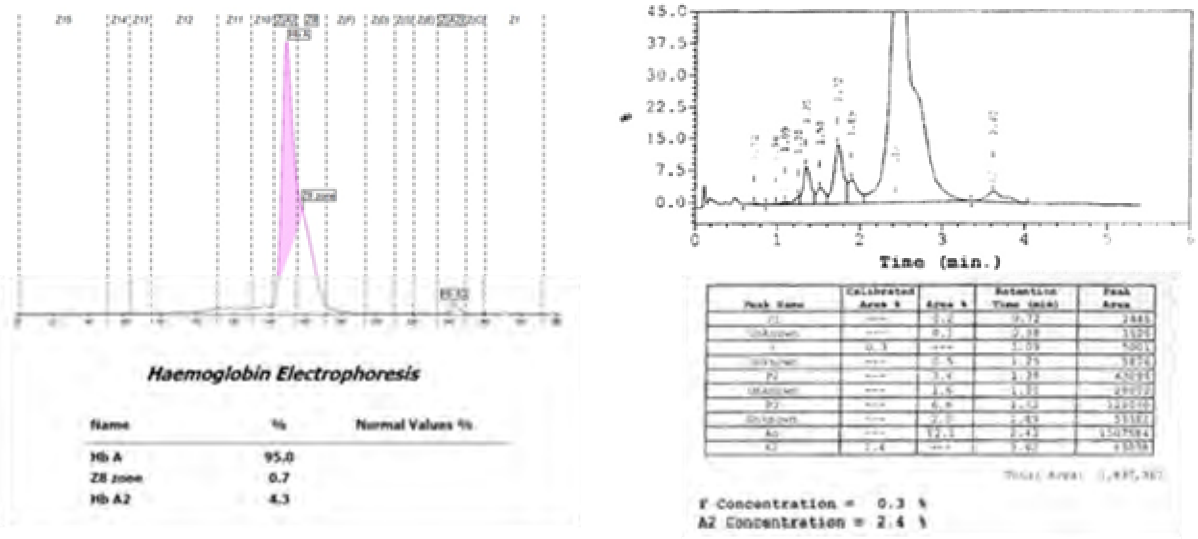


Figure 2. Capillary electrophoresis (A) and Bio-Rad variant II (B) profiles showing the aberrant peak eluting on the downward shoulder of HbA_0 peak.

Capillary electrophoresis was undertaken using a Sebia Capillarys 2 Flex Piercing analyser (Sebia, France). Using the Hb E programme, a slightly elevated HbA_2 of 4.3% (2.2-3.3) and a normal Hb F <1% (<1%) were present. The Hb variant showed as a descending shoulder on the Hb A_0 peak (Figure 2A). Subsequent analysis on a Bio-Rad variant II with a β-Thalassaemia column showed a very obvious shoulder in the descending part of the HbA_0 peak (Figure 2B). The Hb variant did not separate by Hb electrophoresis on cellulose acetate at pH 8.4 and was shown to be stable as the isopropanol flocculation stability test was normal.

Examination of lysate by electrospray mass spectrometry on an Agilent 6230 time-of-flight instrument (4) (Agilent Technologies, Santa Clara, CA,USA) showed a variant a chain with a mass decrease of 16 Da that represented 21% of the total a globin (Figure 3). Further tryptic peptide mapping indicated that the 16 Da decrease in mass was located in peptide α6 (^{41}TYFPHFDLSHGSAQVK^{56}), suggesting possible point mutations of either a42Tyr→Phe, a48Leu→Pro, a49Ser→Ala, or a52Ser→Ala.

DNA sequencing of the coding and non-coding regions of the alpha 1 and 2 globin genes was performed by PCR-based automated fluorescent sequencing (4). Sequencing of the α2 gene showed the presence of a single heterozygous *HBA2*: c.146T>C mutation predicting an α48Leucine→Proline substitution. This mutation (Hb Reading) has been reported only once before as a personal communication (1).

The HbA1c QC survey was processed by 18 laboratories, using a total of 21 analysers comprising of four different HbA1c methods. The methods included: two ion-exchange HPLC methods (D100 (nine) and Variant II Turbo HPLC (one) [Bio-Rad Laboratories, Hercules, California, USA]), two capillary electrophoresis methods (Capillarys 2 Flex Piercing (one), Capillarys 3 Flex Piercing (one) [Sebia, Lisse, France]), one Boronate affinity chromatography method (Primus Premier Hb9210 [Trinity Biotech]) and three immunoassay methods (Cobas 6000 (five), Cobas 8000 (one) [Roche Diagnostics, Indianapolis, IN]), DCA vantage (one) and Dimension Vista 1500 (one) [Siemens Healthineers USA].

The HbA1c results from the QC survey were remarkably consistent between the four different methods employed. The HbA1c values ranged from 39 to 46 mmol/mol (Table 1) with a mean (± SD) of 43 (± 2) mmol/mol. In contrast, from this QC survey data, six out of the 21 analysers detected the aberrant Hb variant. Of the nine Bio-Rad D100's, five detected an aberrant peak, and four of these eluted in the 'E' window. The fifth D100 and the Bio-Rad variant turbo II detected the Hb variant eluting in the 'unknown' window (Table 1).

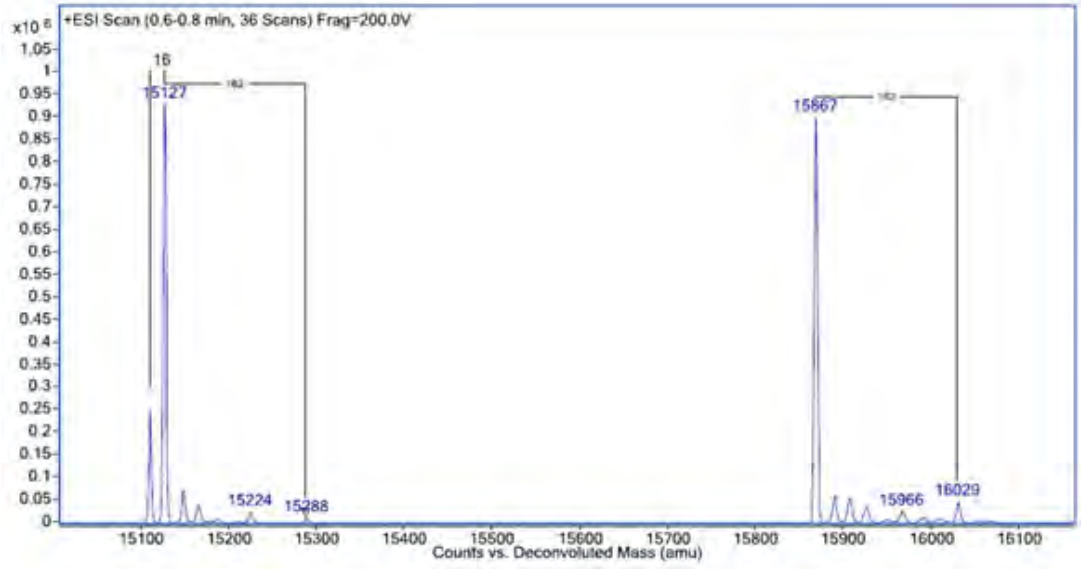


Figure 3. Transformed electrospray mass spectrum of whole lysate from heterozygous carrier of Hb Reading showing normal α and β chains at 15,127 and 15, 867 Da. The new variant a chain at 15,111 Da has a 16 Da decrease in mass and represents 21% of the total a chain material.

Table 1. Summary of HbA1c results and aberrant peak detection on the 21 analysers from the QC HbA1c survey.

HbA1c analyser	Number of analysers	HbA1c results Normal <40 mmol/mol	Hb Reading detected as an aberrant peak	Eluting window of aberrant peak
Bio-Rad D100	9	40,41,43,44,44,45,45,45,46	5	4 in 'E' 1 'unknown'
Bio-Rad Variant II Turbo	1	45	1	1 'unknown'
Sebia Capillarys	2	43,45	0	
Trinity Biotech Primus Hb 9210	1	45	0	
Roche Cobas	6	39,40,42,42,43,44	0	
Siemens DCA Vantage	1	41	0	
Siemens Dimension Vista 1500	1	43	0	

DISCUSSION

Similar to the first documented case of Hb Reading, the present case had no obvious haematological abnormality, a normal full blood count, and normal Hb electrophoresis. Like the first case, this subject also showed the Hb variant as a shoulder on the chromatogram in the descending part of HbA_0. This subject was of NZ Maori descent, unlike the first case that was of British ethnicity. This case verifies that the Hb Reading mutation comprising 21% was on the more highly expressed α2 gene. This cannot be compared to the first case since there was no DNA testing or percentage of variant reported.

There are no previous reports as to whether Hb Reading interferes with HbA1c values, or as to which method of HbA1c (assays based on molecular charge or assays based on molecular structure or mass) can detect the variant. With these aims in mind, the sample containing Hb Reading was sent to 18 laboratories for HbA1c analysis as part of an HbA1c QC survey. Of the 21 analysers across the 18 laboratories, three used assays based on molecular charge: Bio-Rad D100 (HPLC), Bio-Rad Variant II turbo (HPLC) and Sebia Capillary Electrophoresis (Electrophoresis). The other four analysers used assays based on molecular structure or mass: Trinity Biotech Primus Premier Hb9210 (boronate affinity), Roche Cobas, Siemens DCA vantage (immunoassay) and Siemens Dimension Vista 1500 (immunoassay).

Ion-exchange HPLC separates Hb species based on charge differences between HbA1c and other haemoglobins, capillary electrophoresis separates Hb by their electrophoretic mobility according to pH and electro-osmotic flow, boronate affinity assays measure total glycated Hb and immunoassay methods detect structural changes in the N terminal amino acids of Hb using antibodies against the glucose binding sites of β globin chain (5).

Previous studies have documented that the common variants Hb S, Hb C, Hb E, Hb D, as well as other less common or rare variants, can interfere with some HbA1c assay methods (6). It is difficult to study all possible variant haemoglobins with all methods but it is useful to know how the different methods perform with most variants and whether or not the presence of the variant can be detected. In general, Hb variant interference has been shown to be method dependent. If the amino acid substitution causes a change in the net charge of the Hb (as with Haemoglobins S, C, D and E), then it may cause interference with methods such as ion-exchange HPLC or capillary electrophoresis. If there is a substitution at a glycation site, this could alter the rate of glycation and affect certain methods, such as boronate affinity or immunoassays (5).

In this QC study data, there was no significant effect of the method on HbA1c values (Anova test, p=0.09). These findings suggest that Hb Reading does not affect HbA1c values.

However, this is difficult to state categorically without further testing. In contrast, there was significant variation in regards to detection of Hb Reading by the four different HbA1c methods. Previous studies have documented that overall, charge based separation methods have the highest detection rate of Hb variants and derivatives, while assays based on molecular structure or mass have the least detection rate (6). The findings of this QC survey showed similar results. Hb Reading was recognized by two of the assays based on molecular charge, i.e. HPLC (Bio-Rad D100 and Bio-Rad Variant II turbo), through the presence of an abnormal peak, albeit in just over half of the HPLC analysers (6/10). Although the window was different where Hb Reading eluted in, either the 'E' or 'unknown', it was still detected as an aberrant peak. However, this differed in regards to the capillary electrophoresis analysers as none detected the Hb variant. Similar to other studies, all assays based on molecular structure or mass, i.e. Trinity Primus, Roche Cobas, Siemens DCA vantage and Siemens Dimension Vista, did not detect an aberrant Hb. Obviously, detection of this Hb variant by HbA1c analysers is not certain, but is more likely with HPLC.

CONCLUSIONS

The Hb Reading α48Leu>Pro mutation appears to be benign as it is associated with normal red cell indices and normal stability, and the variant α chain is expressed at levels expected for the α2 gene. As shown in this report, Hb Reading does not appear to affect the HbA1c assay, so no apparent difference in HbA1c measurements should be expected. This report highlights the difficulties of detecting Hb Reading with conventional HbA1c methodology. In view of this, the presence of Hb Reading in other subjects is likely to be unrecognised and therefore under reported. However, the detection of Hb variants, especially those affecting HbA1c results are important, given the critical role of HbA1c in the diagnosis and management of diabetes.

ACKNOWLEDGMENTS

We wish to thank Professor Stephen Brennan, Canterbury Health Laboratories, for mutation analysis and the Waikato Hospital Biochemistry Laboratory QC personnel for collection and collation of data from the QC HbA1c survey.

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EDUCATION ARTICLE

An interview with Brandy Gunsolus. First graduate of the Doctorate of Clinical Laboratory Science

Lauren N Eddington

Brandy Gunsolus is the first graduate of the Doctorate of Clinical Laboratory Science (DCLS) programme in the United States, gaining this qualification from Rutgers University in May 2018. This is a relatively new course, available to qualified medical laboratory scientists with a bachelor's degree and at least two years of work experience. It provides the graduate with a generalist laboratory professional doctorate and a more advanced knowledge of laboratory and management subjects. Since then she has been working in Pathology Utilisation and Physician/Laboratory Liaison at Augusta University Health in Atlanta Georgia. I reached out to Brandy by email to learn more about her experiences and her current role, which includes more involvement with clinicians regarding laboratory usage.

Lauren. Hello! Thanks so much for talking to me. First of all, if you can tell me a little about yourself; what is your medical laboratory background?
Brandy. *I initially graduated with a BS degree in Chemistry from Southeastern Louisiana University during a time when the economy was not doing well. The only job I could find was teaching high school science and I quickly realised that it was not for me. I learned about medical laboratory specific education from a neighbour and found a programme that allowed me to complete a 2nd BS degree in CLS in only 15 months. This was a Louisiana State University Health Sciences Center in New Orleans. From there I worked the bench at a large medical center in New Orleans until Hurricane Katrina. I was displaced by the hurricane to North Louisiana and found a position managing a physician office laboratory. There the physicians were able to get to know me and began asking me questions like "what test should I order if I think they have sarcoidosis?" or "how do I interpret this funky test result?" I quickly realised that I did not have enough education to answer the questions they were asking of me. That is when I decided to pursue my master's degree as the DCLS did not exist yet. In my last semester of my master's degree, which I obtained from Rutgers University, the programme director sent me a message stating they had a new degree, the DCLS, explaining what it was and I knew it was so desperately needed. As soon as I graduated from my master's I applied for the DCLS and the rest, as they say, is history.*

Lauren. Why did you decide to do the DCLS programme? Especially as the first person to do it!
Brandy. *I knew from first-hand experience that there was a gap between physicians and the laboratory. Physicians needed help in ordering the right test, at the right time, and interpreting those test results correctly. The DCLS does this and more! The DCLS rounds with patient care teams provided bedside point-of-contact clinical laboratory consultation, aids in diagnosis through Diagnostic Management Teams, develops and monitoring laboratory utilisation programmes, and performs translational research to improve patient outcomes and laboratory service delivery.*

Lauren. What did the programme entail?
Brandy. *I took courses in advanced MLS as well as pharmacology, disease, diagnosis, statistics, clinical correlation, and research. It culminated in a 1-year clinical residency and research project. The DCLS combines chemical, genetic, haematology, immunopathology, and microbiology into one.*

Lauren. Now that you've been qualified for about a year, how have you been able to use your new qualification?
Brandy. *I have developed, implemented, and have been monitoring a system-wide pathology utilisation programme which includes physician education, physician ordering feedback reports, electronic medical record notifications, test algorithm development, and more. I have embarked on a large scale physician education initiative since laboratory medicine has nearly been eliminated from US medical school curriculums. This has been very well received and has made a tremendous impact. The many phone calls, emails, and secure messages I received daily for clinical consultation from physicians has been both great and quite overwhelming. I am so busy that my facility wants to hire more!*

Lauren. What does a usual work day look like for you?
Brandy. *I typically arrive at 0700 and leave around 1700. My day is spent on patient care rounds in the morning, answering consults throughout the day, attending hospital or laboratory quality meetings after lunch, and working on various research/ quality improvement projects in the afternoon. I'm quite busy!*

Lauren: I'm specifically interested in the fact that you attend ward rounds, can you explain how that is an important part of your role?
Brandy: *Much as the clinical pharmacist ensures correct medications and dosages, I ensure correct lab testing, correct timing of testing, and correct interpretation of test results. This reduces both costs and diagnostic errors.*

Lauren. As the first person in this newly developed role you are forging the path for laboratory professionals becoming more integrated into the healthcare team. How has your role been perceived by other hospital staff? And by fellow scientists?
Brandy. *The hospital is thrilled with the results and wants to hire more. The physicians are demanding me to do more and more. They want a DCLS on every patient care team. The negative feedback mainly comes from fellow MLS scientists who did not think the DCLS would be accepted.*

Lauren. What are your thoughts on how the role of the laboratory will develop looking forward?
Brandy. *I think we will see more and more DCLS employed and, much like the clinical pharmacist, see exponential growth as the need is shown and how a DCLS can improve patient outcomes while saving money.*

Lauren. And do you have any words of wisdom for us scientists in New Zealand?
Brandy. *I will leave you with a favourite quote of mine: Knowing is not enough; we must apply. Wishing is not enough; we must do (Johann Wolfgang von Goethe).*

Lauren. A big thank you to Brandy Gunsolus for her time and responses.

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Comment on: An interview with Brandy Gunsolus

Michael Legge

Lauren has provided an insight into the development of the Doctor of Clinical Laboratory Science in the USA with the interview of the first programme graduate. In the previous NZIMLS Journal I provided a brief comparative consideration of how this qualification compared with the RCPA Faculty of Science Fellowship and indicated major points of difference between the two qualifications (1). Reading the interview caused me to consider how the Doctorate might compare with an established programme in the UK, the Physician Associate, which has been running for ten years and is overseen by the Royal College of Physicians. The Masters course is offered by over 30 UK Universities and is a two year full-time programme. Overall the Physician Assistants are an integrated part of a medical team and are responsible for performing medical examinations, ordering and interpreting tests and recommending therapy. The examination is based on a National examination and has a recertification requirement by examination every six years and there is an ongoing CPD requirement. The Faculty of Physician Assistants is part of the Royal College of Physicians where a "managed voluntary register" is maintained and is currently under consideration for Registration by the UK Health and Care Professionals Council.

This programme is part of a wider National Health Service Health Care Practitioners training system, which also provides Masters programmes for Advanced Critical Care Practitioner, Surgical Care Practitioner and Physican's Assistants (Anaesthetics) to work with medical teams. Currently these qualifications are not available in New Zealand.

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OBITUARY

Jim Mann, FNZIMLS
1927—2019

Jim was born in Lower Hutt in 1927 and passed away in Palmerston North in August 2019. I knew Jim as a colleague and friend. He was a long-standing member of the Institute. I first met him in 1964 when I joined the laboratory staff at Palmerston North Hospital. Jim had trained at Waikato Hospital and moved to Palmerston North as charge microbiologist in 1957. It is easy now to forget how different lab work was a generation ago. In the fifties TB was still a major health problem. Concentration and culture of specimens for TB were carried out twice a week. Throat and nasal swabs were still being cultured for diphtheria. Whooping cough was not unusual as this was before nationwide immunisation. Jim introduced staph phage typing which required technical competence and obsessive attention to detail. Jim had both of those attributes.

He described the bulk of the work as routine microbiology. The monitoring of water, milk, cream and ice cream also contributed to the workload. One laboratory assistant was employed full time on media preparation. Jim also had responsibility for the kitchen area where items like petri dishes were autoclaved, rewashed and recycled. Most laboratory material is now discarded after being decontaminated.

Before the age of dipstick, pregnancy testing using live frogs was a responsibility of microbiology. Frenzied chases to catch them when they escaped occurred from time to time. Frogs are almost an endangered species now and frog collecting from the local pond would now not be an acceptable work activity. During Jim's reign, the microbiology department also had the distinction of isolating and identifying a case of cholera, the only time this has occurred in New Zealand I think.

In 1968 Jim joined the New Zealand surgical team at Qui Nhon Hospital in Vietnam. This had a profound effect on him. The possibility of getting malaria, plague, salmonella and working in a war zone was quite frightening. He kept in touch with some of the staff he trained there and at the conclusion of the war he sponsored a couple of Vietnamese to New Zealand. He maintained contact with them until the present day. Over the decade the civilian surgical team was in Vietnam, nine different technologists served about a year each in the laboratory there. In the late 1960's when Jim was there a wider range of tests, including blood transfusion and public health screening, were introduced. Some years after the war several people who had been there before returned for a brief visit.

I am sure that no one could see Jim as a cloth capped union organiser, but during the industrial action of laboratory workers in the 1980's he wrote and distributed material to other laboratories around the country. Of course, there were no faxes or emails then.

He and Dr. Roy Darby had major input into the design of the laboratories in the clinical services block which opened in 1972. At the time this was seen as best practice, a well-lit open plan, this was a dramatic transformation in environment for laboratory staff. He became Principal Technologist that year and an Honorary Member of the NZIMLS in 1987. When he retired he had given 30 years of service to Palmerston North Hospital. I had the honour of succeeding him as Principal Technologist. Jim moved into his house in Rainforth Street in 1964 and the animal house goats, Candy and Anzac, were used to initially tame the section.

Jim was Executive Officer for the Medical Laboratory Science Trust from its establishment in 1987 till it was wound up. With hindsight and in retrospect it was not the greatest year to launch a venture like this with the global financial crisis and share markets crashing. The concept was great, the timing poor.

I want to share some of the comments made by former staff which shows a lot about the man.

- It is the end of an era with the passing of Jim, a kind and caring gentle man.
- A life well-lived, he touched many people in many positive ways.
- He had a wonderful career in medical laboratory science and a well lived life.
- A truly humble gentleman, an old school gentleman who had travelled far and wide.
- From his friend, big Jim in Florida, I will truly miss my best friend and may the railroad in the sky give him peace.
- As a friend, Jim was a lovely human being. He enjoyed entertaining and was a good cook.

For some time, he was a member of the Palmerston North Operatic Society participating in several productions. Jim described himself as a "loco looney", traveling extensively on trains around the world. He was very proud of his Danish heritage with the Danish flag draped on his coffin. Though he never married, he is survived by a number of nieces and nephews.

He was a gentle man and a gentleman.

Contributed by Colvin Campbell. Past President, NZIMLS

Abstracts for the Semester 1, 4th year Otago BMLSc student research projects

Analysis of platelet use over a one-month period at Wellington Hospital

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Objectives: The aim of this study was to analyse patterns of platelet usage in Wellington Hospital (Capital and Coast) and to encourage further study into certain aspects of platelet use. The usage of platelets was also assessed with regard to the New Zealand transfusion guidelines to determine if the indications for platelet use were met.
Methods: Data was collected from Wellington Blood Bank of all platelet issues from the 17th of January 2019 until the 17th of February 2019. Repeat issues where a unit was returned and then reissued to a different patient were discounted, to make the data more reflective of actual platelet use. The pre-transfusion count was assessed with regard to patient condition to determine if the request for a transfusion was appropriate according to the NZ guidelines. A range of other factors were also assessed to give a more holistic view of platelet use.
Results: There were 187 issues of platelets which could be analysed within the specified time period. Twenty-seven units were used for cardiac surgery patients and there was not enough information available to determine if these issues were appropriate. Of the units that could be assessed, 80% were deemed to have been used appropriately. This is consistent with previous studies. The mean platelet increment pre and post transfusion was 12 x10^9/L. Of the platelets used, 48% were given to Haematology patients; 24% to Intensive Care/ Haematology patients and 14% were used for Cardiac Surgery indications. The remaining 14% were used by other departments within the hospital. The study was limited by the fact that not all indications for using platelets could be assessed.
Conclusion: Seventy-nine per cent is a good proportion of appropriately used platelets when factoring in the incomplete analysis of cardiac surgery and some other cases; the true value is likely to be significantly higher.

Clinical stability of K-EDTA plasma ammonia, stored at -20°C and then transported to a central laboratory for analysis

Otto Dove[1] and Sian Horan[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Due to in vitro deamination, the stability of ammonia samples is frequently questioned. Smaller laboratories are often unable to measure ammonia, and instead separate, freeze, and transport samples to a larger laboratory for analysis. The aim of this study was to determine if peripheral laboratory EDTA ammonia samples are clinically stable when they reach the central laboratory.
Methods: A mixture of 25 inpatient and outpatient samples were used to assess the stability of ammonia. Each sample was centrifuged, with the plasma being divided into 7 Hitachi cups. One cup was used to obtain a baseline measurement. The other 6 were stored at -20°C for 24, 48, or 72 hours, after which they were left to stand in an ammonia transportation container for either 1 or 5 hours. Ammonia concentrations were measured on the Cobas c 502 analyser, using an enzymatic method. Bias was calculated by comparing each measurement to the baseline value. Clinical significance was determined by comparing the bias to assay performance criteria established by the Royal College of Pathologists of Australasia.
Results: The average bias did not exceed the allowable limit of performance after 24, 48, and 72 hours of storage at -20°C, for both 1 and 5 hours of transportation. No clear trend existed between the storage time at -20°C and the average bias.
Conclusion: Peripheral laboratory EDTA ammonia samples are clinically stable for up to 72 hours of storage (-20°C) and then, for up to 5 hours of transportation.

Determining the origin of contaminating DNA using QF-PCR: A case report

Jeshyloria Flores[1,2], Kym Drake[2], Kylie Drake[2], Lee-Ann Graham[2], Cheng-Yee Chan[2], and Richard King[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories, Christchurch

Objectives: Genetic testing that cannot be undertaken in New Zealand is referred to overseas reference laboratories. A female sample sent to an international laboratory for whole exome sequencing showed an abnormally large number of variants (n = 46,704 variants) compared to previously observed exome studies ($n \approx 39{,}000$). A large number of variants can be indicative of chimerism, mosaic polyploidy or sample admixture. **Methods:** Chimerism, polyploidy and sample admixture can all be elucidated by quantitative fluorescence- polymerase chain reaction (QF-PCR). QF-PCR was performed by amplification of microsatellite markers using fluorescent-labelled primers, followed by quantitative analysis of the allele peaks on a genetic analyser. A multiplex of 22 primer pairs for loci on each of chromosomes 13, 18, 21, X, and Y was used (Q-STRplusv2, Elucigene, UK).
Results: QF-PCR analysis showed the original DNA sample stored at Canterbury Health Laboratories had a normal chromosome complement, ruling out chimerism or polyploidy as disease mechanisms. Further, the stored DNA was not contaminated, eliminating the DNA extraction process as a source of the potential DNA admixture. The contaminated sample held by the overseas laboratory was repatriated for further analysis, and appeared to have alleles from at least three individuals (of both female and male origin). Sixteen additional samples were sent away to international laboratories the same day this sample was dispatched. These samples were also analysed by QF-PCR and all of them had unique alleles which were not shared with the admixed sample. **Conclusion:** A thorough review of the Sendaway and DNA extraction processes within our laboratory was undertaken and we concluded that sample admixture was unlikely to have occurred at Canterbury Health Laboratories. In the event that a similar case may arise in future, a protocol for investigating potential sample contamination has now been established within our laboratory.

Verifying EUCAST rapid antimicrobial susceptibility testing (RAST) method direct from positive blood cultures

Sarah Grant[1], Gayleen Parslow[2], James Ussher[1, 2] and Simon Dent[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: In November 2018 the European committee on antimicrobial susceptibility testing (EUCAST) released a novel rapid antimicrobial susceptibility testing (RAST) method directly from positive blood culture bottles. The aim of this project was to ratify the RAST method and evaluate the potential for implementation into the laboratory.
Methods: Fifteen patient isolates of *Staphylococcus aureus, Escherichia coli* and *Streptococcus pneumoniae* were inoculated (100–200cfu/mL) into blood culture bottles containing fresh human blood. Bottles were sampled following positive signals from the BD BACTEC system and inoculated onto appropriate media for the disc susceptibility test. Growth inhibition zones were read at 4, 6 and 8 hours with recording fields left blank if zones were unreadable and the retrospective data compared to standard disk diffusion. QC evaluation with 11 staff members and three control microorganisms was also carried out.
Results: The QC evaluated 11 participating staff results, with a passing rate (within the target range) at 4 hours incubation ranging from 47.3-72.7%, 86.4%-100% at 6 hours and 96.6-100% at 8 hours incubation. The categorical agreements for the retrospective study ranged from 35-75% at 4 hours and 90-100% for both 6 and 8 hour incubations, with no very major errors and 5 major errors (2.2%).
Conclusion: The performance of this method improved with longer incubation, with the poor performance of 4-hour incubations partly attributed to the organism growth being too light to accurately read zones. The implementation of this method at 8 hours would provide a lowered turn-around time of 10 hours with the highest degree of accuracy, potentially reducing the likelihood of treatment failure and mortality in sepsis patients. However, careful consideration regarding workflow is recommended before implementation, particularly with the requirement of rapid identification in tandem with this method.

Mismatch repair proteins: A comparison of immunohistochemistry staining quality between Ventanna and Dako manufacturers

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Objectives: The mismatch repair protein panel (MMR panel) is an immunohistochemical staining panel used for the diagnosis of Lynch syndrome. As a disorder characterised by the loss of mismatch repair genes, it is responsible for 15% of colorectal cancer cases worldwide. Cells with malfunctioning mismatch repair genes have an increased chance of proliferating uncontrollably without being able to repair DNA replication errors. The aim of this study was to analyse the staining pattern of two manufacturer's antibodies in the immunohistochemical panel screening for this disorder, Ventanna and Dako.
Methods: Antibodies included in this panel were MLH1, MSH2, MSH6 and PMS2, stained on positive control tissues including tonsil, appendix and colorectal adenocarcinoma. Two slides were assigned to each antibody – a low risk adenocarcinoma with normal MLH1 and PMS2 antigen expression and a high-risk adenocarcinoma with low antigen expression. The quality of the stains was assessed based on intensity of nuclear staining, specificity of staining and presence of sub-optimal staining features. A qualitative score between 1-5 was assigned to each slide from 5 different individuals (pathologists and scientists). **Results:** The results showed that the average score for Dako antibodies was higher than the corresponding Ventanna ones. The difference of average score between Dako and Ventanna MSH2 was low, with n=4.2 and 4.4 for Dako and n=4 and 4.4 for Ventanna. This shows that the staining quality between these 2 antibodies is very similar on average.
Conclusion: The conclusion of the study was that Dako antibodies were superior for MLH1, MSH6 and PMS2, whilst differences in quality in MSH2 was negligible.

An evaluation of the QBC Star dry haematology analyser for point-of-care use

Rafaella Liew[1], Simon Jones[2] and Stephen Robson[2]
[1]University of Otago, Dunedin and [2]Counties Manukau Health Laboratories, Middlemore Hospital, Auckland

Objectives: QBC Star is a point-of-care haematology device designed to give results for nine common blood parameters on a venous or capillary whole blood sample. These include Haematocrit, haemoglobin, total white cell count, MCHC, granulocyte count (absolute and %), lymphocyte + monocyte count (absolute and %) and platelet count. The aim of this study was to assess the comparability between QBC Star and Sysmex XN analysers for eight of these parameters. The effects of platelet clumps, alpha thalassaemia and lipaemia on QBC star performance was also investigated.
Methods: Comparability of the QBC was assessed by comparing 33 K2EDTA samples on both the Sysmex XN analysers and QBC Star, Results were analysed using Bland-Altman and Passing-Bablok statistical analysis.
Results: The results showed that the two methods were comparable for the haematocrit, haemoglobin and platelet parameters. The lymphocyte + monocyte absolute count was not comparable. Two samples with platelet clumps which were run on the XN analysers and subsequently tested on the QBC Star showed normalisation of the platelet count suggesting that the QBC Star was not affected by clumps to the same degree as the XN analysers. Alpha thalassaemia and lipaemia did not appear to affect the comparability of the two analysers. Imprecision for QBC Star was assessed and was adequate for the platelet count. Total white blood cell count precision was above the manufacturer's stated range.
Conclusion: The QBC Star dry haematology analyser provides excellent comparability with the Sysmex XN analysers for the haematocrit, haemoglobin and platelet parameters. It appears to provide a more accurate platelet count in samples with platelet clumps when compared with Sysmex XN analysers. Lipaemia and thalassaemia did not appear to affect comparability of QBC Star results but the sample size was small. Patients requiring an accurate WBC count and differential would benefit from testing on Sysmex XN analysers.

Instrument comparison of the B-type natriuretic peptide (BNP) assay between the Abbot Architect and the Abbot Alinity analysers

Xavier Lozada[1], Heather Hughes[2] and Anne Wilkinson[2]
[1]University of Otago, Dunedin and [2]Canterbury Southern Community Laboratories, Christchurch

Objectives: At Canterbury SCL the immunoassay analyser, Abbot Architect i2000SR was being replaced by an updated immunoassay analyser, Abbot Alinity i-series. The aim of this study was to evaluate the analytical performance of the BNP assay on the Alinity by comparing it against the established instrument, Architect.

Methods: Sixty-three BNP samples were collected 3 weeks before the comparison. A broad range of BNP samples were collected across the clinically significant range of 2.9 - 1000 pmol/L. The samples were stored and frozen. Before the comparison, the samples were thawed at room temperature, vortexed, and centrifuged. The samples were then manually ordered on both instruments and statistical analysis was performed on the Analyse-it programme in Excel.
Results: The Alinity ran BNP controls (3 levels) multiple times and had a coefficient variation (CV) of 3.27 (1st level), 4.76 (2nd level) and 1.46 (3rd level). SCL Architect vs SCL Alinity had a correlation of y = -4.569 + 0.8856 x. The instruments had a mean difference of -20.28 and a standard deviation of 26.58. SCL Alinity and 2 CHL Architect comparisons were performed. The correlation was y = -2.354 + 0.9971 x and y = -5.853 + 1.049. The mean difference was -1.42 and -2.61. The standard deviation was 9.30 and 4.25.
Conclusion: The Architect BNP controls were running erroneously in the lead up to the comparison. The comparison was done knowing that the Architect was producing abnormal controls, to ensure it was not just a QC problem. The statistical analysis proved that not only was it a QC problem, but it also affected patient samples. The pathologist did not sign off the first comparison as the reference method (SCL Architect) was producing erroneous results. The pathologist and the biochemistry head of department decided that we should compare our Alinity BNP assay against two of the Canterbury Health Laboratory (CHL) Architects as a reference method. The comparison was much improved, therefore, the Alinity was authorised by the pathologist for clinical laboratory testing. A few weeks later, Abbot determined that there was an issue with multiple lot numbers of calibrators and controls that were utilised on the SCL Architect during the instrument comparison. Abbot requested an urgent Medical Device Recall for specific lots of the Architect BNP calibrators and controls due to a time dependent, stability drift in patient and control results that has been identified.

Comparison of culture techniques for diagnosis of prosthetic joint infection

Roseanna Olifent[1], Koen Van Der Werff[2] and Maxim Bloomfield[2]
[1]University of Otago, Dunedin and [2] Wellington Southern Community Laboratories

Objectives: The aim of this study was to compare the sensitivity, specificity, and speed of culture techniques for diagnosis of prosthetic joint infection (PJI). Traditional techniques using agar plates and cooked meat broth were compared to an alternative culture technique using blood culture bottles.
Methods: Periprosthetic tissue and fluid specimens were sampled from patients undergoing revision surgery for suspected PJI, and inoculated onto agar plates, into cooked meat broth, and into aerobic and anaerobic blood culture bottles. Media were incubated for a set number of days and monitored for growth, with blood culture bottles incubated in and monitored by the BD BACTEC blood culture system. All microorganism(s) were identified from direct agar plates or from sub-culture onto agar plates by MALDI-TOF-MS. Infectious Diseases Society of America (IDSA) criteria for PJI were used as the gold standard for true infection, to determine the sensitivity and specificity of each media type.
Results: Over the 50-day period, at Wellington Southern Community Laboratories, 17 patients met the study criteria. Seven were determined to have a true infection by IDSA criteria. The sensitivity of blood cultures was 67%, and specificity 86%. The sensitivity of direct plates and cooked meat broth was 58%, and specificity 86%. Blood culture bottles demonstrated a median of two days for microorganism(s) to be observed on agar plates, and the same for direct plates and cooked meat broth.
Conclusion: The blood culture method had increased sensitivity for culture of bacteria causing PJI with no loss of specificity compared to direct plating and cooked meat broth. This method may be attractive to laboratories as it does not require daily reading of multiple plates and manual subculture of enrichment broth.

Effects of storage on spun citrated blood sample prothrombin times and international normalised ratio test results

Constantine Mayo[1] and Robert Allan[2]
[1]University of Otago, Dunedin and [2]Canterbury Southern Community Laboratories, Christchurch

Objectives: The logistics of transporting off-site samples creates unavoidable delays in the testing of these samples that could affect the accuracy and precision of any coagulation test results produced. This study was performed to review the accuracy and precision of delayed coagulation tests. **Methods:** Fifty random citrated blood samples were obtained over a three-week period. Ten INR tests with Innovin were performed on each sample over a period of three days. An initial prothrombin time and international normalised ratio was obtained within 4 hours of sample collection. The samples were stored upright at room temperature from the initial test to the last. Samples were not resuspended but were stored as centrifuged samples. Subsequent delayed results were obtained at 5, 6, 8, 26, 29, 32, 50, 53 and 56 hours after the sample collection time.
Results: The mean INR difference after 56 hours was 0.05 INR units (2.5%) and no INR result exceeded the analytical performance specifications set by the Royal College of Pathologists of Australasia within the three days of testing. Prothrombin times had a few results outside of allowable limits within the lower numbers.
Conclusion: There was no clinically significant change in INR results when testing was delayed for three days if the Royal College of Pathologists of Australasia guidelines were used. However, when expected precision standards set in-house were used for comparison, the results exceeded the limits set by the stricter guidelines.

The use of automated complete blood counts to screen for myelodysplastic syndrome

Isla Revell[1] and Kevin Taylor[2]
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Objectives: Myelodysplastic syndrome involves the proliferation of a clonal myeloid lineage, causing ineffective haematopoiesis (pancytopenia). Recent literature has recommended installing an MDS-CBC screening score on automated haematology analysers, providing a decisive method to stratify patients with high probability of having the disease. The objective of this study was to validate the Myelodysplastic syndrome-complete blood count (MDS-CBC) screening score established in: Boutault R, Peterlin P, Boubaya M, et al. A novel complete blood count-based score to screen for Myelodysplastic syndrome in cytopenic patients. *British Journal of Haematology* 2018; 183: 736–746.
Methods: Retrospective evaluation of 109 samples (patients n=22) diagnosed with myelodysplastic syndrome from January to March 2019 were compared to a 'normal' cohort (n=53). Confidence intervals of 95% from the normal results were used for interpretation. MCV, neutrophil count and Ne-WX values were used to calculate each score; from this, the screens' ability to differentiate patients with myelodysplastic syndrome from normal results was determined.

Results: The MDS-CBC score had a sensitivity of 81% and specificity of 100% regarding the recommended threshold (0.2). High specificity identifies truly negative results in a mixed population, positive scores are probably caused by myelodysplastic syndrome and should be referred for further investigation. Falsely negative scores increase consequently; it is, however, a slow progressing disorder and will likely become positive in time without risk of rapid morbidity/mortality.
Conclusion: The MDS-CBC score is a useful tool when screening for myelodysplastic syndrome against normal samples, prioritizing specific investigation to patients with a higher probability of having the disease. This could improve the rate and efficiency of diagnosis, treatment, and monitoring, thus enhance patient outcome.

Evaluation of the cut off value used in the identification of antibodies to extractable nuclear antigens

Kahla Tyson[1] and Terry Taylor[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories

Objectives: Screening for antibodies to extractable nuclear antigens (ENA) is used to assist in the diagnosis of connective tissue diseases and systemic autoimmune rheumatic diseases. The ENA screen is performed on all patients with an antinuclear antibody (ANA) titre ≥160. Samples above the ENA cut off value of 20 units undergo ENA identification testing with an enzyme immunoassay (EIA). Currently further confirmatory line blot testing is needed if there are no positive antigens detected by the EIA identification. The aim of this investigation was to assess whether ENA screen results between 20 and 50 units with a negative ENA identification EIA will require a confirmation line blot test
Methods: Thirty patient samples were selected, the majority being between the current cut-off of 20 and 50 for the ENA screen. All samples were screened for ENA antibodies with the Bio-Flash chemiluminescence assay followed by the confirmatory Biorad EIA and Euroimmun line blot.
Results: In the confirmatory testing for the samples with an ENA screen value between 20 and 50, there were 15 negatives in both confirmation tests. There were seven confirmation positives and four samples which were negative in the Biorad enzyme immunoassay but positive in the Euroimmun line blot. None of these four positives were of clinical significance.
Conclusion: From this data collected it has been shown that a negative ENA identification EIA with a positive screen between 20 and 50 would not need confirmatory line blot testing.

Verification for qPCR Adenovirus DNA detection in plasma samples

Rachel van Dyk[1], Andrew Strathdee[2] and Trevor Anderson[2]
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Objectives: To ascertain whether the Adenovirus R-gene® commercial kit was suitable for diagnosing Adenovirus disseminating disease and the monitoring of antiviral treatment. Methods: A standard curve was set up, as recommended by the manufacturer, in triplicate to ascertain precision and accuracy for quantitative PCR. External quality control samples from Quality Control for Molecular Diagnostics were run in triplicate to ascertain intra-assay and inter-assay performance. Statistical analysis was performed in R-studio.
Results: Percentage of error of the standard triplicates ranged from -3.2% to 2.4%. Coefficient of variances of the Quality Control for Molecular Diagnostics samples ranged from 0.15 to 2.22%. The standard curve reliably predicted copies/mL of Adenovirus DNA within a sample. Comparison ofour results toother laboratories showed that our results closely matched the laboratories using the Adenovirus R-gene® commercial kit or other commercial kits.
Conclusion: Based upon the results of this study, the Adenovirus R-gene® commercial kit will be suitable for the detection of adenovirus DNA from clinical samples such as plasma and to monitor antiviral treatment related to systemic disseminating disease.

ImmunoCard STAT! EHEC: Improved Screening for Shiga toxin-producing *Escherichia coli* in patient faecal specimens at Canterbury Health Laboratories

Gendi Roberts,[1] Rosie Greenlees and Mary Stevens[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories, Christchurch

Objectives: This study was undertaken to assess the performance of the Immuno*Card* STAT! EHEC (ISE) assay for the improved screening for Shiga toxin-producing *Escherichia coli* (STEC), in patient faecal specimens at Canterbury Health Laboratories.
Method: Retrospective and prospective studies were undertaken, with an overall of 57 tests performed on 42 patient faecal samples. Samples were accepted for testing after visualisation of mauve colonies on CHROMagarTM STEC (STCA) which were identified as *E. coli* by MALDI-TOF. Due to specific laboratory requirements, multiple methods were used. Methods included the manufacturer stated GN (Gram negative) broth and two altered plate culture methods utilising STCA and a blood agar plate (BA). This involved the direct inoculation of specimen onto a STCA plate, where colonies were further sub-cultured on BA for testing. Results were recorded and discrepant samples retested.
Results: Retrospectively, the test performed with a specificity of 100%. However, results varied greatly dependent on whether the GN broth, STCA or BA method was used, with a sensitivity of 33.3%, 75% and 100%, respectively. During the prospective study, the GN broth had a sensitivity of 0% with a specificity of 100%. The BA method performed best with an incomparable result of 100% for both specificity and sensitivity.
Conclusions: It can be concluded that the ISE is a rapid, qualitative, assay which is a clinically beneficial screening test for Shiga toxin producing *E. coli* in patient stool samples. With a specificity of 100% and negative predictive value of 93.3%, this test device accurately reports a negative test result. The test also identifies toxins from a wide variety of serotypes. The BA method was determined to be the most favorable method due to its high sensitivity and specificity. Because of the limited timeframe and number of specimens available, the clinical sensitivity of this test may be greatly underestimated in this study. Further continuation of this study would be valuable.

PorA Opa confirmation test for *Neisseria gonorrhoeae*

Kaitlyn Ustin[1] and Chor Ee Tan[2]
[1]University of Otago, Dunedin and [2]Wellington SCL, Wellington

Objectives: *Neisseria gonorrhoeae* is an oxidase positive, Gram negative diplococcus which causes a sexually transmitted infection, commonly referred to as gonorrhoea. If a sample tests positive and is from a child (≤13-year old) or involved in a medico-legal case, the result must be verified in-house. This is done using the PorA and Opa confirmatory test which currently does not have an incorporated extraction control. The objective was to evaluate the DNA Process Control Kit (Roche) for the

PorA Opa test to provide the assurance that the extraction of the patient's DNA has occurred correctly with no inhibition.
Methods: The Roche LightCycler 480 (LC480) Probe Master (Roche) master-mix was tested against the DNA Extraction Control Kit master mix, to explore which concentration of primer is the most effective, as well as which annealing temperature is the best overall for the three primer concentrations. The best combination of primer and annealing temperature was then tested against the patient samples. A comparison of results was cross referenced with the current LC480 Probe Master. The analytical sensitivity was also compared between the two master mixes.
Results: The study results showed the patient outcomes as well as analytical sensitivity was comparable between the original master mix and the trial master mix.
Conclusion: Test results indicate that a changeover from the LC480 Probe Master to the DNA Process Control Kit will be of equal positive result reliability with the additional security of adding negative result confirmation, and improved sensitivity for low concentrations of the target gene.

Immature platelet fraction as an indicator for platelet recovery in acute myeloid leukaemia patients who received chemotherapy

Jeff Wang[1], Edward Theakston[2] and Farisha Osborne[2]
[1]University of Otago, Dunedin and [2]LabPlus, Auckland

Objectives: Thrombocytopenia is a common feature post extensive chemotherapy, which can lead to a patient being at high risk of bleeding. In terms of internal bleeding prevention, prophylactic platelet packs can be administered to the patient. However, unnecessary platelet transfusion may induce side effects such as alloimmunization and transfusion-induced infections. The aim of this retrospective study was to identify whether the immature platelet fraction (IPF% and IPF#) could be used as a predictive indicator for platelet recovery in post-chemotherapy patients.
Methods: This study focused only on acute myeloid leukaemia patients and compared the IPF values around the point of platelet nadir and platelet recovery. IPF was measured using the PLT-F channel of Sysmex XN20 instruments.
Results: Platelet nadir had a median of IPF% of 0.7% (0.3% to 2.4%) and platelet recovery was 4.2% (2.4% to 9.2%). For IPF#, the median was 0 (0 to 0.2) at platelet nadir and 1.3 (0.8-1.8) at platelet recovery. As this study was performed in a short time frame, the sample population was only six patients with nine cycles of chemotherapy. Hence, this study can only provide an indication of the usefulness of IPF in predicting platelet recovery in post chemotherapy patients, and further prospective studies need to be done to determine true cut-off points for IPF values in patients at platelet nadir and platelet recovery.
Conclusion: This study showed a potential for using IPF as means for predicting platelet recovery in chemotherapy patients.

Validation and comparison of Biomedica's NT-proCNP ELISA kit

Stephanie Watson[1], Jens Peter Gøtze[2] and Peter Dall Mark[2]
[1]University of Otago, Dunedin and [2]Copenhagan University Hospital, Denmark

Objectives: C-type natriuretic peptide (CNP) is an upcoming biomarker of myocardial dysfunction. It is rapidly degraded within the circulation making it hard to detect. Instead a presumably non-bioactive fraction of CNP, NT-proCNP is measured. There are different methods available for the measurement of proCNP. Biomedica's NT-proCNP ELISA kit is a clinical research kit that measures the end terminal of proCNP. The aim of this project was to test the analytical performance of Biomedica's second generation ELISA kit for measurement of NT-proCNP.
Methods: The ELISA kit was validated through the testing of four parameters. These were sensitivity, specificity, precision and accuracy. The kit was also compared to another proCNP method, the processing-independent assay (PIA), via comparison with 254 patient samples to see if there was a correlation between the two methods. Each parameter was assessed using an ELISA immunoassay plate that uses the sandwich principle. The sensitivity was determined via the production of 6 standard curves in order to find the limit of quantification. The specificity was assessed by a cross reactivity study with animal samples. The accuracy was assessed by a serial dilution and a spiking experiment. The precision was calculated by the use of controls present on each plate.
Results: Following the completion of the validation and comparison of the kit, the functional sensitivity was determined to be <10% between the range of 16-128 pmol/l of NT-proCNP. Also, the kit was seen to have accuracy. The precision and comparison aspect failed to show expected results and only a very small correlation was seen between the two methods. The comparison of the ELISA to the PIA showed a modest correlation (R^2 = .01245)
Conclusion: Overall, the ELISA for NT-proCNP measurement performed very well on measures of functional sensitivity, accuracy and precision.

Fragmented red cells (FRC) of the Sysmex XN-3000® cell analyser is a useful parameter for screening thrombocytopenic patients for red cell fragments in blood films

Annabella C Yee[1], Brent R Bishop[2] and Richard Parker[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Red cell fragments (schistocyte) evaluation is an important initial workup for primary thrombotic microangiopathy (TMA) syndromes diagnoses. The lack of standardisation in counting schistocytes by microscopy has prompted the investigation of automated methods. The objective of this study was to determine: (i) a reference interval for schistocytes and; (ii) the usefulness of the fragmented red cell (FRC) parameter of the Sysmex XN-3000® cell analyser in detecting schistocytes during routine haematological screening.
Methods: The FRC reference interval was determined from 51 patients with a normal complete blood count. FRC stability and within-run precision were assessed. FRC-positive samples (50) were analysed for schistocytes using cell analyser and by a standard microscopy method. Blood film results were quality controlled by four qualified medical laboratory scientists.
Results: The FRC reference interval was determined to be 0 to 0.148%. This parameter was stable for up to 24 hours. High Hypo-He resulted in spuriously high FRC (r_s=0.904, P<0.001), therefore samples with Hypo-He > 2% were excluded (n=6). A positive correlation (r_s=0.729, P<0.001) and good agreement (P=0.158) between the two methods were found. At 0.12% FRC cut-off to indicate schistocyte occurrence, the sensitivity and negative predictive value were both at 100%.
Conclusion: FRC may be useful for screening schistocyte occurrence at 0.12% cut-off, but due to its high imprecision at < 1.48% and lack of a true gold standard method, counting on blood films is still recommended.

Acknowledgement: AY conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, wrote the paper. BB provided the project idea. RP provided guidance. Richard Hosking, Katherine Lyon, Caity Dalley and Lauren Eddington have contributed to the skill verification process of schistocyte counting.

Assessment of red cell ABO antibody avidity using chaotropic test methods

Hannah Wong[1], Poppy Zhang[1] and Jim Faed[1,2]
[1]University of Otago, Dunedin and [2]New Zealand Blood Service, Dunedin

Objectives: The purpose of this project was to test blood donor serum/plasma for haemolysins using a chaotropic ion test method and identify cases where chaotropic results do not match the New Zealand Blood Service-assigned haemolysin status of the donor. This test method has been developed by previous BMLSc students as part of an ongoing project.
Methods: Donor serum/plasma samples that had already undergone routine haemolysin screening were tested for haemolysins using chaotropic ion solution phosphate-buffered potassium chloride and A_1B red cells. Samples were determined to be haemolysin positive or negative through grading the agglutination using a 0-12 scale. Grades of 8 or higher signified the presence of haemolysins, while 5 or lower gave the donor a haemolysin negative status.
Results: Of the 394 samples tested, 13.29% of donors given a haemolysin negative status by current New Zealand Blood Service test methods may be haemolysin positive. Conversely 4.98% of haemolysin positive donors gave haemolysin negative results using chaotropic ion methods.
Conclusion: This project found that a significant proportion of donors (8.63%) may be assigned the wrong haemolysin status by current New Zealand Blood Service testing methods. This presents an opportunity for further confirmatory testing and development of the chaotropic ion method, and reason for replacement of the current New Zealand Blood Service testing method.

Comparison of the effect of C reactive protein on activated partial thrombin time using three different reagents

Aysha Willis[1] Rhonda Lucas[2], Brent Bishop[2] and Richard Parker[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: C reactive protein (CRP) is an acute phase protein made by the liver in response to inflammation. CRP has a known affinity for phospholipids. It is this affinity for phospholipids that can prolong activated partial thromboplastin time (APTT) times in a calcium dependant manner. The aim of the study was to compare the effect of elevated CRP has on APTT results using three commonly used reagents.
Methods: Sodium citrated blood samples from 24 patients with elevated levels of CRP and 10 controls (normal CRP) were double centrifuged and platelet-poor plasma was frozen for analysis. Rapidly thawed samples were then tested on a Sysmex CS-2500 automated coagulation analyser using three different APTT reagents in series, Actin FS, TriniCLOT aPTT HS, and Actin FSL. The hypothesis for the study was that there is a significant difference between the reagents APTT times due to the interference of an elevated CRP level (>5mg/L).
Results: When patients with normal and elevated CRP were compared, there was a neglible difference in APTT times for the Actin FS and FSL reagents. However, for TriniCLOT aPTT HS, there was a significant difference ($P < 0.05$). The overall sample size was insufficient for statistical validation.
Conclusion: In this study, elevated CRP affect APTT times when TriniCLOT aPTT HS was used. In view of the small sample size, further investigation is required to confirm this result.

Early antenatal predictors of HDFN-affected fetuses and newborns in pregnant women with anti-D.

Charlotte Wu[1] and Krishna Badami[2]
[1]University of Otago, Dunedin and [2]New Zealand Blood Service, Christchurch

Objectives: Rhesus D incompatibility between mother and fetus is the most common cause of haemolytic disease of the fetus and newborn (HDFN). Managing HDFN can be expensive and invasive. However, there has been little research on determining early antenatal indicators of HDFN-affected fetuses and newborns due to rhesus D incompatibility. The aim of this project was to examine possible early predictive antenatal factors of fetuses and newborns significantly affected by HDFN.
Methods: Antenatal records were accessed from New Zealand Blood Service, Christchurch hospital for pregnant women known to have antenatal immune anti-D between 2008 and 2018. Between the affected pregnancies and unaffected pregnancies, antenatal factors obtained from the mothers and the fetuses or newborns were compared.
Results: Among all the antenatal factors analysed in the 98 pregnancies, fetal R2r phenotype was more frequent in the 23 affected pregnancies than the 75 unaffected pregnancies. Initial anti-D titres were generally higher in the affected pregnancies than the unaffected pregnancies.
Conclusion: It was interesting to find that fetal rhesus phenotype and initial anti-D titre were likely early indicators of severe HDFN. To confirm our findings, this study needs to be repeated on a larger and more complete dataset.

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Thrifty Science: Making the Most of Materials in the History of Experiment

Author: Simon Werrett
Publisher: University of Chicago Press, Chicago, USA, 2019

Simon Werrett is a Senior Lecturer in History at University College, London and has previously written two books relating to historical aspects in science. In his current book "Thrifty Science" he describes how the natural philosophers (the term scientist was not defined until 1833) of the 17th and 18th century conducted their experiments and entered on an age of reuse, recycling and innovative experiments. There are many famous names in the book associated with early discoveries; Francis Bacon, Joseph Banks, Robert Boyle, Margaret Cavendish, Benjamin Franklin, Isaac Newton and so the list goes on. The natural philosophers of their day worked largely in their own homes, utilizing kitchens, cellars and other aspects of their homes as their laboratories, laying the foundations of experimental science as it evolved to modern times. These early (mainly) men experimented with and created new knowledge using everyday utensils, and resources to create new 'natural' knowledge. As well as kitchen utensils, equipment and instruments were made then recycled once the experiments were completed for other experiments. Innovation was a continuing theme of these natural philosophers that often worked by borrowing and gifting equipment. By the 18th century a second hand market existed for equipment and frequently facilitated collaborations as well as attending scientific equipment auctions, often held in fashionable coffee shops.

An underlying concept for the early experimenters was thrift and there were a number of books available during that period expressing frugality linked with "household science". Homes not only served as living space but also doubled as laboratories and storage for scientific equipment. Kitchens were central to the natural philosopher with relatively constant heat where many principles were explored such as distillation, magnetism, medicinal compounds and the observation of the development of 'luminescent' meat while in storage. A home with a cellar was much valued by early anatomists where bodies could be stored and dissections take place. Static electricity was first described when one philosopher observed crackling and sparks when he removed his silk stockings, emphasizing the observational nature of these early researchers.

During this time period communication of experimental results developed with detailed recording of results (often in the kitchen recipe book), scientific meetings and publications. By the end of the 18th century thriving small businesses were evolving of instrument makers, glassmakers and repairers, and the development of business cards. Some activities were more diverse such as the record of shipping 24 tons of "London Urine" to Newcastle for the leather industry and the development of manures from rotting animal carcasses and drained rubbish heap water. As the early 19th century approached, there began the development of dedicated laboratory spaces and permanent instillation of equipment with a focus primarily on chemistry and physics. This began the turning point for "men of science" rather than natural philosophers but the "new" sciences were still a largely male domain. This book provides an excellent insight in to the early days of science, the development of scientific method and the people who helped create many of the basic laws and discoveries we use today.

The Auckland Hospital 'Central' Laboratory 1897-1950

Author: John Buchanan
Publisher: John Buchanan (2019)
ISBN: 978-0-473-48187-2

The author, John Buchanan was a Haematologist at the Auckland Hospital Laboratory where he worked for 34 years, retiring as Head of the Department of Molecular Medicine. Early on in his career the author developed an on-going interest in the history and development of laboratory services in Auckland and the people who were instrumental in developing the facility. This was aided by early written accounts of the pathology laboratory either being available and/or the ability to interview some of the people who worked in the early days of the laboratory and their memories of working with previous colleagues. The book traces not only the history of people and buildings but is an outstanding record of the provision of early pathology services and the knowledge base evolving in the disciplines. Very early in the development of services the primary focus was on 'bacteriology', public health and basic anatomical pathology. Diseases such as typhoid, scarlet fever, syphilis, tuberculosis and meningitis were the norm of the day with the laboratory staff preparing all their own media, reagents and vaccines. Biochemistry started to come into its own with the discovery of insulin and the relationship with blood 'sugar'; although patients were often 'titrated' with insulin use and blood 'sugar' measurements. Haematology gained ground with improved methods and measuring haemoglobin and packed cell volume. The embryonic development of Blood Banking is linked very well with the progressive discovery of blood groups (ABO) and subsequently the Rhesus system. Many of the laboratory staff started as untrained and training was on-the-job, however a limited number of people managed to study for a BSc part-time at night classes. Although there is an element of repetition in the book, this is to be expected when using personal accounts. The book is essentially in three parts, "The History", Picture Gallery' and "Reminiscences" with the latter providing interviews with some of the early laboratory workers including Des Phillips. The author also documents the start of the NZIMLS Journal with a small historical context of the background. Overall, this is a very interesting account of the evolution of pathology services in New Zealand as seen through the development of personalities, skills and knowledge in the early days of diagnostic pathology.

Reviewed by Michael Legge, Deputy-Editor, New Zealand Journal of Medical Laboratory Science

MINUTES OF THE NZIMLS ANNUAL GENERAL MEETING HELD AT THE WAIPUNA HOTEL & CONFERENCE CENTRE, AUCKLAND ON 17 AUGUST 2019 AT 7.35AM

PRESENT

The President presided over approximately 25 members. T Barnett noted that to hold a NZIMLS AGM, 30 members are required to make a quorum. As a number of members who indicated their attendance had not shown, the meeting would be unable to make any motions and/or vote on any issues.

APOLOGIES

Apologies were received from Dianne Webster, Alistair Calvert, Ailsa Bunker and Raylene Sloper

PROXIES

Mary-Ann Janssen has one proxy vote.

MINUTES OF THE AGM HELD

Proposed by T Barnett, seconded John
That the minutes of the Annual General Meeting held on 23 August 2018 be tabled.

Proposed by T Barnett, seconded S Melvin
That the minutes of the Annual General Meeting held on 23 August 2018 be tabled as a true and correct record.

BUSINESS ARISING FROM THE MINUTES

Nil

REMITS AS CIRCULATED

Proposed by T Barnett, seconded S Munroe
"THAT Policy Decision Number 1 be tabled"
Policy Decision No 1 (1971): That all committees and meetings convened under the auspices of the New Zealand Institute of Medical Laboratory Science (Inc) be subject to a standard reference of parliamentary procedure and that this a 'A Guide for Meetings and Organisations' by Renton be tabled.

Proposed by T Barnett, seconded M Legge
"THAT Policy Decision Number 2 be tabled"
Policy Decision No 2 (1989): That all persons wishing to undertake any examination offered by the Institute shall at the time of application and the taking of the examination be financial members of the Institute be tabled.

PRESIDENTS REPORT

Proposed by T Taylor, seconded A Joseph
That the President's Report be tabled.

ANNUAL REPORT

Proposed by T Barnett, seconded S Hemmady
That the Annual Report be tabled.

FINANCIAL REPORT

Proposed by T Barnett, seconded R Hewett
That the Financial Report be tabled.

COUNCIL ELECTIONS:
T Barnett announced that the following were elected unopposed:

President	Terry Taylor
Vice President	Mary-Ann Janssen
Treasurer / Secretary	Tony Barnett
Region 1 Representative:	Sujata Hemmady
Region 2 Representative:	Sean Munroe
Region 4 Representative:	John Sheard
Region 5 Representative:	Sue Melvin

Jane Kendall is elected as the Region 3 Representative.

Ajesh Joseph has been seconded to a new position on Council as a Technician Representative.

HONORARIA
No honoraria to be paid.

AUDITOR
Those present agree that the auditor for 2019/2020 financial year be Nexia New Zealand Limited.

GENERAL BUSINESS

R Siebers, on behalf of the PPTC, thanked the NZIMLS for their annual donation.

2020 Annual Scientific Meeting
Will be held at the Waipuna Hotel & Conference Centre, Auckland 11th – 14th August 2020. The convenor is Tracy Camp from LabPlus.

2021 Annual Scientific Meeting
It is the NZIMLS 75th celebration in 2021 and a tentative venue for this ASM is Wellington.

The meeting closed 7.50am

Fellowship of the NZIMLS

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

Fellowship of the NZIMLS may be gained by thesis, by peer reviewed publications; or by treatise in case of a member holding an appropriate postgraduate or professional qualification.

Fellows may use the nominals FNZIMLS if a current financial member of the Institute.

THESIS

The thesis must be based on the style of Master of Science by thesis requirement of New Zealand universities.

PUBLICATIONS

A minimum of ten peer reviewed publications in international or discipline acknowledged biomedical journals. The candidate must be the 1st or senior author of at least six of these publications. A comprehensive review of the submitted publications is also required.

TREATISE

By submission of a treatise in the form of a dissertation of 3000 5000 words on a medical laboratory science subject. The dissertation may take the form of a review, a scientific study, development of a hypothesis, or any other presentation that meets with the approval of the Fellowship Committee.

Candidates applying for Fellowship by this route must be holders of at least a Master's degree or higher in a medical sciences subject, or have a professional qualification such as Fellowship of the following professional bodies: Australian Institute of Medical Science; Institute of Biomedical Science; Faculty of Science of the Royal College of Pathologists of Australasia, Australiasian Association of Clinical Biochemists; Royal Institute of Biology, London.

For full Fellowship regulations and the application process visit the NZIMLS web site at www.nzimls.org.nz

The current fee for sitting Fellowship is $575.00 (incl. GST)

CURRENT FELLOWS OF THE NZIMLS

Indira Basu
Mark Bevan
Ailsa Bunker
Jennifer Castle
Jan Deroles-Main
Susan Evans
Sheryl Khull
Christine Leaver
Dennis Mok
Maxine Reed
Robert Siebers
Vanessa Thomson
Jacqueline Wright
Sheryl Young
Jenny Bennett
Jillian Broadbent
Julie Creighton
Brett Delahunt (Honorary)
Marilyn Eales
Christine Hickton
Michael Legge
Ron Mackenzie
Howard Potter
Mohammad Shahid
Mary Stevens
Emil Wasef
Rubee Yee

Indira Basu from LabPLus, Auckland and Julie Creighton from Canterbury Health Laboratories have recently been awarded Fellowship of the NZIMLS. Both Indira and Julie gained their Fellowship by the publications route.

Publications by NZIMLS members

This column is to showcase recent peer-reviewed publications by NZIMLS members. If you have had a recent publication please provide full details to the Editor at rob.siebers@otago.ac.nz.

Fitzgerald RP, **Legge M**, Rewi P, Robinson EJ. Excluding indigenous bioethical concerns when regulating frozen embryo storage: an Aotearoa New Zealand case study. *Reproductive Biomedicine & Society Online* 2019; 8: 10-22.

Douglass A, **Legge M**. Regulating surrogacy in New Zealand: Evolving policy and cautious liberalism under the HART Act. In: Perspectives on Commercial Surrogacy in New Zealand: Ethics, Law, Policy and Rights. A Masselot A, R Powell (Editors) 2019 .pp1-24. The Centre for Commercial and Corporate Law, University of Canterbury, New Zealand.

Siebers R, Jones B, Bailey L, Aldridge D, Draper J, Ingham T. Indoor allergen exposure in primary school classrooms in New Zealand. *New Zealand Medical Journal* 2019; 132: 42-47.

Creighton J, Anderson T, Howard J, Dyet K, Ren X, Freeman J. Co-occurrence of mcr-1 and mcr-3 genes in a single Escherichia coli in New Zealand. *Journal of Antimicrobial Chemotherapy* 2019; 74:3113-3116.

Browne AS, Biggs PJ, Wilkinson DA, Cookson AL, Midwinter AC, Bloomfield SJ, Hranac CR, Rogers LE, Marshall JC, Benschop J, Withers H, Hathaway S, **George T**, et al. Use of genomics to investigate historical importation of Shiga toxin-producing Escherichia coli serogroup O26 and nontoxigenic variants into New Zealand. *Emerging Infectious Diseases* 2019; 25: 489-500.

Franz E, Rotariu O, Lopes BS, MacRae M, Bono JL, Laing C, Gannon V, Söderlund R, van Hoek AHAM, Friesema I, French NP, **George T**, et al. Phylogeographic analysis reveals multiple international transmission events have driven the global emergence of Escherichia coli O157:H7. Clinical Infectious Diseases 2018; doi: 10.1093/cid/ciy919. [Epub ahead of print].

Brewer N, Foliaki S, **Bromhead C**, Viliamu-Amusia I, Pelefoti-Gibson L, Jones T, Pearce N, Potter JD, Douwes J. Acceptability of human papillomavirus self-sampling for cervical-cancer screening in under-screened Māori and Pasifika women: a pilot study. New Zealand Medical Journal 2019; 132: 21-31.

Sherman SM, Cohen CR, Denison HJ, **Bromhead C**, Patel H. A survey of knowledge, attitudes and awareness of the human papillomavirus among healthcare professionals across the UK. European Journal of Public Health 2019; pii:ckz113.

Sherman SM, Bartholomew K, Denison HJ, Patel H, Moss EL, Douwes J, **Bromhead C**. Knowledge, attitudes and awareness of the human papillomavirus among health professionals in New Zealand. *PLoS One* 2018; 13: e097648

NAME CHANGE

Taking into consideration a review of NZ Paramedics in terms of regulation and registration, the PPTC Board of Trustees made the decision to seek an alternative to the term "paramedical", which for the last 38 years has appeared in its name title (Pacific Paramedical Training Centre). The importance to retain the acronym "PPTC" was considered an essential factor during the change of name process and therefore the word "Pathology" was considered an appropriate substitute, especially as it is the foundation of our profession. The PPTC is now known as the "Pacific Pathology Training Centre". Its name may have changed but its Terms of Reference will always remain the same, that is" Promoting the advancement of diagnostic pathology knowledge and practice throughout the Asia-Pacific region".

CENTRE BASED TRAINING COURSES

Training courses are held at the Wellington Centre throughout the academic year. Trainees wishing to be accepted must be currently employed in a medical laboratory and have had at least two years' experience before attending. Courses cover all aspects of Medical Laboratory Science. Funding is provided by NZAID, New Zealand Red Cross, Norman Kirk Trust, WHO, PPTC or through the country's Ministry of Health.

Laboratory health & safety; and quality management systems

The above course commenced on 1 July and concluded on 26 July 2019 and four students attended:

- Regina Flood from Kiribati
- Taumate Niu from Samoa
- Crispin De Araujo Afonso from Timor Leste
- Silveiro Aparicio from Timor Leste

Lecturer: Russell Cole

Haematology

This course provided by the PPTC commenced on 5th August and concluded on 13 September at its centre in Wellington. Six students attended:

- Tauli Aperaamo from Samoa
- Caroline Uri from PNG
- Charles Kongs from PNG
- Vanessa Samuelu from Samoa
- Susan Sewen from Vanuatu
- Puaese Falavi from Tuvalu

Lecturer: Phil Wakem. Assisted by Dr Julia Phillips, Elizabeth Tough and Lee Botes

Staff and students. Haematology, 2019.

Microbiology

This course provided by the PPTC commenced on 23 September and concluded on 18 October at its centre in Wellington and11 students attended:

- Mo'unga Tu'inukuafe from Tonga
- Eric Bilo from PNG
- Teweia Toatu from Kiribati
- Lynne Tonar from PNG
- Sanjeshni Autar from Fiji
- Amitesh Prasad from Fiji
- John-Paul Matlam from PNG
- Carolina Da Costa Maia from Timor Leste
- Josefina Domingas Prisca Guterres from Timor Leste
- Ines Imaculada Ximenes Magno from Timor Leste
- Michael Taloifaga from Solomon's

Lecturers: Russell Cole and Navin Karan

Remaining for 2019

Blood Transfusion Science: 4 – 29 November 2019.

Staff and students. Laboratory health & safety; and quality management systems, 2019.

Staff and students. Haematology, 2019.

Staff and Students. Microbiology, 2019.

OVERSEAS TRAVEL: TEACHING AND TRAINING

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

Samoa

29 July – 2 August: Phil Wakem visited the national laboratory in Apia to strengthen haematology in terms of blood cell recognition and blood film interpretation. Dr Vladimir Osipov (Chief Pathologist, SCL Wellington) accompanied Phil to specifically advance the skills of the laboratory's Pathologist Dr Filipina Amosa-Lei Sam in anatomic pathology. Dr Peter Fitzgerald, a very senior and experienced Histo and Cyto Pathologist, and Medical Director for SCL, NZ , who was holidaying in Apia at this time with family, contributed also to Pina's education by committing a great deal of his holiday time viewing cytology slides with Pina in order to extend her skills in cellular identification and disease diagnosis.

Dr Peter Fitzgerald and Dr Vladimir Osipov with Dr Filipina Amosa-Lei Sam

Vanuatu

22 – 26 July: Filipo Faiga made a visit to the National Laboratory in Port Vila to provide technical training to the biochemistry staff including the upskilling of the HOD and senior staff on specific departmental methods, procedures and the general operation of the section.

23 – 27 September: Phil Wakem and Dr Julia Phillips visited the national laboratory in Port Vila to work with both clinical and laboratory staff, strengthening haematology in both the clinical setting and in laboratory diagnosis.

Tonga

15 – 19 July: Phil Wakem visited the national laboratory in Nuku'alofa to strengthen haematology especially in terms of blood cell recognition and blood film interpretation.

14 – 18 October: Vichet Khieng visited the national laboratory in Nuku'alofa Tonga to strengthen biochemistry and install a Sysmex Coagulation CA 560 instrument donated by SCL Kenepuru, Wellington. He was accompanied by Susan Evans the PPTC's Consultant for blood banking who carried out teaching and training in blood banking procedures.

Solomons

12 – 16 August: Vichet Khieng and Navin Karan both visited the national laboratory in Honiara to assess the laboratory's progress towards process improvement in the sections of biochemistry and microbiology as well as offer technical support and advise as required.

CONFERENCES

Noumea (New Caledonia)

8 – 12 July: Vichet Khieng the PPTC's Pacific IT Specialist attended the Pacific Health Information Network meeting on health information and digital health strengthening in the Pacific, the objectives of which were as follows:

- To present global and regional eHealth and digital health resolutions and strategies and its practical application in the Pacific.
- To discuss improvements between CRVS (civil registration and vital statistics) and digital health linkages (based on the CRVS event and health ID landscape analysis).
- To discuss regional data analysis successes and challenges as highlighted in data collection for the Healthy Island Monitoring Framework and other regional reporting obligations.View List

Manila (Philippines)

Sept 9 – 13: Russell Cole, the PPTC's Laboratory Quality Manager attended the WHO Regional Meeting in Manila to Strengthen Public Health Laboratory Systems for Health Security.

This meeting was organized by WHO (Western Pacific Region) to monitor the collective progress and common issues with the implementation of APSED111 (*Asia Pacific Strategy for Emerging Diseases and Public Health Emergencies)* to advance laboratory core capacities and agree on common priority activities for the next two-year period. This meeting was also a forum for discussion and agreement on the way forward for the implementation of the WHO guidance document on the introduction of new technologies in Western Pacific Countries.

A WARM WELCOME

The PPTC is pleased to introduce Dr Juliet Elvy (BMedSci, BMBS, MRCP, FRCPath), Clinical Microbiologist, who will be assisting the PPTC microbiology QAP programme providing clinical guidance and oversight. Juliet is Consultant Clinical Microbiologist for both Wellington SCL and Medlab Nelson Marlborough here in New Zealand. She is also the chair of the RCPA microbiology programme and a key member of the New Zealand National Antimicrobial Testing Committee and the New Zealand Microbiology Network. We thank Juliet for her contribution to the Pacific.

CAN YOU HELP?

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

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

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

Journal Questionnaire

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

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

The site will remain open until Friday 14th February 2020. You must get a minimum of eight questions right to obtain five CPD points. The Editor sets the questions but the CPD Co-ordinator, Jillian Broadbent, marks the answers. Direct any queries to her at cpd@nzimls.org.nz.

NOVEMBER 2019 JOURNAL QUESTIONNAIRE

1. What are the current components and their roles of the Ziehl-Neelsen stain?
2. The S100 calcium-binding protein B belongs to which family of proteins, helps regulate what process, has been identified in high concentrations in which types of cells, and can be detected in which body fluids?
3. How do carbapenemase-producing *Enterobacterales* confer resistance to carbapenem antibiotics?
4. What were the five most common bacteria isolated from students' mobile phones?
5. What conditions could result from a high level of plasma homocysteine, and what could it result in?
6. Hyper-homocysteinemia resulting in endothelial damage has a direct influence on what?
7. Which factors have been identified as important modifiable metabolic syndrome risk factors?
8. The mechanisms through which smoking reduces HDL-C have been linked to alteration of which lipid transport enzymes?
9. Which common haemoglobin variants can interfere with some HbA1c assay methods?
10. Which methodologies have the highest detection rate of haemoglobin variants and derivates, and which have the least detection rate?

AUGUST 2019 JOURNAL QUESTIONNAIRE AND ANSWERS

1. What is the prescribed drug regime during the first two months for a *M. tuberculosis* isolate fully susceptible to first line drugs?
   Daily dose of isoniazid and rifampicin, along with ethambutol and pyrazinamide.
2. What does the WHO define as extensively drug-resistant tuberculosis?
   A multi-drug resistant tuberculosis that is also resistant to any one of the fluoroquinolones and one of the second line injectable drugs.
3. What is the algorithm implemented at LabPlus if there is a high clinical suspicion of tuberculosis and why?
   If there is a high clinical suspicion of TB, a Mycobacteria Growth Indicator Tube (MGIT) culture with a negative immunochromatographic test (ICT) result is further run through Xpert MTB/RIF PCR (with a different target) to rule out the presence of MBTC in the liquid MGIT culture.
4. What are the three most commonly used tuberculosis genotypic methods?
   Insertion sequence 6110 (IS6110) based restriction fragment length polymorphism (RFLP), spacer oligonucleotide typing or spoligotyping, mycobacterium interspersed repetitive units-variable tandem repeat loci (MIRU-VNTR).
5. What strong metabolic indicators are risk factors consistent with metabolic syndrome?
   Glucose intolerance, hyperinsulinaemia, insulin resistance, dyslipidaemia and onset of obesity.
6. What could be the net result of increased plasma free fatty acids and glycerol in spinal cord injured males?
   Increase in hepatic glucose production and a diminished inhibition of glucose production by insulin with the simultaneous increase in skeletal muscle insulin resistance by the elevated free fatty acids.
7. What are some of the well-known factors for recurrent spontaneous miscarriage?
   Infection, chromosome abnormalities, and endocrine, immunological and anatomic factors.
8. The most common autosomal trisomies are in which chromosomes?
   In chromosomes 18, 21 and 13, X and Y.
9. Which techniques are generally used to detect underlying chromosomal abnormalities in spontaneous miscarriage?
   *__Cytogenetic analysis of amniotic fluid, chorionic villus sampling and occasionally fetal blood sampling, or:__
   *__Karyotype analysis, fluorescence in situ hybridization (FISH), quantitative fluorescent PCR (QF PCR) and multiplex ligation-dependent probe amplification (MLPA.__
10. Ibrutinib treatment of relapsed cases of chronic lymphatic leukaemia or small lymphatic lymphoma targets which enzyme, by which mechanism, and what role does this enzyme have?
    Bruton's tyrosine kinase (BTK), by inhibiting this enzyme which plays a role in signalling pathways of B-cell receptor.

*There are two possible answers for question 9. Either answer is correct.

Science Digest

Contributed by Michael Legge

Chocolate and its effects on pregnancy.

The intake of products from *Theobroma cacao* are considered to have potential health benefits resulting from the intake of flavonoids strongly associated with this plant. Chocolate, a product from *Theobroma cacao*, is a source of flavonoids that has been associated with beneficial effects for the cardiovascular system, reduction of insulin resistance, improving lipid profiles and anti-inflammatory effects. These beneficial effects are due primarily to the antioxidant effects of the flavonoids. A publication from Italy investigated the potential effects of chocolate (cacao content ≥70%) in pregnancy (1). After screening for potential pregnancy-related complications 90 women (46 in Group A and 44 in Group B) entered the trail. Group A received 30g of ≥70% chocolate per day and Group B were not given a daily chocolate supplement.

The trial was initiated at first clinic visit (11 to 13 weeks gestation) and continued until delivery at term. Group B received the equivalent energy supplements to Group A. Diets and additional chocolate consumption was monitored by a dietician and any additional chocolate was classified as >60% or < 60% cacao. While there were no significant changes in the haematological parameters in Group A, chocolate had a positive effect on glycaemic and liver parameters and was effective in reducing both blood pressure and cholesterol during the pregnancies. The authors concluded that a moderate amount of high cocoa content chocolate might be a valuable supplement during pregnancy.

What do you know about sunscreens?

Sunscreens are a prominent factor in helping to prevent UV exposure-induced skin cancers and have been strongly advocated for this preventative measure. The use of sunscreens varies and is usually related to the length of time an individual is exposed to UV in sunlight and the area of the body covered. While historically there have been concerns relating to the composition of certain sunscreens, it is generally accepted that the current over the counter (OTC) sunscreens are safe to use and are strongly advocated for reducing the risk of skin cancer. A recent publication from the USA has provided new research information relating to the absorption of photo-protective agents through the skin and at detectable concentrations in the blood (2).

In this research four OTC sunscreens (two sprays, one lotion and one cream from different manufacturers, but not named) containing the active chemicals, avobenzone, oxybenzone, octocrylene and ecamsule were tested. Four groups of volunteers (three men and three women in each group) used one of the four sunscreens applying a set amount every two hours over 75% of their body surface area outside of normal swim wear four times a day for four days. Sequential blood samples were collected for seven days. All blood samples were analysed by liquid chromatography and tandem mass spectrometry.

All four photo-protective chemicals were found in the blood samples. Plasma avobenzone was detected from all four products and exceeded 0.5ng/ml (see below) from day one of use usually within six hours of application. Plasma oxybenzone was identified in only three products and the use of the three containing oxybenzone exceeded 0.5ng/ml within two hours of application and peaked at around 100ng/ml for the duration of the trail and a slow decrease for three days after cessation of use. Plasma octocrylene exceeded 0.5ng/ml peaking at around 5ng/ml through to day seven. Only one product contained ecamsule and plasma concentrations peaked at 1.5ng/ml but generally remained below 1.0ng/ml. While the authors recognized the limitations of this relatively small trail the levels of the compounds detected exceeded the FDA guidelines to trigger a systematic testing of chemicals with health related issues (0.5ng/ml). This concentration was exceeded by day one of the trail. The health impact of these four compounds in this research is not known.

Does phototherapy for neonatal jaundice affect oxidant/antioxidant status?

Hyperbilirubinaemia is a common problem in neonatal care, which may result from many causes. However, because of the potential neurotoxicity of unconjugated bilirubin in neonates due to an immature blood brain barrier and due to the lipid solubility, it can be absorbed in the brain resulting in the most severe outcomes of yellow staining of the brain (kernicterus). Historically neonatal hyperbilirubinaemia has been shown to equate with poor neonatal/paediatric outcomes resulting in loss of hearing and failure to achieve milestones. Phototherapy is an effective, non-invasive, technique for treating what is often a transitory elevation of bilirubin. The principle of phototherapy is that bilirubin photo-oxidises at 420 to 490nm into water-soluble isomers that are excreted.

Recent research from Egypt has investigated the effect of phototherapy on neonatal oxidant/antioxidant status (3). In this research 120 neonates requiring phototherapy (but with normal full-term birth weights) were randomly divided into three groups of 40 and each exposed to one of three phototherapy techniques: conventional (CP), intensive (IP) and LED (LEDP). All neonates had blood taken before treatment and 24 hours post-treatment. Neonatal complications other than hyperbilirubinaemia were excluded from this trial. Besides the biophysical parameters being monitored and plasma unconjugated and conjugated bilirubin, the researchers measured malondialdehyde (MDA), nitric oxide (NO), total antioxidant capacity (TAC) as well as zinc, iron and copper, which all have the potential to mediate free radical reactions.

While all three phototherapy techniques reduced circulating bilirubin to safe concentrations, the researchers found that they differed in the range of oxidant/antioxidant parameters measured. The generation of MDA, NO and TAC had the lowest response to LEDP exposure followed by CP, and IP was third. Copper and iron (pro-oxidants) were more responsive in the IP treatment, with zinc (anti-oxidant) being highest in the LEDP group. While the authors concluded that all phototherapy techniques induced oxidative stress to the neonates, the LEDP treatment had less overall effect than either CP or IP treatments.

Converting blood group A to blood group O.

The use of the ABO blood group O in blood transfusion is acknowledged as a “universal donor” in that conceptually it can be transfused to other recipients with dissimilar ABO blood groups i.e. A, B and AB. While the reverse is not true as group A contains antibodies against B antigen and group B contains antibodies against A antigen. The ability to use group O as a “universal donor” is due to the red blood cells (RBCs) lacking certain carbohydrate structures on their cell surface. Group A has α-1,3-linked-N-acetylgalactosamine (GalNAC) and group B galactose, which confer the specificity of the blood group system. Historically attempts to remove the carbohydrates using enzymes has demonstrated the possibility of converting

blood groups A and B to O. However, the technology is limiting and the quantities of enzymes required is large/unit (mg to gm), rendering this approach impractical.

Research from Canada has reported overcoming some of the major hurdles in conversion of blood groups A and B to O (4). The authors considered that that the gut microbiome should have enzymes capable of removing GalNAC as this carbohydrate is present in gut mucins as a bacterial protective agent. A meta-genomic library was established from faecal samples from a blood group AB positive male donor. Library screening and biochemical analysis identified eleven hits with A antigen cleaving activity and one with B antigen activity. The eleven fosmids were sequenced and the sequences identified the bacteria the fosmids were obtained from. The genes were cloned and expressed in *E. coli* and the resulting proteins purified and characterised.

Two highly active enzymes were identified from *Flavonifractor plautii* which cleaved both A and B antigens. Using bioinformatics analysis, FpGalNAC deacetylase preliminary structure was determined followed by x-ray crystallography, which provided the protein structure and the catalytic domain of the enzyme. When purified enzyme was incubated with group A RBCs the enzyme removed all A antigenicity at a concentration down to 3mg/ml. Using fluorescence activated cell sorting and conventional agglutination techniques the converted group A RBCs were tested against anti-A and anti-H (present on group O RBCs). A complete conversion of A antigens to H antigens was demonstrated. To further confirm this result, blood from 26 blood group A donors were treated with FpGalNAC deacetylase in-vitro and all were converted to H antigens. Finally the researchers demonstrated that the enzymes could be removed by washing the RBCs and centrifugation. They considered that the use of the enzymes demonstrated a possible cost-effective process that would fit current blood transfusion systems.

Evolutionary loss of a gene predisposes humans to atherosclerosis.

Humans are the most prone species to cardiovascular disease (CVD) and atherosclerosis and despite the identity of risk factors, approximately 15% of first time heart attacks occur in the absence of the risk factors. Other animal species with similar risk factors, e.g. carnivores, do not develop CVD. While there is no doubt about the multiplicity of risk factors in humans, the puzzle has been the lack of development in other animal species.

Research from the USA may have provided a new understanding on the biology of atherosclerosis (5). About 2 to 3 million years ago humans lost the ability to synthesis N-glycolylneuramic acid due to the loss of the enzyme cytidine monophosphate –N-acetylneuraminic acid (Neu5Ac) hydrolase (*CMAH*) which results in an excess of sialic acid (Neu5Ac), the precursor for Neu5Gc in the glycocalyx in human cells.

Deficiency of Neu5Gc is implicated in glucose intolerance and hyperactive macrophages. In addition Neu5Gc is obtained from red meat and once absorbed is incorporated into glycolipids and glycoproteins. Although the amounts are small they accumulate in the epithelium and endothelium, initiating a xeno-antigen response, which in turn promotes local chronic inflammation (most humans have circulating xeno-antigens to Neu5Gc).

Using a mouse model for human atherosclerosis the authors inactivated the *Cmah* gene and demonstrated development of atherosclerosis. They then gave the mice non-human Neu5Gc (equating with red meat consumption) and demonstrated a high immune response and a diabetic phenotype. The mice also demonstrated an increase in foam cells and a decrease of phagocytosis activity. The authors conclude that the lack of the *CMAH* gene in humans helps to explain the significant difference between humans and other species for the susceptibility to atherosclerosis and CVD but indicate that further research is required relating to the metabolism of extrinsic Neu5Gc and its potential for initiating an immune response.

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Journal Reviewers August 2018—July 2019

The listed persons below reviewed submitted articles to the Journal between August 2018 and July 2019, some more than once. All submitted articles undergo peer review in order that the Journal maintains its high standard. Additionally, thoughtful comments and suggestions made by reviewers help authors in ensuring that their articles, if accepted, are put in front of the reader in the best possible light. The Editors thanks the reviewers for their valuable time and effort, and A/Prof Nevil Pierse, Wellington for statistical review of submitted articles.

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